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KIR and HLA Genotypes Predictive of Low-Affinity Interactions Are Associated with Lower Relapse in Autologous Hematopoietic Cell Transplantation for Acute Myeloid Leukemia

John Marra,* Justin Greene,* Jimmy Hwang,† Juan Du,* Lloyd Damon,* Tom Martin,* and Jeffrey M. Venstrom*

Killer cell Ig–like receptors (KIRs) bind cognate HLA class I ligands with distinct affinities, affecting NK cell licensing and inhibition. We hypothesized that differences in KIR and HLA class I genotypes predictive of varying degrees of receptor–ligand binding affinities influence clinical outcomes in autologous hematopoietic cell transplantation (AHCT) for acute myeloid leukemia (AML). Using genomic DNA from a homogeneous cohort of 125 AML patients treated with AHCT, we performed KIR and HLA class I genotyping and found that patients with a compound KIR3DL1+ and HLA-Bw4-80Thr, HLA-Bw4-80lle* genotype, predictive of low-affinity interactions, had a low incidence of relapse, compared with patients with a KIR3DL1+ and HLA-Bw4-80lle* genotype, predictive of high-affinity interactions (hazard ratio [HR], 0.22; 95% confidence interval [CI], 0.06–0.78; p = 0.02). This effect was influenced by HLA-Bw4 copy number, such that relapse progressively increased with one copy of HLA-Bw4-80lle (HR, 1.6; 95% CI, 0.84–3.1; p = 0.15) to two to three copies (HR, 3.0; 95% CI, 1.4–6.5; p = 0.005) and progressively decreased with one to two copies of HLA-Bw4-80Thr (p = 0.13). Among KIR3DL1+ and HLA-Bw4-80lle+ patients, a predicted low-affinity KIR2DL2/3+ and HLA-C1/C1 genotype was associated with lower relapse than a predicted high-affinity KIR2DL1+ and HLA-C2/C2 genotype (HR, 0.25; 95% CI, 0.09–0.73; p = 0.01). Similarly, a KIR3DL1+ and HLA-Bw4-80lle+ genotype, or lack of KIR3DL1+ and HLA-Bw4-80lle+ genotype, rescued KIR2DL1+ and HLA-C2/C2 patients from high relapse (p = 0.007). These findings support a role for NK cell graft-versus-leukemia activity modulated by NK cell receptor–ligand affinities in AHCT for AML. The Journal of Immunology, 2015, 194: 000–000.

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Abbreviations used in this article: AHCT, autologous hematopoietic cell transplantation; allogeneic hematopoietic cell transplantation (alloHCT); AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CI, confidence interval; GVL, graft-versus-leukemia; HR, hazard ratio; KIR, killer-cell Ig-like receptor; NRM, nonrelapse mortality.

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therapy, particularly in the heterogeneous group of AML patients stratified as intermediate-risk, comprising 40–50% of AML patients and characterized by significant variability in survival outcomes (7). Identifying novel biomarkers that can improve patient stratification, therefore, remains a critical unmet need in AML.

The myeloablative chemotherapy used in AHCT induces a period of pancytopenia and immunodeficiency (8). Before recovery of a potent adaptive immune system, the innate immune system recovers, with neutrophils, monocytes, and NK cells (9–11). During this period, innate immunity provides an immediate response to foreign pathogens and may provide early protection from leukemia relapse, as suggested in HLA-identical alloHCT, in which NK cells contribute to graft-versus-leukemia (GVL) effects (12–17). Although NK cells are ready to kill as soon as they are formed, NK cell immunity is variable and regulated by genetic polymorphisms among receptors and ligands (18–20). Because the immunogenetic factors that influence NK cell function may similarly influence antileukemia reactivity in the posttransplant period, variation in NK cell receptor and ligand combinations among AML patients may affect AHCT outcomes.

The highly polymorphic killer-cell Ig-like receptor (KIR) gene region encodes an array of inhibitory and activating receptors expressed by NK cells (21–23); KIR ligands are expressed by a similarly polymorphic HLA class I gene region, with HLA gene products expressed by all nucleated cells (21, 22). Interindividual variability in KIR and KIR ligands gives rise to a vast diversity of NK cell repertoires and differences in responsiveness among NK cell subsets (24). The “licensing” or NK cell education model predicts that NK cells with inhibitory KIR for self-HLA class I
acquire effector function, whereas NK cells lacking inhibitory KIR for self-HLA class I are hyporesponsive (25), and this model has been supported and elaborated by findings of gene dose effects among receptors and ligands. Individual NK cells with multiple copies of inhibitory KIR for self-HLA class I acquire progressively increased effector function (26, 27), and increasing copy number of HLA-Bw4-80Ile and HLA-C2 alleles confers increased antitumor responsiveness to cognate KIR3DL1- and KIR2DL1-expressing NK cells (28, 29). Similarly, an increasing number of distinct MHC-encoded ligands confers increased responsiveness, presumably through a collective increase in licensed NK cell subsets (27).

Because the same KIR and HLA interactions that license NK cells also serve to inhibit their cytotoxic functions (30–34); licensed NK cells are inhibited from self-reactivity in the steady state. Importantly, changes in the environment in various disease settings result in the recruitment of specific NK cell subsets, allowing for greater disease control among certain patients (30, 35–38). In AHCT for neuroblastoma, the presence of a “missing ligand” for inhibitory KIR was associated with improved survival, suggesting that normally hyporesponsive unlicensed NK cells are specifically recruited to control disease in this setting (39). In HLA-matched T-cell–depleted alloHCT for hematologic malignancies, both unlicensed hyporesponsive inhibitory KIR-expressing NK cell subsets and licensed normally inhibited NK cell subsets in donor and recipient gain responsiveness after transplantation (37).

We hypothesize that an NK cell GVL mechanism exists in AHCT for AML, such that certain compound KIR and HLA genotypes confer increased NK cell responsiveness and better clinical outcomes. Beyond gene dose effects, the dichotomy of licensed versus unlicensed NK cells is further modulated by differences in binding affinity and specificity among KIR and HLA pairs, resulting from polymorphisms within both the KIR and HLA genes (27, 28, 30, 32, 40). Of the KIR3DL1 ligands, HLA-Bw4 molecules with threonine at position 80 (HLA-Bw4-80Thr) bind KIR3DL1 with lower affinity than HLA-Bw4 molecules with isoleucine at position 80 (HLA-Bw4-80Ile) (32, 33, 41), conferring lower responsiveness but weaker NK cell inhibition (32, 33). Similarly, certain HLA-C1 alleles weakly bind KIR2DL2/3, although the clinical implications are unclear (29, 30, 42, 43).

In this report, we evaluated the clinical effects of KIR and HLA genotypes predictive of high- and low-affinity receptor–ligand interactions in a homogeneous cohort of AML patients treated with AHCT. As suggested by findings in mice (27), we hypothesized that NK cell receptor–ligand affinities modulate NK cell antileukemic activity triggered by high-dose chemotherapy and thereby influence the probability of relapse following AHCT.

Materials and Methods

Patient cohort

Genomic DNA samples linked to clinical outcomes were available for 125 patients receiving a first AHCT for AML between 1986 and 2010, as described previously (Table I) (1, 2). Patient information and clinical outcomes were obtained from medical records. Data quality was validated through third-party audits, computerized checks for errors, and a review of clinical information by transplant physicians. Research was approved by the University of California, San Francisco Committee on Human Research (NCT00002768, NCT01048827).

DNA extraction

Genomic DNA was extracted from peripheral blood or bone marrow samples using the DNeasy Blood & Tissue Kit (Qiagen).

KIR genotyping

KIR typing was performed using PCR with sequence specific primers (44, 45) with minor modifications. Primers for KIR genes and internal control genes were purchased from Integrated DNA Technologies (San Diego, CA). DNA samples from volunteers with known KIR typing were used as positive and negative controls.

HLA class I genotyping and KIR ligand assignments

HLA class I genotyping was performed by the University of California, San Francisco Immunogenetics and Transplantation Laboratory using PCR with sequence specific primers and sequence specific oligonucleotides. HLA typing was used along with KIR typing to stratify patients into groups according to predicted KIR–ligand interactions and binding affinities (Table II) (30, 42, 46). HLA-Bw4 alleles were divided based on the presence of an isoleucine at position 80 (predicted high-affinity KIR3DL1 ligand) or a threonine at position 80 (predicted low-affinity ligand), HLA-Bw4-80Ile+ (40), HLA-A23:01, HLA-A*24:02, and HLA-A*32:01 alleles were considered high-affinity KIR3DL1 ligands (HLA-Bw4-80Ile+), based on the presence of the HLA-Bw4-80Ile motif and recent NK cell cytotoxicity data validating KIR3DL1+ NK cell inhibition by these specific HLA-A ligands (46–48). In patients with both HLA-Bw4-80Ile and HLA-Bw4-80Thr (n = 13), the presence of HLA-Bw4-80Ile was given precedence, based on a dominant effect of a high-affinity interaction seen in mice (27). HLA-C alleles were similarly divided based on the presence of a predicted low-affinity HLA-C2Lys80 binding motif for KIR2DL2/3 or a predicted high-affinity HLA-C2Thr80 binding motif for KIR2DL1 (30, 46).

Statistical analysis

Probabilities of AML relapse were calculated and plotted using cumulative incidence functions to accommodate the competing risk of nonrelapse mortality (NRM) (49–53). To assess and control for other factors associated with relapse, multivariate competing risk regression analyses based on the approach of Fine and Gray were performed (53), incorporating cytogenetic risk status (better-risk versus intermediate-risk versus poor-risk versus unknown risk), age (≥60 versus <60), and disease subtype (AML or acute promyelocytic leukemia [APL]) as covariates. Two-sided p values were derived using the Fine and Gray method for the primary analyses and subgroup analyses involving sufficient patients and events; two-sided p values were similarly derived using the Gray method for subgroup analyses involving small sample size (n < 25) (50, 53).

Results

Patient characteristics

Characteristics of the 125 patients are listed in Table I. Median follow-up was 118 mo. The three main genotype subgroups examined (KIR3DL1+ and HLA-Bw4-80Ile+; KIR3DL1+ and HLA-Bw4-80Thr+, HLA-Bw4-80Ile+; and HLA-Bw4-480Ile+ or KIR3DL1+ genotype) were similar with respect to age at transplant, AML subtype (AML versus APL), remission status (Complete Remission-1 versus Complete Remission-2), and cytogenetic risk status (better-risk versus intermediate-risk versus poor-risk). Frequencies of KIR and HLA class I ligand status were similar to those in other reports (54–56).

Compound KIR3DL1+ and HLA-Bw4-80Thr+, HLA-Bw4-80Ile+ genotype predictive of low-affinity interactions is associated with lower relapse in AHCT

To test the hypothesis that KIR3DL1 and HLA-Bw4 genotypes predictive of different receptor–ligand affinities (Table II) affect outcomes, we stratified patients according to possession of a KIR3DL1+ and HLA-Bw4-80Ile+ genotype, predictive of high-affinity interactions; a KIR3DL1+ and HLA-Bw4-80Thr+; HLA-Bw4-80Ile+ genotype, predictive of low-affinity interactions; or a missing ligand or receptor (HLA-Bw4-480Ile+ or KIR3DL1+). Possession of a KIR3DL1+ and HLA-Bw4-80Thr+, HLA-Bw4-80Ile+ genotype was associated with lower relapse than a KIR3DL1+ and HLA-Bw4-80Ile+ genotype (hazard ratio [HR], 0.22; 95% confidence interval [CI], 0.06–0.78; p = 0.02), whereas a missing ligand or receptor genotype was associated with intermediate relapse (Fig. 1). The effects of predicted high- and low-affinity KIR3DL1 and HLA-Bw4 interactions were influenced by gene dose of HLA-Bw4, with relapse progressively increasing among KIR3DL1+ patients with one copy of HLA-Bw4-80Ile (HR, 1.6; 95% CI, 0.84–3.1; p = 0.15) to
two to three copies (HR, 3.0; 95% CI, 1.4–6.5; \( p = 0.005 \); Fig. 2A), and progressively decreasing among patients with one copy of HLA-Bw4-80Ile to two copies (Fig. 2B). Similar findings were obtained when excluding APL patients and when restricting to patients with busulfan and etoposide chemotherapy conditioning (data not shown).

These findings are consistent with an NK cell GVL mechanism in AHCT for AML and suggest that the net effect of KIR3DL1 and HLA-Bw4 interactions is specific to the HLA-Bw4 ligand subtype. Although high-affinity HLA-Bw4-80Ile ligands may endow greater “missing-self” killing and protect from HIV progression in the setting of downregulated HLA-B expression (28, 35, 57), in the absence of HLA downregulation, as in AML (58), low-affinity KIR and HLA interactions may provide the optimal balance of licensing (28) without potent HLA class I–mediated inhibition (30, 32, 34, 42).

**Compound KIR3DL1, KIR2DL1/2/3, and HLA genotypes combine to influence relapse in AHCT for AML.**

Similar to HLA-Bw4 ligands and their cognate KIR3DL1 receptors, the HLA-C ligands inhibit, license, and bind with variable affinity to KIR2DL1/2/3 receptors (20, 30, 42) and may similarly affect AHCT outcomes (14, 59). The presence of a KIR2DL1+ and HLA-C2+ genotype was associated with higher relapse, compared with HLA-C1/C1 (data not shown), although this was not statistically significant and was confounded by an unequal distribution of KIR3DL1 and HLA-Bw4 interactions: 61% of HLA-C2+ patients possessed the KIR3DL1+ and HLA-Bw4-80Ile+ genotype, compared with 32% of HLA-C1/C1 patients (\( p = 0.004 \); Table III). Conversely, HLA-C1/C1 patients were more likely to lack KIR3DL1 and HLA-Bw4 interactions: 52% of HLA-C1/C1 patients possessed a missing ligand or receptor genotype, compared with 20% of HLA-C2+ patients (\( p = 0.0006 \)).

Because KIR3DL1 and KIR2DL1/2/3 receptor–ligand interactions combine to influence aggregate NK cell function, we stratified patients according to compound KIR3DL1, KIR2DL1/2/3, and HLA genotypes. Among patients with a KIR3DL1+ and HLA-Bw4-80Ile+ genotype, predictive of high-affinity interactions, relapse increased with increasing number of predicted KIR2DL1 and HLA-C2 interactions, such that two copies of HLA-C2 with KIR2DL1 were associated with higher relapse than two copies of HLA-C1 (HR, 4.0; 95% CI, 1.4–11; \( p = 0.01 \); Fig. 3A). Although the patient numbers were small, among 17 patients with a KIR2DL1+ and HLA-C2/C2 genotype, a KIR3DL1+ and HLA-Bw4-80Ile+ genotype, predictive of high-affinity interactions, or a lack of a KIR3DL1+ and HLA-Bw4-80Ile+ genotype, rescued KIR2DL1+ and HLA-C2/C2 AML patients from high relapse (\( p = 0.007 \); Fig. 3B). Similar results were obtained when excluding APL patients and when restricting to patients with busulfan and etoposide chemotherapy conditioning (data not shown). These findings suggest that KIR3DL1 and KIR2DL1/2/3 receptor–ligand interactions combine to influence the NK cell GVL mechanism in AHCT for AML and that KIR2DL1 and HLA-C2 interactions are highly inhibitory in the setting of AHCT for AML, consistent with findings in alloHCT for AML (14, 59) and in hepatitis C (30, 36).

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>56</th>
<th>20</th>
<th>49</th>
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<td>55</td>
<td>47</td>
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<tr>
<td>Median</td>
<td>46</td>
<td>50</td>
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<tr>
<td>Mean</td>
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<td>23–72</td>
<td>20–69</td>
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<tr>
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<td>15</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age distribution, n (%)</td>
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<tr>
<td>&lt;60</td>
<td>45 (80)</td>
<td>16 (80)</td>
<td>44 (90)</td>
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</tr>
<tr>
<td>≥60</td>
<td>11 (20)</td>
<td>4 (20)</td>
<td>5 (10)</td>
<td></td>
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<tr>
<td>Disease, n (%)</td>
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<tr>
<td>AML</td>
<td>47 (84)</td>
<td>18 (90)</td>
<td>41 (84)</td>
<td>0.78</td>
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<tr>
<td>APL</td>
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<td>8 (16)</td>
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<td>Complete remission (CR) status, n (%)</td>
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<td>CR-1</td>
<td>49 (88)</td>
<td>19 (95)</td>
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<tr>
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<td>1 (2)</td>
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<td>Cytogenetic risk status(^a), n (%)</td>
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<td></td>
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<tr>
<td>Better</td>
<td>18 (32)</td>
<td>4 (20)</td>
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<td>Intermediate</td>
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<td>12 (60)</td>
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<td>—</td>
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<td>Race, n (%)</td>
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<td>1 (5)</td>
<td>5 (10)</td>
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<td>3 (6)</td>
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<tr>
<td>White</td>
<td>41 (73)</td>
<td>16 (80)</td>
<td>36 (73)</td>
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<tr>
<td>Other or unknown</td>
<td>6 (11)</td>
<td>3 (15)</td>
<td>3 (6)</td>
<td></td>
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</table>

\( ^{a} \)The \( p \) values are by \( \chi^2 \) test, except for age distribution, disease, remission status (Fisher exact test), and age (t test).

\( ^{b} \)Cytogenetic risk was assigned as better-risk for inv(16), t(16;16), t(8;21), or t(15;17); intermediate-risk for normal cytogenetics, trisomy 8 alone, t(9;11) or other undefined cytogenetics; poor-risk for complex abnormalities, del5, del7, 11q23 (non t9;11), inv3, t(6;9), t(9;22), or FLT3-ITD mutation.
Of 21 patients salvaged with alloHCT after AHCT relapse (Supplemental Table I), patients lacking both HLA-Bw4-80Ile and HLA-C2 experienced the lowest relapse (1 [25%] of 4 relapse, 1 NRM, 2 censored) compared with patients with one predicted high-affinity allele (HLA-Bw4-80Ile+ or HLA-C2+; 4 [44%] of 9 relapse, 1 NRM, 1 censored) or both predicted high-affinity alleles (HLA-Bw4-80Ile+ and HLA-C2+; 6 [75%] of 8 relapse, 1 NRM, 1 censored), suggesting similar biology in alloHCT (Supplemental Fig. 1).

Similar benefit of KIR3DL1+ and HLA-Bw4-80Thr+, HLA-Bw4-80Ile− genotype is suggested in intermediate-risk AML patients

Approximately 40–50% of AML patients have a normal karyotype or cytogenetic abnormalities not associated with well-defined risk subgroups and are therefore classified as intermediate-risk (6, 60). Applying KIR and HLA immunogenetics as a novel biomarker to inform postremission strategies may be most useful among intermediate-risk AML patients, as outcomes are variable, and the use of alloHCT is controversial within this subgroup (6). Although the patient numbers were small, there was a suggestion of a benefit of a KIR3DL1+ and HLA-Bw4-80Thr+, HLA-Bw4-80Ile− genotype among AML patients with intermediate-risk cytogenetics, with lower relapse relative to patients with a KIR3DL1+ and HLA-Bw4-80Ile+ genotype (HR, 0.23; 95% CI, 0.05–1.0; \( p = 0.06 \)) and a missing ligand or receptor genotype (HR, 0.32; 95% CI, 0.06–1.6; \( p = 0.17 \); Supplemental Fig. 2).

### Discussion

The biological impact of genetic polymorphisms among genes encoding receptors and ligands of innate immune cells is supported

---

Table II. KIR ligand assignments

<table>
<thead>
<tr>
<th>KIR3DL1</th>
<th>HLA-Bw4-80Ile</th>
<th>HLA-Bw4-80Thr</th>
<th>KIR2DL1</th>
<th>HLA-Bw6</th>
<th>KIR2DL2/3</th>
<th>HLA-C2</th>
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<td>B*07, 07:02</td>
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<td>B*39, 39:06</td>
<td>C*16:02</td>
<td>C*14</td>
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<td>B*41, 41:01</td>
<td>C*17:01</td>
<td>C*16, 16:01</td>
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<td>B*46, 46:01</td>
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<td>B*57, 57:01</td>
<td>B*48:01</td>
<td>B*50:01</td>
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<td>B*60</td>
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</table>

Alleles present in cohort.

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**FIGURE 1.** A predicted low-affinity KIR3DL1 and HLA-Bw4 genotype is associated with lower relapse than a predicted high-affinity KIR3DL1 and HLA-Bw4 genotype in AHCT for AML. KIR and HLA typing were used to stratify AML patients according to the presence of a predicted high-affinity KIR3DL1 and HLA-Bw4 genotype (KIR3DL1+ and HLA-Bw4-80Ile+), low-affinity genotype (KIR3DL1+ and HLA-Bw4-80Thr+, HLA-Bw4-80Ile−), or missing ligand or receptor genotype (HLA-Bw4− or KIR3DL1−). Probabilities of relapse were calculated and compared using a multivariate competing risk regression model to accommodate the competing risk of NRM. Probabilities of relapse (black curves) and NRM (red curves) are shown. Patients with a predicted low-affinity KIR3DL1 and HLA-Bw4 genotype had lower relapse than patients with a predicted high-affinity KIR3DL1 and HLA-Bw4 genotype, and patients with a missing ligand or receptor genotype had intermediate relapse.
by multiple studies in humans (26, 32, 33, 47, 48, 61, 62) and mice (25, 27, 28), although the clinical significance of these differences is unclear. AHCT is an ideal setting for evaluating the clinical importance of immunogenetic polymorphisms influencing the innate immune system because innate immune cells recover early, in the absence of immunosuppression and before the reconstitution of adaptive immunity (8–11). In a cohort of patients with AML treated uniformly with AHCT, we found that differences in highly polymorphic KIR and HLA genotypes predictive of differences in NK cell receptor–ligand interaction affinities are associated with relapse. Patients with KIR and HLA class I genotypes predictive of low-affinity interactions and weak NK cell licensing and inhibition (26, 30, 32) experience lower relapse than patients with KIR and HLA genotypes predictive of high-affinity interactions and potent NK cell licensing and inhibition. These findings are consistent with an autologous NK cell GVL effect (39, 63) and support a model of NK cell reactivity whereby low-affinity inhibitory KIR and HLA interactions tip the balance in favor of licensing and activation, whereas high-affinity inhibitory KIR and HLA interactions tip the balance in favor of inhibition.

The affinity of a receptor–ligand interaction as a modulator of NK cell immune responses has been proposed in mice and human viral infections (27, 30, 64); we now show an association between AML relapse and inhibitory KIR and HLA genotypes predictive of different affinity interactions. Our findings are consistent with the protective effect of the KIR2DL3+ and HLA-C1/C1 compound genotype in hepatitis C and in influenza A, attributed to weaker HLA-C1-mediated inhibition of NK cells expressing KIR2DL3, compared with HLA-C2–mediated inhibition of NK cells expressing KIR2DL1 (30, 36). Along a continuum of weak to strong KIR and HLA interactions, our data suggest that high-affinity KIR3DL1 and HLA-Bw4-80Ile interactions confer not only strong NK cell licensing but also strong inhibition, resulting in weak NK cell antileukemic activity in AHCT for AML. Patients lacking HLA-Bw4 for KIR3DL1 had an intermediate incidence of relapse compared with patients with predicted high- or low-affinity KIR3DL1 and HLA-Bw4 interactions. A missing ligand benefit in AHCT for AML is consistent with increased reactivity of unlicensed NK cell subsets following HLA-matched alloHCT (37) and is consistent with the clinical benefit of a missing ligand genotype observed in AHCT for neuroblastoma.
To our knowledge, we are the first to report a benefit of a predicted low-affinity KIR3DL1 and HLA-Bw4 interaction beyond that of missing ligand. This effect may have been concealed in previous association studies that did not separate HLA-Bw4-80Thr and HLA-Bw4-80Ile alleles and did not consider the predicted KIR3DL1 ligand status of HLA-A Bw4-80Ile alleles, present in 33% of patients in our cohort. Lacking the HLA-Bw4 ligand for KIR3DL1, therefore, may provide a NK cell subset that is neither enhanced by a favorable KIR3DL1 and HLA-Bw4-80Thr interaction nor inhibited by an unfavorable KIR3DL1 and HLA-Bw4-80Ile interaction to improve or antagonize the clinical benefit of AHCT for AML.

Beyond HLA polymorphism within the Bw4 binding motif, other factors influence NK cell phenotype and function and may similarly influence AHCT outcomes (18, 19, 33, 61). KIR3DL1 allelic variants can influence NK cell responses in unexpected ways. Emerging data suggest that two polymorphisms within the KIR3DL1*004 allele (Leu 86 and Ser 182) negatively affect protein folding, resulting in significantly reduced cell surface expression (18, 61, 65–67).

### Table III. Distribution of KIR3DL1 and HLA-Bw4 genotypes with KIR2DL1/2/3 and HLA-C genotypes

<table>
<thead>
<tr>
<th>KIR3DL1 and HLA-Bw4 Subtype</th>
<th>n</th>
<th>KIR3DL1* and HLA-Bw4-80Ile*</th>
<th>KIR3DL1* and HLA-Bw4-80Thr*, HLA-Bw4-80Ile*</th>
<th>HLA-Bw4* or KIR3DL1*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-C1/C1 and KIR2DL2/3*</td>
<td>56</td>
<td>18 (32%)</td>
<td>9 (16%)</td>
<td>29 (52%)</td>
</tr>
<tr>
<td>HLA-C2* and KIR2DL1*</td>
<td>51</td>
<td>31 (61%)</td>
<td>10 (20%)</td>
<td>10 (20%)</td>
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<tr>
<td>p value*</td>
<td>0.004</td>
<td>0.80</td>
<td>0.0006</td>
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</tbody>
</table>

*Alleles present in cohort.

*The p values were by Fisher exact test.

FIGURE 3. Combined KIR3DL1 and KIR2DL1/2/3 ligand subtypes influence relapse in AHCT for AML. (A) Patients with AML and a predicted high-affinity KIR3DL1* and HLA-Bw4-80Ile* genotype were stratified according to KIR2DL1 status and HLA-C2 copy number. Probabilities of relapse were calculated and compared using multivariate competing risk regression analysis. Probabilities of relapse (black curves) and NRM (red curves) are shown. Among KIR3DL1* and HLA-Bw4-80Ile* patients, relapse increased progressively with increasing copy number of HLA-C2 with KIR2DL1. (B) Conversely, KIR2DL1* and HLA-C2/C2 patients were stratified according to possession or lack of a predicted high-affinity KIR3DL1* and HLA-Bw4-80Ile* genotype. Probabilities of relapse were calculated and compared using a univariate competing risk regression analysis model. Probabilities of relapse (black curves) and NRM (red curves) are shown. Among KIR2DL1* and HLA-C2/C2 patients, the lack of a predicted high-affinity KIR3DL1* and HLA-Bw4-80Ile* genotype was associated with lower relapse. No NRM events were observed among the 17 KIR2DL1* and HLA-C2/C2 patients.
alleles are functional despite low surface expression, and small amounts of transiently expressed KIR3DL1*004 molecules at the cell surface may be sufficient for NK cell licensing (67). Lower KIR3DL1 cell surface expression can, however, result in weaker inhibition, particularly if cis interactions with HLA-Bw4 further diminish the presence of KIR3DL1 available for interacting with target cells, as suggested by findings in mice (68, 69). Indeed, NK leukemia cell lines expressing KIR3DL1*004 are only weakly inhibited by HLA-Bw4-80le, compared with NK leukemia cell lines expressing KIR3DL1*002 (67). The retained portion of the KIR3DL1*004 protein may, alternatively, have intracellular function, as suggested by findings in HIV (70), and the degree of cell surface expression or intracellular retention of KIR3DL1 may conceivably contribute to a separation of NK cell licensing from NK cell inhibition. Beyond the KIR3DL1*004 variant, there appears to be a hierarchy of KIR3DL1 expression among different KIR3DL1 alleles, characterized by dominant expression of KIR3DL1*001, and these differences in expression may similarly affect NK cell function and clinical outcomes (71, 72).

Simple dichotomies in NK cell receptor-ligand binding affinities and definitions of cognate KIR–ligand interactions have also been questioned and further limit our ability to predict in vivo NK cell function (20, 73). Differences between KIR3DL1*002 and KIR3DL1*007 alter ligand-binding strength and inhibition of lysis of the NK-sensitive, HLA class I-deficient 721.221 cell line that is transfected with HLA-Bw-4 alleles in a model system using NK leukemia cell lines transduced with distinct KIR3DL1 alleles (33). In HLA class I tetramer-binding studies, certain KIR3DL1 alleles show preferential binding of HLA-Bw4-80le over HLA-Bw4-80Thr, whereas other KIR3DL1 alleles interact equally well with both HLA-Bw4 molecules (19). HLA-B*13 and HLA-A*25 fail to engage KIR3DL1 and inhibit NK cell cytotoxicity despite the presence of an HLA-Bw4 sequence motif (47, 48, 62). Future studies probing how allele-driven differences in KIR3DL1 surface expression and ligand binding affect both NK cell licensing and NK cell inhibition will be essential in refining these immunogenetic-driven prediction algorithms for use in patient stratification (18, 61, 65, 66).

There are numerous reports associating KIR3DL1 and HLA-Bw4-80le combinations with better AIDS outcomes (35, 38). In HIV, unlike in other settings, the benefits of heightened NK cell responsiveness attributed to high-affinity KIR3DL1 and HLA-Bw4-80le interactions may come without the cost of strong inhibition because of the unique and selective downregulation of HLA-A and -B expression on HIV-infected target cells by the HIV-encoded Nef protein (74). Greater NK cell licensing in HIV may lead to greater “missing-self” killing against HIV-infected targets lacking HLA-A and -B expression (27, 64), whereas greater NK cell licensing in AHCT for AML may lead to greater inhibition in a highly inflammatory state in which inhibitory HLA-A and -B ligand expression is increased by cytokines (31, 33, 75).

Similar KIR and HLA combinations have been associated with susceptibility for solid tumors and hematologic malignancies. Possession of a KIR3DL1* and HLA-Bw4 genotype was associated with increased incidence of kidney cancer (76), and possession of a KIR3DL1/3DL1 and HLA-Bw4 genotype was associated with increased incidence of chronic lymphocytic leukemia (77). Among individuals with KIR3DL1 and HLA-Bw4, patients with colon cancer were enriched for HLA-Bw4-80le, whereas patients with other solid tumors were enriched for HLA-Bw4-80Thr (76). Possession of a KIR2DL3* and HLA-C1/C1 genotype was previously associated with a decreased incidence of non-small-cell lung carcinoma and kidney cancer (76), whereas possession of a KIR2DL2/3 and HLA-C1* genotype was associated with a decreased incidence of myeloid leukemia (77). In our cohort of patients with AML, however, we did not find an increased frequency of KIR3DL1 and HLA-Bw4 subtypes, as compared with estimates of KIR and HLA haplotype frequencies in U.S. population studies (data not shown).

AHCT has weaker antileukemic activity but lower rates of transplant-related mortality and graft-versus-host disease, compared with alloHCT (78). The durable remissions seen in patients with AML with predicted low-affinity KIR and HLA genotypes treated with AHCT challenges the clinician to consider AHCT to capture autologous NK cell GV activity without the risk of graft-versus-host disease. The inflammatory conditions of high-dose chemotherapy may trigger enhanced NK cell function and lead to memory-like NK cell features similarly seen in mice (79). Differences in genes regulating NK cell function through differences in receptor–ligand affinities may modify the clinical benefit and be useful in selecting appropriate candidates for AHCT. These results merit further phenotype and functional studies and prospective evaluation to validate the consideration of host immunogenetics to complement age, comorbidities, and tumor-intrinsic factors in stratifying patients with AML for postinduction therapy.

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Disclosures
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