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Donor killer-cell immunoglobulin-like receptor *B* haplotypes, recipient HLA-C1 and HLA-C mismatch enhance the clinical benefit of unrelated transplantation for acute myelogenous leukemia[1]

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Abstract

Killer cell immunoglobulin-like receptors (KIR) interact with HLA class I ligands to regulate NK cell development and function. These interactions affect the outcome of unrelated donor (URD) hematopoietic cell transplantation (HCT). We have shown previously that donors with *KIR B* vs. *KIR A* haplotypes improve the clinical outcome for patients with acute myelogenous leukemia (AML) by reducing the incidence of leukemic relapse and improving leukemia free survival (LFS). Both centromeric and telomeric *KIR B* genes contribute to the effect, but the centromeric

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Sarah Cooley, Daniel J. Weisdorf, Lisbeth A. Guethlein, Peter Parham and Jeffrey S. Miller designed this study, analyzed data and wrote the manuscript.
John P. Klein and Tao Wang performed the biostatistical analysis for this study and wrote the manuscript.
Steven G.E. Marsh, Stephen Spellman, and Michael D. Haagenson provided clinical data integration, assisted with data interpretation and assisted in writing the manuscript.
Martha Ladner, Koy Saeteurn and Elizabeth Trachtenberg performed all *KIR* genotyping, were involved in data analysis and assisted in writing the manuscript.

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genes are dominant. They include the genes encoding inhibitory KIR that are specific for the C1 and C2 epitopes of HLA-C. We used an expanded cohort of 1532 T-cell replete transplants to examine the interaction between donor *KIR B* genes and recipient Class I HLA KIR ligands. The relapse protection associated with donor *KIR B* is enhanced in recipients who have one or two C1-bearing HLA-C allotypes, compared to C2 homozygous recipients, with no effect based on donor HLA. The protective interaction between donors with ≥2 vs. 0–1 *KIR B*-motifs and recipient C1 was specific to transplants with class I mismatch at HLA-C (RR of LFS 0.57 [0.40–0.79]; P=0.001) irrespective of the KIR ligand mismatch status of the transplant. The survival advantage and relapse protection in C1/x recipients compared to C2/C2 recipients was similar irrespective of the particular donor *KIR B* genes. Understanding the interactions between donor KIR and recipient HLA class I can be used to inform donor selection to improve outcome of URD HCT for AML.

Introduction

The interactions of variable killer-cell immunoglobulin-like receptors (*KIR*) with polymorphic HLA class I ligands form an extraordinary immunogenetic system that influences NK cell biology, human susceptibility to disease, and the success of hematopoietic cell transplantation (HCT) as therapy for acute myelogenous leukemia (AML). A key feature of this system is that the *KIR* and *HLA* genes are on different chromosomes and thus segregate independently in human populations. This serves to increase the functional diversity of the system and has important consequences for HCT. Unrelated donors (URD) and recipients who are HLA-identical almost never have identical KIR genes. In fact, even in families, only 25% of HLA-identical siblings are also KIR identical (1).

KIR recognize four polymorphic epitopes of HLA-A, B and C molecules. These epitopes, defined by amino-acid substitutions in residues 76–83 of the α_1 helix of the HLA class I heavy chain, are also called KIR ligands. The C1 and C2 epitopes are carried by different subsets of HLA-C allotypes, the Bw4 epitope is carried by subsets of HLA-A and –B allotypes and the A3/11 epitope is carried by the HLA-A*03 and –A*11 allotypes. Each of the four epitopes is recognized by different inhibitory KIR which are encoded by polymorphic genes. The C2 epitope is also recognized by the activating receptor encoded by *KIR2DS1*. Additional members of the *KIR* gene family encode proteins whose functions are yet to be determined. In addition to the polymorphism of individual genes, the *KIR* locus exhibits haplotypic gene-content variation. The basis for this component of *KIR* variation is the presence of two groups of *KIR* haplotype: *KIR-A* and *KIR-B* haplotypes in all human populations. The *KIR-A* haplotypes have conserved gene content and encode mainly inhibitory receptors, whereas *KIR-B* haplotypes have varied gene content that includes a variety of activating receptors of unknown function. Further details of *HLA* and *KIR* immunogenetics are provided in the Materials and Methods section.

The potential value of NK cell responses in HCT was first demonstrated by Ruggeri et al(2). These investigators observed that certain HLA-B and -C incompatibilities reduce relapse and improve the survival of AML patients receiving a haploidentical, T-cell depleted transplant from a related family members(3, 4). For these transplants, in which donor and

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recipient share one HLA haplotype but are mismatched for the other haplotype, a beneficial alloreactive response occurs when the donor has a KIR ligand, Bw4, C1 or C2, not present in the recipient. In this situation, subsets of donor-derived NK cells can attack and kill recipient cells because they are missing self-HLA class I. Velardi and colleagues proposed that reduced relapse was due to NK-cell killing of residual leukemia cells that had survived the myeloablative conditioning regimen. They also proposed that the reduced graft-versus-host disease (GVHD) they observed was caused by NK-cell killing of recipient dendritic cells(5). These pioneering observations led to investigations of various other types of transplant examining the effects of alloreactive NK cells and the HLA-A, -B and –C epitopes recognized by KIR(6–10). A general observation emerging from these subsequent studies is that NK cell effects in HCT are principally seen in patients transplanted for AML. A second observation is that the nature of NK cell effects varies considerably and is influenced by factors that include the intensity of the preparative regimen, the extent of HLA match, donor type (sibling or URD) and the source, processing method and T-cell content of the stem cell graft (8, 9). Third, it has been reported that C2/C2 homozygous patients with AML have more relapse (11, 12).

Whereas other studies concentrated on the polymorphic HLA class I ligands that are recognized by KIR, we have studied variation of the *KIR* gene family and its effect on HCT. For AML patients transplanted with a T-cell replete transplant from an unrelated donor (URD), we found that clinical outcome was better when the donors have one or two *KIR-B* haplotypes (*KIR-B/x* donors) than for donors who have two *KIR-A* haplotypes (*KIR-A/A* donors). With a *KIR-B/x* donor, relapse was reduced and leukemia-free survival (LFS) was increased(13). In a subsequent study we sought to determine whether the protective effect of *KIR-B* could be mapped to either the centomeric or the telomeric region of the *KIR* locus. The centromeric region contains genes encoding the inhibitory receptors for the C1 and C2 epitopes of HLA-C, whereas the telomeric region contains genes encoding the inhibitory receptors for the Bw4 and A3/11 epitopes and the activating C2 receptor. We found that both the centromeric and telomeric regions of *KIR-B* correlated with protective effect, but the much stronger association was with the centromeric region(14). The beneficial effects associated with *KIR B* haplotype donors were consistent in both HLA-matched and HLA-mismatched transplants. Because the genes encoding the inhibitory C1 and C2 receptors are located in the centromeric regions, we have investigated the influence of the C1 and C2 epitopes on the protection provided by donor *KIR-B* haplotypes in HCT.

Materials and Methods

Patient cohort

We studied 1532 patients with AML, including 1086 previously analyzed(14), who received myeloblative preparation for an unrelated donor (URD) HCT facilitated by the National Marrow Donor Program (NMDP) between 1988 and 2009. DNA samples were obtained from the NMDP Research Sample Repository. Outcome data were obtained from the Center for International Blood and Marrow Transplant Research (CIBMTR). Complete high-resolution *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1* and *HLA-DQB1* typing data were obtained from the NMDP retrospective typing project. The demographics, *KIR* genotypes, and

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multivariate statistical analysis of the clinical data have been described(13, 14). *KIR* genotyping using MALDI-TOF mass spectroscopy was performed as previously described(15, 16). DNA samples and clinical data were obtained with informed consent and approval from the NMDP and University of Minnesota Institutional Review Boards.

HLA and KIR immunogenetics

Complete high-resolution, allele-level *HLA-A, HLA-B, HLA-C, HLA-DRB1* and *HLA-DQB1* typing data were obtained from the NMDP retrospective typing project. *KIR* typing at the level of *KIR* gene content was performed using MALDI-TOF mass spectroscopy as described previously(15, 16). Four epitopes of HLA-A, -B and –C molecules are recognized by KIR. The epitopes, also called KIR ligands, are situated on the upward face of the HLA class I molecule and involve the amino-terminal part of the α_1 helix and the carboxy-terminal parts of the bound peptide and the α_2-helix(17). The epitopes are mutually exclusive, such that each HLA-A, -B or –C molecule either carries one of the four epitopes or no epitope at all. Every HLA-C allotype is a KIR ligand whereas only 43% of HLA-A and 35% of HLA-B allotypes are KIR ligands. The KIRs are named according to the number of extracellular Ig-like domains, either 2 or 3, and the length of the cytoplamic tail, either long (L) or short (S), correlating respectively with inhibitory and activating signaling function(18). The C1 and C2 epitopes carried by HLA-C are distinguished by lysine and methionine residues at position 80, respectively(17). The C1 epitope is recognized by the inhibitory KIR2DL2/3 receptor, whereas the C2 epitope is recognized by inhibitory KIR2DL1 and activating KIR2DS1 receptors. The Bw4 epitope, carried by 27% of HLA-A and 35% of HLA-B allotypes, is recognized by inhibitory KIR3DL1(19, 20). The A3/11 epitope, carried by 16% of HLA-A allotypes is recognized by inhibitory KIR3DL2. The C1, C2 and Bw4 epitopes play major roles in NK-cell regulation. Such a role has not been demonstrated for the A3/11 epitope(21) which is unusually dependent upon the sequence of the peptide bound to HLA-A*03 or HLA-A*11(22). For this reason the A3/11 epitope was not included in the analyses of the transplant donors and recipients studied here. In the studies described in this paper, the impact of recipient C1 on transplant outcome dominated C2. In some analyses, recipients with *C2/C2* genotype were compared to recipients with either *C1/C1* or *C1/C2* genotypes. The combined group of *C1/C1* or *C1/C2* recipients was designated *C1/x*.

The *KIR* locus is part of the leukocyte receptor complex on human chromosome 19 and segregates independently of the HLA class I genes in the MHC on chromosome 6. A *KIR* haplotype is the set of *KIR* genes that are linked together on the same chromosome. Haplotypes contain 7–15 *KIR* genes and are 129–215kb in length(23). Every individual has a maternally inherited and a paternally inherited *KIR* haplotype that together form his or her *KIR* genotype. Conserved genes in the center of the locus and at the two ends divide the locus into centromeric (*Cen*) and telomeric (*Tel*) regions, each of which exhibits variable content of *KIR* genes. In both regions there are two distinctive types of variable gene-content motif. These are designated *Cen-A*/*Cen–B*, and *Tel-A/Tel-B*. Further variations within these four motifs are differentiated by numbers; e.g. *Cen-B1* and *Cen-B2*. The *A* motifs are shorter, more conserved and consist mainly of genes for inhibitory KIR that recognize the C1, C2 and Bw4 epitopes. The *B* motifs are longer, more variable and contain one or more

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of seven *KIR B*-specific genes(23). These comprise *KIR2DS2* and *KIR2DL2* in *Cen-B*, *KIR2DS1* and *KIR3DS1* in *Tel-B*, and *KIR2DS3/5* and *KIR2DL5* in either *Cen-B* or *Tel-B*, or both. Of the KIR encoded by the *B*-specific genes, only KIR2DL2 (C1-specific) and KIR2DS1 (C2-specific) recognize HLA class I. Haplotypes that consist of a *Cen-A* motif and a *Tel-A* motif are called *KIR A* haplotypes and haplotypes consisting of a *Cen-B* and a *Tel-B* motif are called *KIR B* haplotypes. Recombinant haplotypes, which consist of either *Cen-A* and *Tel-B* or *Cen-B* and *Tel-A*, are also included in the *KIR B* haplotypes because of the dominant effect of the *B* motifs in disease, transplantation and other clinical associations.

In this study we characterized transplant donors according to their *KIR B*-motif content, a parameter that varies from 0–4 and is simply the sum of the number of *Cen-B* and *Tel-B* motifs in the donor's *KIR* genotype. Based upon the results of our previous study (2), donors are classified as "Neutral" (0–1 *KIR B*-motifs),"Better" (≥2 B-motifs without *Cen-B/B*), or "Best" (≥ 2 B-motifs with *Cen-B/B*)(14). In some analyses, the "Better" and "Best" groups were combined to form the *KIR-Better/Best* donor group (with ≥ 2 B-motifs) (Table I).

Statistical Analysis

We considered five clinical outcomes of HCT: leukemia free survival (LFS), relapse, treatment-related mortality (TRM), grade II–IV or III–IV acute graft-versus-host disease (aGVHD) and chronic graft-versus-host disease (cGVHD). Kaplan-Meier curves were used to evaluate LFS, whereas cumulative incidence functions were used to evaluate the other outcomes. Unadjusted comparisons between *KIR* genotypes were made on either the hazard rates for LFS or the crude hazard rates for relapse, TRM, aGVHD and cGVHD. In the cohort we studied, the completeness of follow-up at three years after transplantation was over 98.8%. At this time, 90% of events had occurred. Cox proportional hazard models were used to adjust for important clinical factors. Proportional hazards were checked in a time-dependent covariate model. Factors that violated proportional assumptions were adjusted via stratification. Forward stepwise regression modeling was used to identify clinical and patient factors that needed adjustment using a 5% significance level. Adjusted factors include patient age, cytogenetic risk, sex match, HLA matching, graft source, CMV serostatus, race, Karnofsky score, GVHD prophylaxis, the use of total body irradiation (TBI), time from diagnosis to transplant, disease status and year of transplantation. Cases were excluded from some models if data for the outcome or significant covariates were missing. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

Results

KIR-Better/Best donors improve recipient survival and reduce relapse in patients transplanted for AML

The cohort of myeloablative, T-cell replete unrelated donor (URD) transplants we studied included adult and pediatric patients with early, intermediate and advanced AML. Fifty-six percent (n=856) of the donor-recipient pairs were 10/10 *HLA*-allele matched for *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, and *HLA-DQB1;* the rest (n=676) had varying degrees of *HLA* mismatch: 407 had one mismatch, 173 had two mismatches, and 85 had three or more

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mismatches. Whereas 357 (53%) of the *HLA*-mismatched transplants involved an *HLA-C* mismatch, only 110 of them were also KIR ligand mismatched in the graft-versus-host direction. Approximately 53% (n=810) of the recipients received bone marrow (BM) grafts, whereas the others received grafts of stem cells present in peripheral blood (PB) mobilized with granulocyte-colony stimulating factor (G-CSF). Additional information describing the transplant characteristics and *HLA* matching of the cohort is provided in Supplemental Tables I and II.

Transplant donors were typed for the presence or absence of each of the 15 *KIR* genes. From these *KIR* genotype data, donors were assigned as either *A/A* or *B/x* based upon the absence or presence of *KIR B*-specific genes(13). Each donor was also assigned to one of three groups based on the number of centromeric and telomeric *B*-motifs in the *KIR* genotype: "Neutral" (0 or 1 *B*-motif), "Better" (≥2 *B*-motifs without *Cen-B/B*) or "Best" (≥2 *B*-motifs with *Cen-B/B*) (Table I)(23). Analysis of clinical outcome for this cohort of myeloablative transplants confirmed our previous observations that improved LFS and protection from relapse are associated with transplant donors having ≥2 *B*-motifs. These comprise the combination of the "Better" and "Best" donor groups; *KIR-Better/Best*(13, 14). Compared to *KIR-Neutral* donors who have one or no *KIR B*-motifs, a 30% reduction in the risk of relapse (RR 0.70 [0.57–0.86]; P=0.0005) was associated with *KIR-Better/Best* donors, which gave improved LFS (RR 0.79 [0.69–0.91]; P=0.001) (Table II: A and B). The magnitude of the protection was similar for HLA-matched and HLA-mismatched transplants. Compared to *KIR-neutral* donors, the *KIR-Better/Best* donors improved LFS (RR 0.83 [0.69–1.01]; P=0.063 and RR 0.76 [0.62–0.93]; P=0.0078, for HLA-matched and HLA-mismatched transplants, respectively) and decreased the risk of relapse (RR 0.72 [0.55–0.94]; P=0.016 and RR 0.49 [0.57–0.93]; P=0.016, respectively).

Recipient C1 contributes to the benefit provided by a KIR B donor by decreasing the likelihood of relapse

We investigated the mechanism underlying the beneficial effect of *KIR B* donors in URD transplantation for AML. Multivariate analyses tested the involvement of interactions between donor KIR and the Bw4, C1 and C2 epitopes of donor or recipient HLA class I. In these analyses we distinguished between *C2/C2* individuals (N=238), who lack the C1 epitope, and *C1/x* individuals (N=1294) who are hetrozygous or homozygous for HLA-C bearing the C1 epitope. *C1/x* recipients had improved LFS when transplanted with grafts from *KIR-Better/Best* compared to *KIR-Neutral* donors (RR 0.78 [0.67–0.91]; P=0.0015; Table III, Figure 1:A1). A similar beneficial effect was not observed for *C2/C2* recipients (RR 0.93 [0.63–1.37]; P=0.71; Table III, Figure 2: A1). Factors that may contribute to the improved LFS are reductions in leukemic relapse, treatment related mortality (TRM) and GVHD, either singly or in combination. Our analyses demonstrate that reduced incidence of leukemia relapse is the predominant protective effect. No significant correlations were observed with the risks of TRM, acute GVHD or chronic GVHD. Thus, *C1/x* recipients paired with *KIR-Better/Best* donors experienced significantly less relapse than *C1/x* recipients with *KIR-Neutral* donors (RR 0.70 [0.56–0.87]; P=0.0018; Table III, Figure 1: B1). Five years after transplantation, the frequencies of relapse in *C1/x* recipients based on donor *KIR* were 27% vs. 38%, respectively. Although a 4% absolute relapse protection was

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observed in *C2/C2* recipients receiving grafts from *KIR-Better/Best* vs. *KIR-Neutral* donors, this trend was not statistically significant (Table III, Figure 2: B1). In all these analyses of the interactions between donor KIR and the Bw4, C1 and C2 epitopes of HLA class I, significant benefits were observed only with C1 epitopes of recipient HLA-C. No significant interactions with donor KIR were demonstrated with recipient C2 and Bw4 or with donor Bw4, C1 and C2.

An HLA-C mismatch further reduces relapse for transplants with KIR B donors and C1/x recipients

Our study cohort consisted of similar numbers of HLA-matched (57%) and HLA-mismatched (43%) transplants. This balance enabled a robust evaluation of the effects of HLA mismatch on the interactions of donor KIR with recipient HLA class I. In this analysis, the effects of *KIR-Better/Best* donors were always compared to those of *KIR-Neutral* donors (Table III). For HLA-matched transplantation, *KIR-Better/Best* donors increased LFS and reduced relapse for *C1/x* recipients compared to *C2/C2* recipients (Table III, Figure 1: A2 and B2, Figure 2: A2 and B2) but the difference was not significant. For HLA-mismatched transplants, a stronger, statistically significant effect was observed involving *KIR-Better/Best* donors and *C1/x* recipients. Compared to *KIR-Neutral* donors, LFS was enhanced (RR 0.70 [0.56–0.88]; P=0.003) and relapse was reduced (RR 0.61 [0.43–0.88]; P=0.008; Table III, Figure 1: A3 and B3). Again, *C2/C2* recipients derived no significant benefit from a *KIR-Better/Best* donor (Table III, Figure 2: A3 and B3).

Having demonstrated the beneficial effect of an HLA mismatch on the interaction between donor KIR B and recipient C1, further analyses were performed on the set of 676 HLA-mismatched transplants to determine which HLA genes were involved. We first compared the effects of HLA class I and II mismatch. Improved LFS and relapse protection were observed for *C1/x* recipients receiving transplants from *KIR-Better/Best* donors in the subset of 457 HLA-class I mismatched transplants (RR 0.69 [0.54–0.88]; P=0.0029, and RR 0.62 [0.42–0.92]; P=0.019, respectively: Table IV A), but not in the subset of 81 HLA class II mismatched transplants (Table IVA). No differences between HLA class I and class II mismatched transplants were seen in the *C2/C2* recipients (data not shown). To identify the specific HLA class I gene responsible for the interaction, we next compared the outcomes for transplants mismatched at HLA-A, or –B (N = 180) or at HLA-C (N=277). The added benefit of an HLA mismatch for a transplant involving a *KIR-Better/Best* donor and a *C1/x* recipient was observed only for HLA-C mismatched transplants (RR 0.57 [0.40–0.79]; P=0.001, and RR 0.54 [0.33–0.88]; P=0.013, respectively, Table IV B). Again, no differences were observed in the *C2/C2* recipients (data not shown). We next determined whether the benefit of an HLA-C mismatch is the consequence of a KIR-ligand mismatch between transplant donor and recipient. In the circumstance of KIR-ligand mismatch, when the donor expresses C1 or C2 ligand which is lacking in the recipient, donor NK cells can respond alloreactively to the recipient's cells because they are missing self inhibitory signals. We compared LFS and relapse risk between transplants which included mismatches at HLA-C (n= 460) vs. those with KIR-ligand mismatches based on C1 and C2 (n=60) for the *C1/x* recipient group. In this small subset, KIR-ligand mismatched transplants were not associated with additional protection (data not shown), demonstrating that KIR-ligand

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mismatch does not contribute added benefit to other types of HLA-C mismatch. In previous analyses of HLA alone, the degree of HLA-matching correlates with better transplant outcomes(24). Consideration here of the interaction between the *HLA* and *KIR* gene systems has shown a benefit for mismatching HLA-C in the particular context of transplantation involving *KIR-Better/Best* donors and *C1/x* recipients.

All KIR B genes contribute to improved clinical outcome associated with KIR B/x donors

Next we examined the extent to which each of the seven *KIR B* genes individually affected the outcomes associated with the HLA-C1, C2 and Bw4 KIR-ligand status of the recipients. The relative risks for each outcome were determined for 7 groups of *KIR B/x* donors in comparison to *KIR A/A* donors. Each of these groups corresponded to the subset of donors carrying one of the 7 *KIR* genes specific to the *KIR-B* haplotype. Because most *KIR B/x* donors have more than one *KIR B* haplotype-specific gene, each donor is represented in more than one of the 7 groups.

In the full cohort of transplants, *C1/x* recipients benefited from increases in LFS associated with all 7 *KIR B/x* donor groups compared to *KIR A/A* donors (Table V:A). Only *KIR2DS1* and *KIR3DS1* were associated with about a 20% reduction against relapse (p=0.052 and 0.044 respectively). A striking difference was noted based on the HLA match status of the transplant, as significantly improved LFS and relapse protection were seen only for HLA-mismatched transplants. There was no effect in matched transplants (Table V: B, C). In the HLA-mismatched transplants, multivariate analyses showed that each of the seven *KIR B* genes contributed clinical benefit of similar magnitude; RR ranged from 0.65–0.80 (P=0.0032–0.055) for LFS and from 0.57–0.70 (P=0.0038–0.036) for relapse (Table V: C). In contrast, for transplantation of *C2/C2* recipients, the clinical outcomes were similar for all 7 groups of *KIR B/x* donors, where *KIR B/x* donor group has no effect on survival or relapse protection (Table VI). Consequently, no particular donor *KIR B* haplotype genes interact with recipient C1 to increase LFS or reduce relapse.

Discussion

In the present study, which was designed to determine whether the differential clinical effects of donor centromeric and telomeric encoded KIR could involve interactions with HLA-Bw4, HLA-C1 and HLA-C2, we analyzed a cohort of patients who received HLA-matched and mismatched URD grafts without T-cell depletion following myeloablative preparative regimens. In this large cohort, *KIR B* donors reduced relapse and improved LFS in both HLA-matched and mismatched transplants. We now demonstrate a significantly protective interaction between donor KIR and recipient C1. In *C1/x* recipients, *KIR-Better/Best* donors were associated with improved LFS, attributed to an 11% reduction in relapse rate. The protective effect of this interaction was strongest in the HLA-mismatched transplants, specifically those with class I mismatch for at the C locus. Thus, we have demonstrated that donor *KIR B*, recipient C1, and an HLA-C mismatch between donor and recipient are all factors that interact to reduce leukemia relapse and increase the LFS after URD HCT as treatment for AML. The correlation of donor *KIR B* and recipient C1 with protection from relapse raises the strong possibility that interactions between C1 epitopes

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and the C1-reactive KIR encoded by *KIR B* haplotypes is a molecular mechanism underlying the improved transplant outcome. Inhibitory KIR2DL2 is the only C1 receptor encoded by *KIR B*. Moreover, the *KIR2DL2* gene, in combination with the *KIR2DS2* gene, defines the common *Cen-B* motif that in homozygous form defines the *KIR-Best* transplant donors(14). These correlations are consistent with the interaction of C1 with KIR2DL2 being an important contributor to the observed clinical benefits.

While the data support a model in which an interaction between recipient C1 and donor KIR2DL2 enhances NK cell education and improves clinical outcome, we must consider the alternatives. Three *KIR* genes encode receptors that discriminate HLA-C1 and HLA-C2. These comprise the inhibitory C1 receptor KIR2DL2/3, the inhibitory C2 receptor KIR2DL1 and the activating C2 receptor KIR2DS1. Although KIR2DL2 and KIR2DL3 are both inhibitory receptors that recognize C1, KIR2DL2 is specific to *Cen-B* haplotypes and KIR2DL3 is specific to *Cen-A* haplotypes. These receptors differ in four potentially important ways. First, KIR2DL2 has higher avidity for C1 than KIR2DL3, which can affect the education of NK cells mediated by the C1 ligand(25). Second, KIR2DL2 has crossreactivity with C2(25, 26) which can alter NK cell education and produce NK cells that are educated by and responsive to both C1 and C2. The presence of the *KIR2DL2* gene causes a major reduction in frequency of NK cells expressing KIR2DL1(27). This mechanism is independent of the presence or absence of the C1 or C2 epitope and is a potential mechanism by which *KIR B* and *Cen-B* can mediate beneficial clinical effects in the absence of C1. Fourth, the *KIR2DL1* alleles that are in linkage disequilibrium with the *KIR2DL2* gene are functionally weaker in ligand-binding affinity or signaling function(28) than those in linkage disequilibrium with *KIR2DL3* (23, 28, 29).

The differences between KIR2DL2 and KIR2DL3 may not account for all the beneficial clinical effect associated with *KIR B* donors. KIR2DS1 is specific to *KIR B* haplotypes, and the KIR2DL1 allotypes carried by *B* haplotypes expressed at lower frequencies by NK cells(27). We have previously demonstrated a benefit, albeit less significant, associated with *Tel-B* in the absence of *Cen-B*(14). That finding is consistent with this analysis of individual *KIR-B* specific genes. We have shown that all seven genes contributed equally to the clinical benefit, specifically in *C1/x* but not *C2/C2* recipients. It has previously been reported that *C2/C2* homozygous patients with AML had more relapse after HLA-C matched URD HCT (12) and HLA-matched sibling HCT(11).

We also observed significant relapse protection associated with the telomeric *KIR2DS1* and *KIR3DS1* in the total cohort, consistent with other reported associations with those KIR and improved transplant outcome(30, 31). Venstrom et al have reported that donor *KIR2DS1* is associated with reduced relapse and better LFS for AML patients who received an URD HCT that is HLA matched or has a single HLA mismatch(30). Based on a mechanism described by Fauriat et al in which NK cells expressing only KIR2DS1 from healthy *C2/C2* donors are hyporesponsive (32), Venstrom et al propose that those cells recognize leukemia and improve LFS via recognition of missing self when transplanted into *C1/x* recipients.

Because of the compact arrangement of genes in the *KIR* locus and the extensive linkage disequilibrium between *KIR* genes, these data should be interpreted with caution(33). For

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example, *KIR2DS2* and *KIR2DL2* in *Cen-B* haplotypes are in almost complete linkage disequilibrium. Several characteristics of the *KIR* system, including the coordinated transcription of the *KIR* genes, their variegated expression, and the haplotypic gene-content variation support a model in which the *KIR* genotype reduces the effect of individual genes. Additionally, *KIR* gene content analyses alone could be misleading given that the differences between KIR allotypes affect the affinity for HLA class I ligands as well as signaling function(25, 28, 34, 35). Application of high resolution typing of *KIR* alleles will investigate this possibility. It is also important to emphasize that we can address only the modulation of NK cell education and function by polymorphic KIR and HLA and not by the contributions of the many conserved receptors and ligands that affect these processes(36). Lastly, one must consider the possibility that allogeneic disparity contributes to the graft-versus-leukemia protection mediated by T cells. This could be mediated directly, by an allogeneic response that provides T cell help to NK cells, or indirectly through reciprocal activation of dendritic cells and NK cells that function to bridge the innate and adaptive immune response(37). With those caveats, we propose that the many differences between centromeric and telomeric *KIR A and B* haplotype receptors result in substantial influences on NK-cell education and repertoire development, which in turn alters NK-cell mediated graft-versus-leukemia reactions following URD HCT for AML.

Independent of the underlying molecular mechanism, there is a general consensus that *KIR B/x* donors improve outcome for AML patients receiving T-cell containing, myeloablative HCT. We have demonstrated that interactions with HLA-C1 augment the effect of a *KIR B/x* donor, specifically by enhancing relapse protection, most significantly in transplants mismatched at HLA-C. For the 15% of recipients who are *C2/C2*, our analysis did not detect additional improvements in survival or reductions in relapse based in interactions with *KIR B* donors. Larger studies will be needed to test the validity of this result. Understanding the interactions between *KIR B*/x donors and recipient HLA-C1 is particularly important because a considerable majority (~85%) of US transplant recipients are *HLA-C1/x*. The findings presented here are being further tested in our ongoing multi-center prospective study incorporating *KIR* genotyping into URD selection for AML coordinated through the National Marrow Donor Program and Center for International Blood and Marrow Transplant Research (clinicaltrials.gov NCT01288222).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Shilling HG, Young N, Guethlein LA, Cheng NW, Gardiner CM, Tyan D, Parham P. Genetic control of human NK cell repertoire. J Immunol. 2002; 169:239–247. [PubMed: 12077250]
2. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A. Effectiveness of donor natural killer cell

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alloreactivity in mismatched hematopoietic transplants. Science. 2002; 295:2097–2100. [PubMed: 11896281]

3. Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, Urbani E, Negrin RS, Martelli MF, Velardi A. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999; 94:333–339. [PubMed: 10381530]
4. Ruggeri L, Mancusi A, Capanni M, Urbani E, Carotti A, Aloisi T, Stern M, Pende D, Perruccio K, Burchielli E, Topini F, Bianchi E, Aversa F, Martelli MF, Velardi A. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood. 2007; 110:433–440. [PubMed: 17371948]
5. Ruggeri L, Mancusi A, Burchielli E, Aversa F, Martelli MF, Velardi A. Natural killer cell alloreactivity in allogeneic hematopoietic transplantation. Curr Opin Oncol. 2007; 19:142–147. [PubMed: 17272987]
6. Hsu KC, Gooley T, Malkki M, Pinto-Agnello C, Dupont B, Bignon JD, Bornhauser M, Christiansen F, Gratwohl A, Morishima Y, Oudshoorn M, Ringden O, van Rood JJ, Petersdorf E. KIR ligands and prediction of relapse after unrelated donor hematopoietic cell transplantation for hematologic malignancy. Biol Blood Marrow Transplant. 2006; 12:828–836. [PubMed: 16864053]
7. Malmberg KJ, Schaffer M, Ringden O, Remberger M, Ljunggren HG. KIR-ligand mismatch in allogeneic hematopoietic stem cell transplantation. Mol Immunol. 2005; 42:531–534. [PubMed: 15607809]
8. Giebel S, Locatelli F, Lamparelli T, Velardi A, Davies S, Frumento G, Maccario R, Bonetti F, Wojnar J, Martinetti M, Frassoni F, Giorgiani G, Bacigalupo A, Holowiecki J. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. Blood. 2003; 102:814–819. [PubMed: 12689936]
9. Farag SS, Bacigalupo A, Eapen M, Hurley C, Dupont B, Caligiuri MA, Boudreau C, Nelson G, Oudshoorn M, van Rood J, Velardi A, Maiers M, Setterholm M, Confer D, Posch PE, Anasetti C, Kamani N, Miller JS, Weisdorf D, Davies SM. 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; 12:876–884. [PubMed: 16864058]
10. Leung W. Use of NK cell activity in cure by transplant. Br J Haematol. 2011; 155:14–29. [PubMed: 21812770]
11. Cook MA, Milligan DW, Fegan CD, Darbyshire PJ, Mahendra P, Craddock CF, Moss PA, Briggs DC. The impact of donor KIR and patient HLA-C genotypes on outcome following HLA-identical sibling hematopoietic stem cell transplantation for myeloid leukemia. Blood. 2004; 103:1521–1526. [PubMed: 14504099]
12. Giebel S, Locatelli F, Wojnar J, Velardi A, Mina T, Giorgiani G, Krawczyk-Kulis M, Markiewicz M, Wylezol I, Holowiecki J. Homozygosity for human leucocyte antigen-C ligands of KIR2DL1 is associated with increased risk of relapse after human leucocyte antigen-C-matched unrelated donor haematopoietic stem cell transplantation. Br J Haematol. 2005; 131:483–486. [PubMed: 16281939]
13. Cooley S, Trachtenberg E, Bergemann TL, Saeteurn K, Klein J, Le CT, Marsh SG, Guethlein LA, Parham P, Miller JS, Weisdorf DJ. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. Blood. 2009; 113:726–732. [PubMed: 18945962]
14. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, Marsh SG, Geraghty D, Spellman S, Haagenson MD, Ladner M, Trachtenberg E, Parham P, Miller JS. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010; 116:2411–2419. [PubMed: 20581313]
15. Houtchens KA, Nichols RJ, Ladner MB, Boal HE, Sollars C, Geraghty DE, Davis LM, Parham P, Trachtenberg EA. High-throughput killer cell immunoglobulin-like receptor genotyping by MALDI-TOF mass spectrometry with discovery of novel alleles. Immunogenetics. 2007; 59:525–537. [PubMed: 17464504]
16. Hollenbach JA, Ladner MB, Saeteurn K, Taylor KD, Mei L, Haritunians T, McGovern DP, Erlich HA, Rotter JI, Trachtenberg EA. Susceptibility to Crohn's disease is mediated by KIR2DL2/

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KIR2DL3 heterozygosity and the HLA-C ligand. Immunogenetics. 2009; 61:663–671. [PubMed: 19789864]

17. Boyington JC, Sun PD. A structural perspective on MHC class I recognition by killer cell immunoglobulin-like receptors. Mol Immunol. 2002; 38:1007–1021. [PubMed: 11955593]
18. Valiante NM, Uhrberg M, Shilling HG, Lienert-Weidenbach K, Arnett KL, D'Andrea A, Phillips JH, Lanier LL, Parham P. Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. Immunity. 1997; 7:739–751. [PubMed: 9430220]
19. Gumperz JE, Litwin V, Phillips JH, Lanier LL, Parham P. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. J Exp Med. 1995; 181:1133–1144. [PubMed: 7532677]
20. Gumperz JE, Barber LD, Valiante NM, Percival L, Phillips JH, Lanier LL, Parham P. Conserved and variable residues within the Bw4 motif of HLA-B make separable contributions to recognition by the NKB1 killer cell-inhibitory receptor. J Immunol. 1997; 158:5237–5241. [PubMed: 9164941]
21. Thananchai H, Gillespie G, Martin MP, Bashirova A, Yawata N, Yawata M, Easterbrook P, McVicar DW, Maenaka K, Parham P, Carrington M, Dong T, Rowland-Jones S. Cutting Edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J Immunol. 2007; 178:33–37. [PubMed: 17182537]
22. Hansasuta P, Dong T, Thananchai H, Weekes M, Willberg C, Aldemir H, Rowland-Jones S, Braud VM. Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific. Eur J Immunol. 2004; 34:1673–1679. [PubMed: 15162437]
23. Pyo CW, Guethlein LA, Vu Q, Wang R, Abi-Rached L, Norman PJ, Marsh SG, Miller JS, Parham P, Geraghty DE. 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:e15115. [PubMed: 21206914]
24. Petersdorf EW. Optimal HLA matching in hematopoietic cell transplantation. Curr Opin Immunol. 2008; 20:588–593. [PubMed: 18674615]
25. 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; 180:3969–3979. [PubMed: 18322206]
26. Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. J Immunol. 1998; 161:571–577. [PubMed: 9670929]
27. Schonberg K, Sribar M, Enczmann J, Fischer JC, Uhrberg M. Analyses of HLA-C-specific KIR repertoires in donors with group A and B haplotypes suggest a ligand-instructed model of NK cell receptor acquisition. Blood. 2011; 117:98–107. [PubMed: 20935255]
28. Bari R, Bell T, Leung WH, Vong QP, Chan WK, Das Gupta N, Holladay M, Rooney B, Leung W. Significant functional heterogeneity among KIR2DL1 alleles and a pivotal role of arginine 245. Blood. 2009; 114:5182–5190. [PubMed: 19828694]
29. Yawata M, Yawata N, Draghi M, Partheniou F, Little AM, Parham P. MHC class I-specific inhibitory receptors and their ligands structure diverse human NK-cell repertoires toward a balance of missing self-response. Blood. 2008; 112:2369–2380. [PubMed: 18583565]
30. Venstrom JM, Pittari G, Gooley TA, Chewning JH, Spellman S, Haagenson M, Gallagher MM, Malkki M, Petersdorf E, Dupont B, Hsu KC. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. N Engl J Med. 2012; 367:805–816. [PubMed: 22931314]
31. Venstrom JM, Gooley TA, Spellman S, Pring J, Malkki M, Dupont B, Petersdorf E, Hsu KC. Donor activating KIR3DS1 is associated with decreased acute GVHD in unrelated allogeneic hematopoietic stem cell transplantation. Blood. 2010; 115:3162–3165. [PubMed: 20124216]
32. Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaelsson J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. Blood. 2010; 115:1166–1174. [PubMed: 19903900]
33. Gourraud PA, Meenagh A, Cambon-Thomsen A, Middleton D. Linkage disequilibrium organization of the human KIR superlocus: implications for KIR data analyses. Immunogenetics. 2010; 62:729–740. [PubMed: 20878401]

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34. Frazier WR, Steiner N, Hou L, Dakshanamurthy S, Hurley CK. Allelic variation in KIR2DL3 generates a KIR2DL2-like receptor with increased binding to its HLA-C ligand. J Immunol. 2013; 190:6198–6208. [PubMed: 23686481]

35. Hilton HG, Vago L, Older Aguilar AM, Moesta AK, Graef T, Abi-Rached L, Norman PJ, Guethlein LA, Fleischhauer K, Parham P. Mutation at positively selected positions in the binding site for HLA-C shows that KIR2DL1 is a more refined but less adaptable NK cell receptor than KIR2DL3. J Immunol. 2012; 189:1418–1430. [PubMed: 22772445]

36. Bjorkstrom NK, Riese P, Heuts F, Andersson S, Fauriat C, Ivarsson MA, Bjorklund AT, Flodstrom-Tullberg M, Michaelsson J, Rottenberg ME, Guzman CA, Ljunggren HG, Malmberg KJ. Expression patterns of NKG2A, KIR, and CD57 define a process of CD56dim NK-cell differentiation uncoupled from NK-cell education. Blood. 2010; 116:3853–3864. [PubMed: 20696944]

37. Andrews DM, Scalzo AA, Yokoyama WM, Smyth MJ, Degli-Esposti MA. Functional interactions between dendritic cells and NK cells during viral infection. Nat Immunol. 2003; 4:175–181. [PubMed: 12496964]

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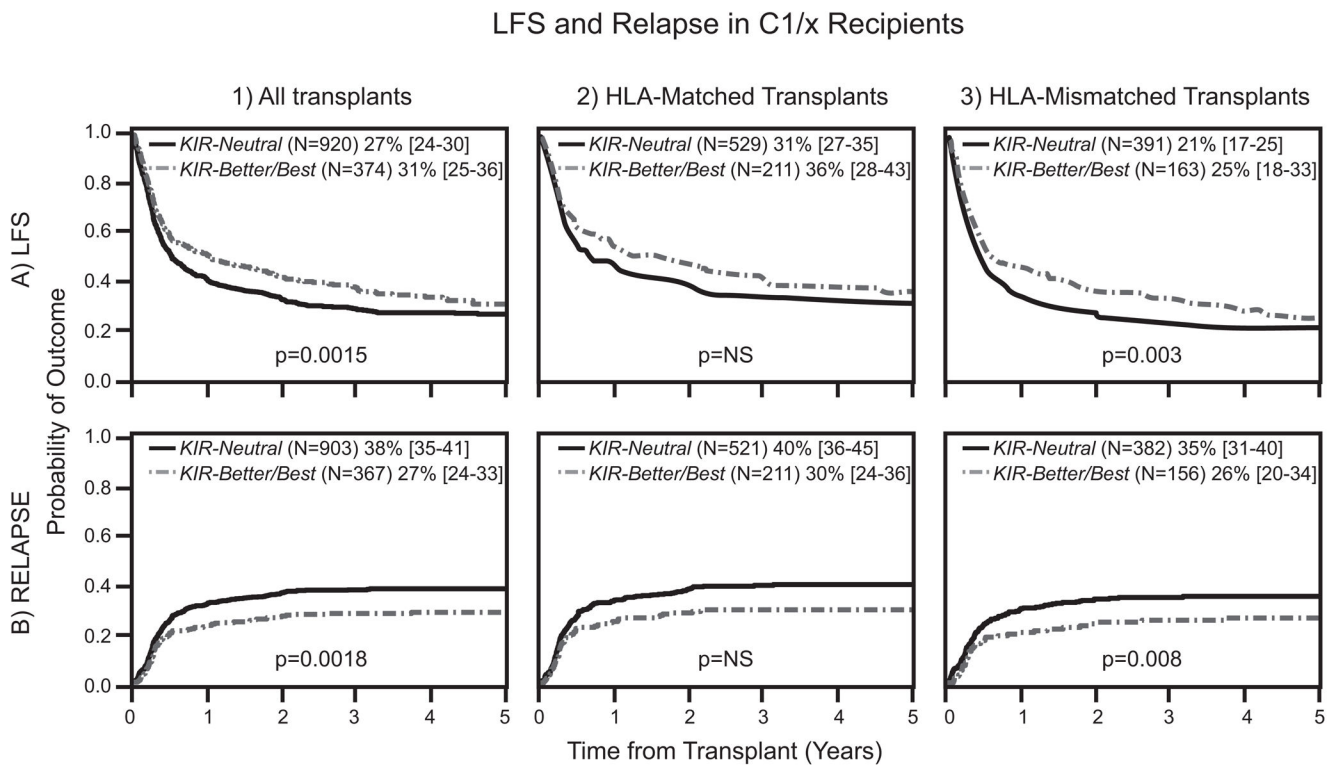

Figure 1. Interactions between *KIR-Better/Best* donors and recipient HLA-C1 improve LFS and protect against relapse, especially in HLA-mismatched transplants

Donors were assigned to *KIR-Neutral* and *KIR-Better/Best* groups based on *KIR* genotyping. Probabilities of LFS are provided by Kaplan Meier curves (A) and cumulative incidence probabilities are shown for relapse (B). Each outcome is shown comparing *KIR-Neutral* donors with *KIR-Better/Best* donors in *HLA-C1/x* recipients for all transplants (1), HLA-matched transplants (2) and the HLA-mismatched transplants (3). The estimated rates are presented for LFS and relapse at 5 years. P values were calculated from multivariate analyses comparing relative risks of outcomes for *KIR-Neutral* and *KIR-Better/Best donor* groups.

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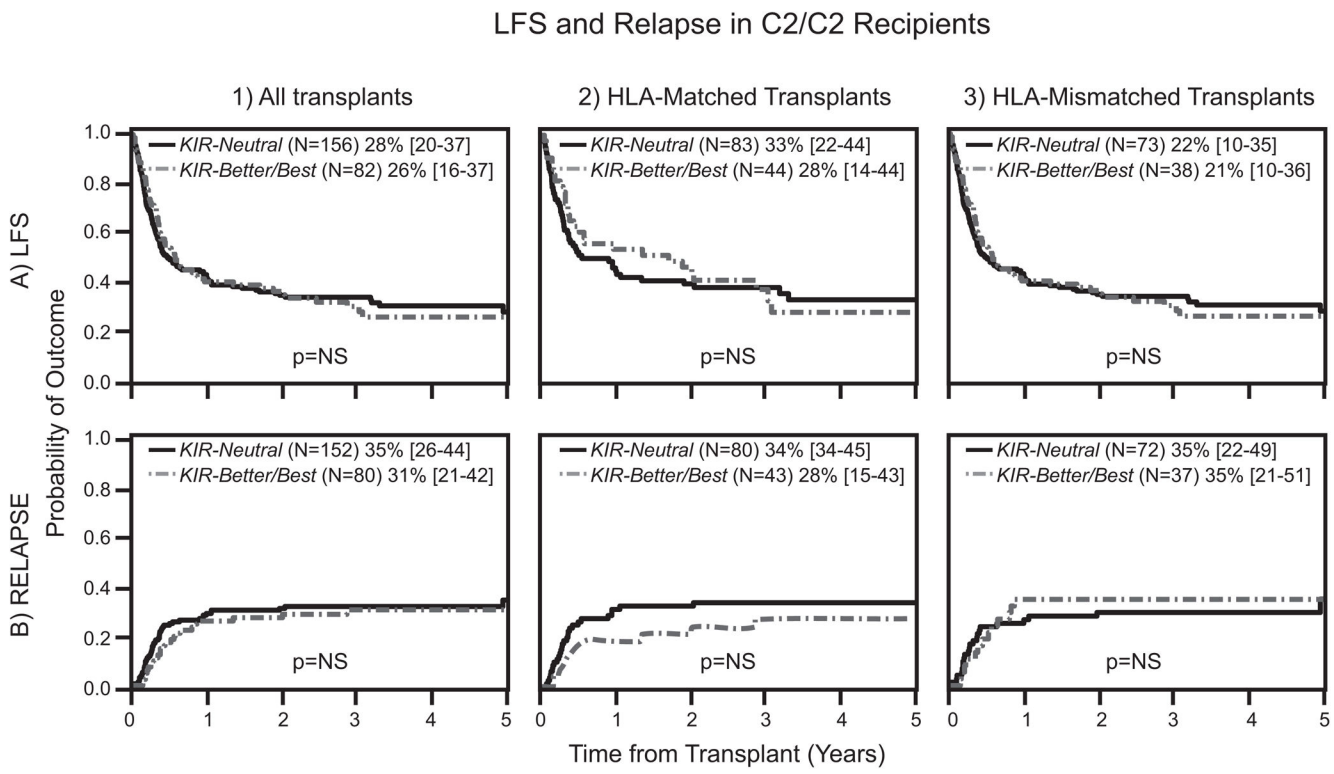

Figure 2. HLA-C2/C2 recipients do not experience enhanced protection from *KIR-Better/Best* donors

Donors were assigned to *KIR-Neutral* and *KIR-Better/Best* groups based on *KIR* genotyping and recipients were designated based on their HLA-C allotypes (C1/x and C2/C2). Probabilities of LFS are provided by Kaplan Meier curves (A) and cumulative incidence probabilities are shown for relapse (B). Each outcome is shown comparing *KIR-Neutral* donors with *KIR-Better/Best* donors in *HLA-C2/C2* recipients for all transplants in all transplants (1), HLA-matched transplants (2) and the HLA-mismatched transplants (3). The estimated rates are presented for LFS and relapse at 5 years. P values were calculated from multivariate analyses comparing relative risks of outcomes for *KIR-Neutral* and *KIR-Better/Best donor* groups.

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Table I

KIR Haplotype Group Nomenclature

***KIR* Genotype**	***KIR B-Motif* Content**	**Centromeric Haplotypes**	**Telomeric Haplotypes**	**KIR Donor Group**	
A/A	*0*	*A/A*	*A/A*	“Neutral”	*KIR-Neutral (0–1 B-motif)*
B/x	*1*	*A/A*	*A/B*		
		A/B	*A/A*		
	2	*A/A*	*B/B*	“*Better”	*KIR-Better/Best (≥2 B-motifs)*
		A/B	*A/B*		
		B/B	*A/A*	*“Best”	
	3	*A/B*	*B/B*	“Better”	
		B/B	*A/B*	“Best”	
	4	*B/B*	*B/B*		

*”Better” *KIR donors have* ≥2 *B-motifs* without *Cen-B/B*, and ”Best” *KIR* donors have ≥2 *B-motifs* with *Cen-B/B*

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TABLE II

Effect of Donor *KIR* Genotype on Outcome

A. All Transplants		**LFS**	
Donor KIR Content Group	**N**	**RR (CI)**	**P**
KIR B=0 *[i.e. KIR A/A]*	478	1.00	
KIR B ≥1 *[i.e. KIR B/x]*	964	0.89 (0.78–1.01)	0.075
KIR B=1	535	0.98 (0.85–1.14)	0.80
KIR B=2	325	0.79 (0.66–0.94)	0.008
KIR B=3+4	104	0.76 (0.59–0.99)	0.043
KIR B=0 or 1 *[i.e. Neutral Donors]*	1013	1.00	
B≥2 (non Cen B/B) *[i.e. Better Donors]*	290	0.86 (0.73–1.00)	0.055
B≥2 (Cen B/B) *[i.e. Best Donors]*	139	0.67 (0.54–0.85)	0.0007
B≥2 *[i.e. Better/Best Donors]*	429	0.79 (0.69–0.91)	0.001

B. All Transplants		**Relapse**	
Donor KIR B Content Group	**N**	**RR (CI)**	**P**
KIR B=0 *[i.e. KIR A/A]*	499	1.00	
KIR B ≥1 *[i.e. KIR B/x]*	1003	0.88 (0.73–1.06)	0.18
KIR B=1	556	1.03 (0.84–1.26)	0.77
KIR B=2	339	0.73 (0.57–0.94)	0.014
KIR B=3+4	108	0.64 (0.43–0.97)	0.033
KIR B=0 or 1 *[i.e. Neutral Donors]*	1055	1.00	
B≥2 (non Cen B/B) *[i.e. Better Donors]*	302	0.82 (0.65–1.02)	0.078
B≥2 (Cen B/B) *[i.e. Best Donors]*	145	0.46 (0.31–0.68)	0.0001
B≥2 *[i.e. Better/Best Donors]*	447	0.70 (0.57–0.86)	0.0005

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Table III

KIR-Better/Best Donors Improve Outcomes for *C1/x* Recipients

	Outcome	Donor *KIR* Group	All Transplants			HLA-Matched			HLA-Mismatched		
			N	RR	*P*	N	RR	*P*	N	RR	*P*
Recipient HLA *C1/x*	LFS	*KIR-Neutral*	865	1.00		502	1.00		363	1.00	
		KIR-Better/Best	354	0.78 (0.67–0.91)	0.0015	201	0.86 (0.69–1.07)	0.17	153	0.70 (0.56–0.88)	0.003
	Relapse	*KIR-Neutral*	903	1.00		521	1.00		382	1.00	
		KIR-Better/Best	367	0.70 (0.56–0.87)	0.0018	211	0.79(0.59–1.06)	0.11	156	0.61 (0.43–0.88)	0.008
	TRM	*KIR-Neutral*	893	1.00		516	1.00		377	1.00	
		KIR-Better/Best	363	0.89 (0.72–1.09)	0.26	207	1.02 (0.75–1.40)	0.90	156	0.82 (0.62–1.10)	0.19
Recipient HLA *C2/C2*	LFS	*KIR-Neutral*	148	1.00		77	1.00		71	1.00	
		KIR-Better/Best	75	0.93 (0.63–1.37)	0.71	42	0.81 (0.45–1.43)	0.47	33	1.28 (0.67–2.45)	0.45
	Relapse	*KIR-Neutral*	152	1.00		80	1.00		72	1.00	
		KIR-Better/Best	80	0.80 (0.47–1.36)	0.41	43	0.64 (0.29–1.42)	0.27	37	1.20 (0.51–2.83)	0.68
	TRM	*KIR-Neutral*	150	1.00		79	1.00		71	1.00	
		KIR-Better/Best	78	0.94 (0.57–1.55)	0.81	42	0.89 (0.42–1.89)	0.76	37	0.98 (0.45–2.16)	0.96

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Table IV

C1/x Recipients Receiving Transplants with Mismatch at HLA-C Benefit from Enhanced Protection with *KIR-Better/Best* Donors

A: Transplants with Mismatch at HLA Class I or Class I and Class II vs. Mismatch at HLA Class II							
	Donor *KIR* Group	**Includes Class I Mismatch**			**Class II Mismatched Only**		
		N	**RR**	***P***	**N**	**RR**	***P***
LFS	*KIR-Neutral*	310	1.00		53	1.00	
	KIR-Better/Best	131	0.69 (0.54–0.88)	0.0029	22	1.31 (0.57–3.02)	0.53
Relapse	*KIR-Neutral*	323	1.00		59	1.00	
	KIR-Better/Best	134	0.62 (0.42–0.92)	0.019	22	1.45 (0.47–4.50)	0.52

B: HLA Class I Mismatch Transplants at HLA-C vs. HLA-A and/or HLA-B							
	Donor *KIR* Group	**HLA-C Mismatched**			**HLA-A,B Mismatched**		
		N	**RR**	***P***	**N**	**RR**	***P***
LFS	*KIR-Neutral*	185	1.00		125	1.00	
	KIR-Better/Best	83	0.57 (0.40–0.79)	0.001	48	0.97 (0.63–1.49)	0.89
Relapse	*KIR-Neutral*	192	1.00		131	1.00	
	KIR-Better/Best	85	0.54 (0.33–0.88)	0.013	49	0.68 (0.32–1.45)	0.32

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Table V

Impact of Individual Donor *KIR B* Genes on LFS and Relapse in *C1/x* Recipients

	A. All Transplants					
	n	**RR**	***P***	**n**	**RR**	***P***
Donor KIR Group		**LFS**			**Relapse**	
KIR A/A	404	1.00		422	1.00	
KIR B/x with 2DS2+	615	0.85 (0.73–0.99)	0.041	639	0.86 (0.69–1.06)	0.15
KIR B/x with 2DS5+	382	0.85 (0.72–1.01)	0.068	397	0.83 (0.66–1.05)	0.12
KIR B/x with 2DL2+	610	0.86 (0.74–1.00)	0.056	634	0.87 (0.70–1.07)	0.19
KIR B/x with 2DS1+	463	0.84 (0.71–0.99)	0.036	483	0.80 (0.64–1.00)	0.052
KIR B/x with 3DS1+	448	0.83 (0.70–0.98)	0.027	469	0.79 (0.63–0.99)	0.044
KIR B/x with 2DS3+	306	0.81 (0.67–0.97)	0.025	321	0.85 (0.66–1.09)	0.20
KIR B/x with 2DL5+	584	0.86 (0.74–1.01)	0.061	608	0.87 (0.71–1.08)	0.20

	B. HLA Matched					
	n	**RR**	***P***	**n**	**RR**	***P***
Donor KIR Group		**LFS**			**Relapse**	
KIR A/A	233	1.00		240	1.00	
KIR B/x with 2DS2+	344	0.96 (0.77–1.19)	0.71	361	1.04 (0.79–1.38)	0.77
KIR B/x with 2DS5+	221	0.96 (0.76–1.21)	0.73	231	1.04 (0.76–1.41)	0.82
KIR B/x with 2DL2+	341	0.96 (0.77–1.19)	0.70	358	1.04 (0.79–1.37)	0.79
KIR B/x with 2DS1+	274	0.96 (0.77–1.20)	0.71	287	0.98 (0.73–1.32)	0.90
KIR B/x with 3DS1+	267	0.93 (0.74–1.17)	0.54	281	0.97 (0.72–1.31)	0.86
KIR B/x with 2DS3+	178	0.97 (0.75–1.25)	0.78	189	1.07 (0.77–1.50)	0.67
KIR B/x with 2DL5+	341	0.99 (0.80–1.23)	0.95	358	1.09 (0.82–1.44)	0.55

	C. HLA Mismatched					
	n	**RR**	***P***	**n**	**RR**	***P***
Donor KIR Group		**LFS**			**Relapse**	
KIR A/A	171	1.00		182	1.00	
KIR B/x with 2DS2+	271	0.78 (0.62–0.99)	0.037	278	0.68 (0.49–0.95)	0.023

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	C. HLA Mismatched					
	n	**RR**	***P***	**n**	**RR**	***P***
Donor KIR Group		**LFS**			**Relapse**	
KIR B/x with 2DS5+	161	0.75 (0.58–0.99)	0.040	166	0.59 (0.40–0.88)	0.0090
KIR B/x with 2DL2+	269	0.80 (0.63–1.01)	0.055	276	0.70 (0.51–0.98)	0.036
KIR B/x with 2DS1+	189	0.73 (0.56–0.94)	0.015	196	0.59 (0.41–0.85)	0.0052
KIR B/x with 3DS1+	181	0.73 (0.57–0.95)	0.018	188	0.57 (0.39–0.83)	0.0038
KIR B/x with 2DS3+	128	0.65 (0.48–0.86)	0.0032	132	0.58 (0.38–0.88)	0.0098
KIR B/x with 2DL5+	243	0.75 (0.59–0.96)	0.020	250	0.63 (0.44–0.88)	0.0076

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Table VI

Impact of Individual Donor *KIR B* Genes on LFS and Relapse in *C2/C2* Recipients

	A. All Transplants					
	n	**RR**	***P***	**n**	**RR**	***P***
Donor KIR Group		**LFS**			**Relapse**	
KIR A/A	74	1.00		77	1.00	
KIR B/x with 2DS2+	113	1.09 (0.73–1.63)	0.69	119	1.17 (0.67–2.07)	0.58
KIR B/x with 2DS5+	75	1.22 (0.78–1.92)	0.38	76	0.89 (0.48–1.65)	0.72
KIR B/x with 2DL2+	112	1.07 (0.71–1.61)	0.74	117	1.16 (0.65–2.08)	0.61
KIR B/x with 2DS1+	92	1.16 (0.75–1.80)	0.51	96	0.99 (0.56–1.77)	0.97
KIR B/x with 3DS1+	90	1.10 (0.72–1.69)	0.65	94	1.06 (0.60–1.87)	0.84
KIR B/x with 2DS3+	60	1.26 (0.75–2.10)	0.38	63	1.06 (0.56–2.01)	0.85
KIR B/x with 2DL5+	117	1.09 (0.73–1.61)	0.68	121	0.98 (0.57–1.69)	0.93
	B. HLA Matched					
	n	**RR**	***P***	**n**	**RR**	***P***
Donor KIR Group		**LFS**			**Relapse**	
KIR A/A	37	1.00		39	1.00	
KIR B/x with 2DS2+	66	1.13 (0.63–2.02)	0.68	68	0.94 (0.43–2.05)	0.88
KIR B/x with 2DS5+	36	1.26 (0.62–2.54)	0.52	36	0.81 (0.31–2.12)	0.66
KIR B/x with 2DL2+	65	1.08 (0.60–1.95)	0.79	66	0.86 (0.39–1.91)	0.71
KIR B/x with 2DS1+	45	1.25 (0.63–2.47)	0.52	46	0.90 (0.37–2.21)	0.82
KIR B/x with 3DS1+	44	1.15 (0.58–2.28)	0.69	45	1.00 (0.41–2.43)	0.99
KIR B/x with 2DS3+	33	0.95 (0.43–2.13)	0.91	34	0.85 (0.34–2.12)	0.72
KIR B/x with 2DL5+	60	1.18 (0.63–2.20)	0.60	61	0.82 (0.36–1.84)	0.63
	C. HLA Mismatched					
	n	**RR**	***P***	**n**	**RR**	***P***
Donor KIR Group		**LFS**			**Relapse**	
KIR A/A	37	1.00		38	1.00	
KIR B/x with 2DS2+	47	1.57 (0.71–3.48)	0.27	51	1.62 (0.56–4.72)	0.38
KIR B/x with 2DS5+	39	1.07 (0.49–2.33)	0.86	40	0.92 (0.36–2.36)	0.86

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	C. HLA Mismatched					
	n	RR	*P*	n	RR	*P*
Donor KIR Group		**LFS**			**Relapse**	
KIR B/x with 2DL2+	47	1.53 (0.69–3.40)	0.30	51	1.81 (0.59–5.55)	0.30
KIR B/x with 2DS1+	47	1.18 (0.56–2.47)	0.67	50	1.26 (0.51–3.08)	0.62
KIR B/x with 3DS1+	46	1.21 (0.58–2.49)	0.61	49	1.27 (0.52–3.08)	0.60
KIR B/x with 2DS3+	27	2.33 (0.73–7.47)	0.15	29	1.31 (0.42–4.08)	0.64
KIR B/x with 2DL5+	57	1.13 (0.58–2.24)	0.72	60	1.09 (0.45–2.62)	0.85