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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

Sarah Cooley,* Daniel J. Weisdorf,* Lisbeth A. Guethlein,† John P. Klein,‡ Tao Wang,§ Steven G. E. Marsh,∥ Stephen Spellman,‡ Michael D. Haagenson,‡ Koy Saetern,∥ Martha Ladner,∥ Elizabeth Trachtenberg,∥ Peter Parham,‖ and Jeffrey S. Miller*"t

Killer cell Ig-like receptors (KIRs) interact with HLA class I ligands to regulate NK cell development and function. These interactions affect the outcome of unrelated donor hematopoietic cell transplantation (HCT). We have shown previously that donors with KIR B versus KIR A haplotypes improve the clinical outcome for patients with acute myelogenous leukemia 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 genes are dominant. They include the genes encoding inhibitory KIRs 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 with C2 homozygous recipients, with no effect due to donor HLA. The protective interaction between donors with two or more, versus none or one, KIR B motifs and recipient C1 was specific to transplants with class I mismatch at HLA-C (RR of leukemia-free survival, 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 with 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 unrelated donor hematopoietic cell transplantation for acute myelogenous leukemia. The Journal of Immunology, 2014, 192: 4592–4600.

The online version of this article contains supplemental material.

Abbreviations used in this article: AML, acute myelogenous leukemia; Cen, centromeric; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; KIR, killer cell Ig-like receptor; LFS, leukemia-free survival; RR, relative risk; Tel, telomeric; TRM, treatment-related mortality.

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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 are present
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 Materials and Methods.

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 haploidential,
T cell–depleted transplant from a related family member (3, 4).
For these transplants, in which donor and 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
(5) proposed that reduced relapse was due to NK cell killing of
residual leukemia cells that had survived the myeloablative condi-
tioning regimen. They also proposed that the reduced graft-versus-
host disease (GVHD) they observed was caused by NK cell killing
of recipient dendritic cells. These pioneering observations led to
investigations of various other types of transplant examining the
effects of allogeneic NK cells and the HLA-A,-B, and -C epitopes
recognized by KIRs (6–10). A general observation emerging from
these subsequent studies is that NK cell effects in HCT are prin-
cipally 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 KIRs, 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 a URD,
we found that clinical outcome was better when the donors have
one or two KIR B haplotypes (KIR B/a donors) than for donors
who have two KIR A haplotypes (KIR A/a donors). With a KIR B/a
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
centromeric or the telomeric region of the KIR locus. The cen-
tromic region contains genes encoding the inhibitory receptors for
the C1 and 2 epitopes of HLA-C, whereas the telomeric region
contains genes encoding the inhibitory receptors for the Bw4 and
A3/A11 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 in-
hibitory 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 myeloablative preparation for a URD HCT facilitated by
the National Marrow Donor Program between 1988 and 2009. DNA
samples were obtained from the National Marrow Donor Program Re-
search Sample Repository. Outcome data were obtained from the Center
for International Blood and Marrow Transplant Research. The demo-
graphics, KIR genotypes, and multivariate statistical analysis of the clinical
data have been described (13, 14). DNA samples and clinical data were
obtained with informed consent and approval from the National Marrow
Donor Program 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 National
Marrow Donor Program retrospective typing project. KIR typing at the
level of KIR gene content was performed using MALDI-TOF mass spec-
trosopy as described previously (15, 16). Four epitopes of HLA-A,-B,
and -C molecules are recognized by KIRs. The epitopes, also called KIR
ligands, are situated on the upward face of the HLA class I molecule
and involve the N-terminal part of the a helix and the carboxyl-terminal
parts of the bound peptide and the a 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 two or three, and the length of the cytoplasmic tail, either long (L) or short (S), correlating respectively with inhibitory and activating signaling functions (18). The C1 and C2 epitopes carried by HLA-C are distinguished by different amino acids, at position 80 (17). The C1 epitope is recognized by the inhibitory KIR2DL2/3 receptor, whereas the C2 epitope is recog-
nized by inhibitory KIR2DL1 and activating KIR2DS1 receptors. The Bw4
epitope, carried by 27% of HLA-A and 35% of HLA-B allotypes, is rec-
nogized 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 on the sequence of the peptide bound to HLA-A^a03 or
HLA-A^a11 (22). For this reason the A3/11 epitope was not included in the
analyses of the transplant donors and recipients studied. In the present
studies, the impact of recipient C1 on transplant outcome dominated C2.
In some analyses, recipients with C2/C2 genotype were compared with
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 leucocyte 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 129–215 kb in length (23). Every individual has a ma-
ternally inherited and a paternally inherited KIR haplotype that together
form his or her KIR genotype. Conserved genes in the center of the locus
and 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; for example, Cen-B1
and Cen-B2. The A motifs are shorter, more conserved, and consist
mainly of genes for inhibitory KIRs that recognize the C1, C2, and Bw4
epitopes. The B motifs are longer, more variable, and contain one or more
of seven KIR B–specific genes (23). These comprise KIR2DS2
and KIR2DS2 in Cen-B, KIR2DS1 and KIR3DS1 in Tel-B, and KIR2DS3/5
and KIR2DL5 in either Cen-B or Tel-B, respectively. KIRs encoded by the
B-specific genes, only KIR2DL2 (C1-specific) and KIR2DS2 (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 hap-
lotypes, 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 applications.

In this study we characterized transplant donors according to their KIR B
motif content, a parameter that varies from none to four and is simply
the sum of the number of Cen-B and Tel-B motifs in the donor’s KIR genotype.
Based on the results of our previous study (2), donors are classified as
“Neutral” (none or one KIR B-motifs), “Better” (two or more B motifs without
Cen-B or Tel-B), or “Best” (two or more B motifs with Cen-B or Tel-B) (14).
In some analyses, the Better and Best groups were combined to form the KIR-
Better/Best donor group (with two or more B motifs) (Table I).

Statistical analysis

We considered five clinical outcomes of HCT: LFS, relapse, treatment-
related mortality (TRM), grades II–IV or III–IV acute GVHD, and
chronic GVHD. 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, acute GVHD, and chronic GVHD. In the cohort we studied, the completeness of follow-up at 3 y after transplantation was >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, time from diagnosis to transplant, disease status, and year of transplantation. Cases were excluded from some models when 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 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 of 10 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 mismatches. Whereas 357 (53%) of the KIR-selected 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 grafts, whereas the others received grafts of stem cells present in peripheral blood mobilized with 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 on 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 (none or one B motif), Better (two or more B motifs without Cen-B/B), or Best (two or more 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 two or more 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 (relative risk [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). 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 heterozygous or homozygous for HLA-C bearing the C1 epitope. C1/x recipients had improved LFS when transplanted with grafts from KIR-Better/Best compared with KIR-Neutral donors (RR 0.78 [0.67–0.91], p = 0.0015; Fig. 1A1, Table III). A similar beneficial effect was not observed for C2/C2 recipients (RR 0.93 [0.63–1.37], p = 0.71; Fig. 2A1, Table III). Factors that may contribute to the improved LFS are reductions in leukemic relapse, 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 did C1/x recipients with KIR-Neutral donors (RR 0.70 [0.56–0.87], p = 0.0018; Fig. 1B1, Table III). Five years after transplantation, the frequencies of relapse in C1/x recipients based on donor KIR were 27 versus 38%, respectively. Although a 4% absolute relapse protection was observed in C2/C2 recipients receiving grafts from KIR-Better/Best versus KIR-Neutral donors, this trend was not statistically significant (Fig. 2B1, Table III). 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 KIRs 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 KIRs with recipient HLA class I. In this

Table I. KIR haplotype group nomenclature

<table>
<thead>
<tr>
<th>KIR Genotype</th>
<th>KIR B Motif Content</th>
<th>Centromeric Haplotypes</th>
<th>Telomeric Haplotypes</th>
<th>KIR Donor Groupa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>0</td>
<td>A/A</td>
<td>A/A</td>
<td>Neutral</td>
</tr>
<tr>
<td>B/x</td>
<td>1</td>
<td>A/A</td>
<td>A/B</td>
<td>KIR-Neutral (zero or one B motif)</td>
</tr>
<tr>
<td>B/x</td>
<td>1</td>
<td>A/A</td>
<td>A/B</td>
<td>KIR-Neutral (zero or one B motif)</td>
</tr>
<tr>
<td>B/x</td>
<td>2</td>
<td>A/B</td>
<td>B/B</td>
<td>KIR-Better/Best (two or more B motifs)</td>
</tr>
<tr>
<td>B/x</td>
<td>2</td>
<td>A/B</td>
<td>A/B</td>
<td>KIR-Better/Best (two or more B motifs)</td>
</tr>
<tr>
<td>B/x</td>
<td>3</td>
<td>A/B</td>
<td>B/B</td>
<td>KIR-Better/Best (two or more B motifs)</td>
</tr>
<tr>
<td>B/x</td>
<td>3</td>
<td>B/B</td>
<td>A/B</td>
<td>KIR-Better/Best (two or more B motifs)</td>
</tr>
<tr>
<td>B/x</td>
<td>4</td>
<td>B/B</td>
<td>B/B</td>
<td>KIR-Better/Best (two or more B motifs)</td>
</tr>
</tbody>
</table>

aBetter KIR donors have two or more B motifs without Cen-B/B, and Best KIR donors have two or more B motifs with Cen-B/B.
analysis, the effects of KIR-Better/Best donors were always compared with 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 with C2/C2 recipients (Figs. 1A2, 1B2, Table III), 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; Fig. 1A3, 1B3, Table III). Again, C2/C2 recipients derived no significant benefit from a KIR-Better/Best donor (Fig. 2A3, 2B3, Table III).

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

<table>
<thead>
<tr>
<th>Donor KIR content group</th>
<th>n</th>
<th>RR (CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR B = 0 (KIR A/A)</td>
<td>478</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>KIR B = 1 (KIR B/x)</td>
<td>964</td>
<td>0.89 (0.78–1.01)</td>
<td>0.075</td>
</tr>
<tr>
<td>KIR B = 2</td>
<td>535</td>
<td>0.98 (0.85–1.14)</td>
<td>0.80</td>
</tr>
<tr>
<td>KIR B = 3 + 4</td>
<td>325</td>
<td>0.79 (0.66–0.94)</td>
<td>0.008</td>
</tr>
<tr>
<td>KIR B = 0 or 1 (Neutral donors)</td>
<td>1013</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>B ≥ 2 (non–Cen-B/B) (Better donors)</td>
<td>290</td>
<td>0.86 (0.73–1.00)</td>
<td>0.055</td>
</tr>
<tr>
<td>B ≥ 2 (Cen-B/B) (Best donors)</td>
<td>139</td>
<td>0.67 (0.54–0.85)</td>
<td>0.0007</td>
</tr>
<tr>
<td>B ≥ 2 (Better/Best donors)</td>
<td>429</td>
<td>0.79 (0.69–0.91)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

CI, confidence interval.

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 (no. 1), HLA-matched transplants (no. 2), and the HLA-mismatched transplants (no. 3). The estimated rates are presented for LFS and relapse at 5 y. The p values were calculated from multivariate analyses comparing relative risks of outcomes for KIR-Neutral and KIR-Better/Best donor groups.
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), but not in the subset of 81 HLA class II–mismatched transplants (Table IV). 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 V). 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 that 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 that included mismatches at HLA-C (n = 460) versus 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 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 in the present study 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.

### Table III. KIR-Better/Best donors improve outcomes for C1/x recipients

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Outcome</th>
<th>Donor KIR Group</th>
<th>All Transplants</th>
<th>HLA Matched</th>
<th>HLA Mismatched</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>RR</td>
<td>p</td>
<td>n</td>
</tr>
<tr>
<td>HLA C1/x</td>
<td>LFS</td>
<td>KIR-Neutral</td>
<td>865</td>
<td>1.00</td>
<td>502</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR-Better/Best</td>
<td>354 [0.67–0.91]</td>
<td>0.0015</td>
<td>201 [0.69–1.07]</td>
</tr>
<tr>
<td></td>
<td>Relapse</td>
<td>KIR-Neutral</td>
<td>903</td>
<td>1.00</td>
<td>521</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR-Better/Best</td>
<td>367 [0.56–0.87]</td>
<td>0.0018</td>
<td>211 [0.59–1.06]</td>
</tr>
<tr>
<td></td>
<td>TRM</td>
<td>KIR-Neutral</td>
<td>893</td>
<td>1.00</td>
<td>516</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR-Better/Best</td>
<td>363 [0.72–1.09]</td>
<td>0.26</td>
<td>207 [0.75–1.40]</td>
</tr>
<tr>
<td>HLA C2/C2</td>
<td>LFS</td>
<td>KIR-Neutral</td>
<td>148</td>
<td>1.00</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR-Better/Best</td>
<td>75 [0.63–1.37]</td>
<td>0.71</td>
<td>42 [0.45–1.43]</td>
</tr>
<tr>
<td></td>
<td>Relapse</td>
<td>KIR-Neutral</td>
<td>152</td>
<td>1.00</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR-Better/Best</td>
<td>80 [0.47–1.36]</td>
<td>0.41</td>
<td>43 [0.29–1.42]</td>
</tr>
<tr>
<td></td>
<td>TRM</td>
<td>KIR-Neutral</td>
<td>150</td>
<td>1.00</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KIR-Better/Best</td>
<td>78 [0.57–1.55]</td>
<td>0.81</td>
<td>42 [0.42–1.89]</td>
</tr>
</tbody>
</table>

### 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 (no. 1), HLA-matched transplants (no. 2), and the HLA-mismatched transplants (no. 3). The estimated rates are presented for LFS and relapse at 5 y. The p values were calculated from multivariate analyses comparing relative risks of outcomes for KIR-Neutral and KIR-Better/Best donor groups.
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, HLA-C2, and Bw4 KIR ligand status of the recipients. The relative risks for each outcome were determined for seven groups of KIR B/x donors in comparison with KIR A/A donors. Each of these groups corresponded to the subset of donors carrying one of the seven 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 seven groups.

In the full cohort of transplants, C1/x recipients benefited from increases in LFS associated with all seven KIR B/x donor groups compared with KIR A/A donors (Table VI). Only KIR2DS1 and KIR3DS1 were associated with 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 VI). In the HLA-mismatched transplants, multivariate analyses showed that each of the seven KIR B genes contributed clinical benefit of similar magnitude: RR from 0.65 to 0.80 (p = 0.0032–0.055) for LFS and from 0.57 to 0.70 (p = 0.0038–0.036) for relapse (Table VI). In contrast, for transplantation of C2/C2 recipients, the clinical outcomes were similar for all seven groups of KIR B/x donors, where the KIR B/x donor group has no effect on survival or relapse protection (Table VII). 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 depleting 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 KIRs 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 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 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.

Although 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 does KIR2DL3, which can affect the education of NK cells mediated by the C1 ligand (25). Second, KIR2DL2 has cross-reactivity 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 KIR2DL1 (27) may not account for all the beneficial clinical effect associated with KIR B

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Table IV. Transplants with mismatch at HLA class I or class I and class II versus mismatch at HLA class II

<table>
<thead>
<tr>
<th>Donor KIR Group</th>
<th>Includes Class I Mismatch</th>
<th>Class II—Mismatched Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>RR</td>
</tr>
<tr>
<td>LFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR-Neutral</td>
<td>310</td>
<td>1.00</td>
</tr>
<tr>
<td>KIR-Better/Best</td>
<td>131</td>
<td>0.69</td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR-Neutral</td>
<td>323</td>
<td>1.00</td>
</tr>
<tr>
<td>KIR-Better/Best</td>
<td>134</td>
<td>0.62</td>
</tr>
</tbody>
</table>

---

Table V. HLA class I mismatch transplants at HLA-C versus HLA-A and/or HLA-B

<table>
<thead>
<tr>
<th>Donor KIR Group</th>
<th>HLA-C Mismatch</th>
<th>HLA-A/B Mismatch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>RR</td>
</tr>
<tr>
<td>LFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR-Neutral</td>
<td>185</td>
<td>1.00</td>
</tr>
<tr>
<td>KIR-Better/Best</td>
<td>83</td>
<td>0.57</td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR-Neutral</td>
<td>192</td>
<td>1.00</td>
</tr>
<tr>
<td>KIR-Better/Best</td>
<td>85</td>
<td>0.54</td>
</tr>
</tbody>
</table>
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 KIRs and

<table>
<thead>
<tr>
<th>Donor KIR Group</th>
<th>LFS p</th>
<th>Relapse p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>RR</td>
</tr>
<tr>
<td>All transplants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIR A/A</td>
<td>404</td>
<td>1.00</td>
</tr>
<tr>
<td>KIR B/x with 2DS2</td>
<td>615</td>
<td>0.85 (0.73–0.99)</td>
</tr>
<tr>
<td>KIR B/x with 2DS3</td>
<td>342</td>
<td>0.85 (0.72–1.01)</td>
</tr>
<tr>
<td>KIR B/x with 2DL2</td>
<td>610</td>
<td>0.86 (0.74–1.00)</td>
</tr>
<tr>
<td>KIR B/x with 2DS1</td>
<td>463</td>
<td>0.84 (0.71–0.99)</td>
</tr>
<tr>
<td>KIR B/x with 3DS1</td>
<td>448</td>
<td>0.83 (0.70–0.98)</td>
</tr>
<tr>
<td>KIR B/x with 2DS3</td>
<td>306</td>
<td>0.81 (0.67–0.97)</td>
</tr>
<tr>
<td>KIR B/x with 2DL5</td>
<td>584</td>
<td>0.86 (0.74–1.01)</td>
</tr>
</tbody>
</table>

| HLA matched     |       |           |       |     |
| 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 2DS3 | 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 (0.77–1.20) | 0.71 | 287 | 0.98 (0.73–1.32) | 0.90 |
| KIR B/x with 2DS1 | 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 |

| HLA mismatched  |       |           |       |     |
| 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 |
| KIR B/x with 2DS3 | 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) | 0.052 |
| KIR B/x with 2DS3 | 181 | 0.73 (0.57–0.95) | 0.018 | 188 | 0.57 (0.39–0.83) | 0.0038 |
| KIR B/x with 2DL2 | 269 | 0.65 (0.48–0.86) | 0.0032 | 321 | 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 |

Table VI. Impact of individual donor KIR B genes on LFS and relapse in C1/x recipients

Table VII. Impact of individual donor KIR B genes on LFS and relapse in C2/C2 recipients
The effect of individual genes. Additionally, 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 alleles affect the affinity for HLA class I ligands as well as signaling function (25, 28, 34). Application of high-resolution typing of KIR alleles will investigate this possibility. Importantly, note 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 these 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 alter 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 United States transplant recipients are HLA-C1/a. The findings presented in the present study are being further tested in our ongoing multicenter 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 (http://www.clinicaltrials.gov, NCT01288222).

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Disclosures

The authors have no financial conflicts of interest.

References


