Donors with Group B KIR Haplotypes Improve Relapse-Free Survival after Unrelated Hematopoietic Cell Transplantation for Acute Myelogenous Leukemia

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ABSTRACT

Survival after unrelated donor (URD) hematopoietic cell transplantation (HCT) for Acute Myeloid Leukemia (AML) is limited by toxicity and relapse. Killer-cell immunoglobulin-like receptors (KIR) control NK cell alloreactivity after HCT. Hypothesizing that donor KIR genotype (A/A: 2 A KIR haplotypes; B/x: ≥ 1 B haplotype) would affect outcomes, we genotyped donors and recipients from 448 URD transplantations for AML. Three year overall survival was significantly higher after transplantation from a KIR B/x donor (31% [95% CI: 26-36] vs. 20% [95% CI: 13-27]; p = 0.007). Multivariate analysis demonstrated a 30% better relative risk of relapse-free survival with B/x vs. A/A donors (RR 0.70 [95% CI 0.55-0.88]; p=.002). B/x donors were associated with more chronic GVHD (RR 1.51 [95% CI 1.01-2.18]; p=.03), but not more acute GVHD, relapse or treatment-related mortality. This analysis demonstrates that HCT from unrelated donors with KIR B haplotypes confers significant survival benefit for patients with AML.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) from related or unrelated donors (URD) is standard treatment for many patients with hematologic malignancies who are unlikely to be cured by chemotherapy alone. While the most important variable for donor selection is the match for HLA class I and class II, other relevant factors include donor sex, parity, CMV serostatus, and age[1]. Alloreactive donor-derived natural killer (NK) cells have been correlated with improved survival after HCT for acute myelogenous leukemia, and are thought to promote engraftment, reduce graft vs. host disease (GVHD), and decrease leukemic relapse[2,3]. NK cell function is determined by the net effect of signaling through several receptor families, including activating and inhibitory killer-cell immunoglobulin-like receptors (KIR). While the ligands for activating KIR remain elusive, the interactions between inhibitory KIR on the donor-derived NK cells and HLA class I molecules on the recipient's healthy and leukemic cells determine NK cell alloreactivity.   All HLA-C allotypes have either the C1 epitope, the ligand for *KIR2DL2/3*, or the C2 epitope, the ligand for *KIR2DL1*. Analogously, all HLA-B allotypes have either the Bw4 or Bw6 epitope, but only the Bw4 epitope is a ligand for KIR: its cognate inhibitory receptor being *KIR3DL1*[4]. In healthy individuals, interactions of inhibitory KIR with cognate HLA class I prevent NK cells from attacking healthy cells[5]. In the setting of allogeneic transplantation, donor NK cells attack the allogeneic cells if the recipient HLA class I ligands do not sufficiently engage their inhibitory receptors.

The KIR genes on chromosome 19 segregate independently of HLA, with the important consequence for unrelated allogeneic transplantation that matching for HLA does not match for KIR. Diverse KIR haplotypes can be simplified into two biologically distinct groups, A and B. Group A haplotypes have a fixed number of genes that encode inhibitory receptors with the exception of *2DS4* , while group B haplotypes have variable gene content including additional activating receptor genes. These KIR haplotypes have been associated with reproductive success, responses to viral infections such as HIV and HCV, susceptibility to autoimmune

disease and outcomes of HCT [6-8]. All individuals can be categorized as having one of two KIR genotypes: A/A which is homozygous for group A KIR haplotypes, or B/x, which contains either one (A/B heterozygotes) or two (B/B homozygotes) group B haplotypes. Previous HCT studies have reported varied and sometimes inconsistent associations between KIR haplotype and clinical outcome[9-16]. The results were confounded by the small size and heterogeneity of the cohorts studied. In aggregate these results suggested the hypothesis that combining KIR and HLA genotyping could help selection of transplant donors and improve the outcome of transplantation. To test this hypothesis we analyzed the effects of KIR genotype in a homogeneous cohort of 448 AML patients transplanted from unrelated donors.

MATERIALS & METHODS

Population

Collection of DNA samples from 448 donor/recipient pairs from URD HCT performed to treat AML was facilitated by the National Marrow Donor Program (NMDP) Research Sample Repository. Outcome data was obtained from the Center for International Blood and Marrow Transplant Research (CIBMTR). Samples and clinical data were obtained after informed consent and approval from the NMDP and University of Minnesota Institutional Review Boards according to the Declaration of Helsinki. To decrease the cohort's clinical heterogeneity, samples from 3 groups (HLA matched/KIR-ligand matched, HLA mismatched/KIR-ligand matched and HLA-mismatched/KIR-ligand mismatched) were selected using an algorithm which matched on important demographic and clinical variables (see Table 1). KIR ligand matching status was determined using high-resolution HLA-B and HLA-C genotypes[17], following the algorithm available on the KIR-ligand calculator tool maintained by the HLA Informatics Group of the Anthony Nolan Research Institute (http://www.ebi.ac.uk/ipd/kir/)[18].

Procedures

KIR Genotyping: The presence or absence of 16 KIR genes (*KIR2DL5A* and *KIR2DL5B* were considered together) was determined using a high-throughput SNP-based Sequenom MassARRAY™ system and the Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) platform for the large scale KIR genotyping of DNA samples as previously validated[19].

Haplotype Assignment: Detection of at least one of the KIR B haplotype-defining loci (*KIR2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS5* or *3DS1*) in a sample dictated that the genotype contains at least one B haplotype. Such samples were assigned the genotype designation B/x. Samples lacking all KIR B loci were assigned the genotype A/A.

Statistical Analysis

We considered six outcomes in the analysis: (survival, relapse-free survival (RFS), relapse, treatment-related mortality (TRM), and grade II-IV acute and chronic GVHD). Overall survival (OS) and RFS were summarized by Kaplan-Meier curves, while the other four events were summarized by cumulative incidence functions. OS and RFS were compared between KIR genotype groups using the log rank test. Other outcomes were compared using Gray's test[20]. The completeness of follow-up was over 90% at three years post follow-up and 95% of events occurred by three years post HCT.

For OS and RFS a Cox proportional hazards model was used to adjust for important clinical factors. All variables were tested for proportional hazards using a time-dependent covariate approach. The multivariate model accounted for HLA matching, and other covariates listed in Table 1 were examined using backward stepwise selection. Variables were removed when the multiple degree of freedom test p-value exceeded 0.10. A second model that stratified on HLA type gave similar results.

For the other events (relapse, TRM, acute and chronic GVHD) the Cox model was replaced by the model of Fine and Gray for the sub-distributional hazard based on the cumulative incidence function[21]. Relapse and death were considered as competing risks.

RESULTS & DISCUSSION

Results

The 448 AML patients received URD myeloablative, T-cell replete transplantations facilitated by the National Marrow Donor Program between 1988 and 2003. The cohort consisted of three HLA match groups (see Table 1). Approximately half (47%) were 10/10 HLA-allele matched (and thus KIR-ligand matched) at HLA-A, B, C, DRB1, DQB1. The HLA mismatched group (53%) was further divided into KIR-ligand mismatched (29%) and KIR-ligand matched (71%) groups. KIR genotyping for the presence of 16 KIR genes show that the donors and recipients in this predominantly Caucasian population had similar and typical KIR frequencies. In this cohort 29.2% of donors and 34.8% of recipients had the A/A KIR genotype, the remainder having the B/x genotype. There were no significant differences in KIR gene or haplotype frequencies between the three HLA match groups, neither did they vary for transplants performed at different times during the period 1988-2002: in any given year ~66% of donors were B/x.

We analyzed the effect of donor and recipient KIR genotype on outcomes after URD HCT for AML. Whereas the recipient KIR haplotype content had no effect on relapse-free survival (p = .75), KIR B/x donors were associated with significantly better 3 year relapse-free survival (Donor KIR B/x: 28% [95% CI: 23-33] vs. Donor KIR A/A 17% [95% CI: 11-24]; p = .003) (Figure 1). In univariate analysis, 3 year overall survival was also significantly higher after transplantation from a B/x donor (31% [95% CI: 26-36] vs. 20% [95% CI: 13-27]; p = 0.007) (Figure 2). Stratifying patients by HLA matching, we found that the 3 year relapse-free survival was higher with KIR B/x vs. KIR A/A donors after 10/10 allele HLA matched/KIR-ligand matched (32% vs. 20%) and HLA mismatched but KIR-ligand matched transplantations (29% vs. 14%), but not in the cohort who received HLA mismatched/KIR-ligand mismatched transplants (15% vs. 18%) (Figure 3).

To explore further the influence of donor KIR haplotypes, a multivariate Cox proportional hazards model was constructed adjusting for statistically significant (p < 0.10) clinical and demographic factors including HLA match group, disease status (1st or greater complete remission [CR] vs. primary induction failure [PIF] or relapse), time from diagnosis to transplant, age, race, and Karnofsky Performance Status (KPS). After adjustment, the benefit of transplantation from KIR B/x donors (compared to A/A) remained highly significant, with a favorable RR of 3 year relapse-free survival of 0.70 (95% CI: 0.55-0.88; p=.002). Graft type (peripheral blood progenitor cell [PBPC] vs. marrow) was not a significant covariate and was not included in the multivariate model. There was no significant interaction between graft type and the donor KIR haplotype effect and the conclusions were unchanged in a subset analysis which excluded those patients (11% of total) who received PBPC; RR with B/x donors of 3 year relapse-free survival of 0.71 (95% CI: 0.56-0.92; p=.008).

As expected, older and less fit patients being treated for intermediate and advanced leukemia or receiving grafts from HLA-mismatched donors had poorer relapse-free survival (Table 2). The multivariate analysis confirmed that donor KIR B/x genotype conferred a significant relapse-free survival benefit (RR 0.69 [95% CI: 0.54-0.90), p = 0.005) for all KIR-ligand matched transplantations (HLA matched and HLA mismatched). We also analyzed the group with 1 or 2 HLA allele mismatches only (n= 185), excluding those with greater degrees of HLA mismatch. In this partially HLA-matched group the 3 year estimate of relapse-free survival using KIR B/x genotype donors (26% [95% CI: 19-34]) was comparable to the 3 year relapse-free survival estimate for all (donor KIR A/A or B/x) 10/10 HLA allele matched transplantations (29% [95% CI: 23-35]). A multivariate Cox model comparing these groups showed no difference (RR 1.07 [95% CI: 0.76-1.50]), suggesting that favorable donor KIR genotype can, at least in part, overcome the adverse effect of partial HLA mismatch.

After establishing that B/x haplotype donors conferred a survival advantage over A/A donors we performed further analyses to explore the underlying mechanism. The influence of individual donor KIR genes on survival outcomes was examined (see Table 3). The two KIR showing independent effects on overall survival were *KIR2DL2* (p = .07) and *KIR2DS2* (p = .02), which are in strong linkage disequilibrium with each other. Of the 448 donors, 246 had both *KIR2DL2* and *KIR2DS2*, 195 had neither, and only seven had just one of them (6 *2DS2+/2DL2-* and 1 *2DS2-/2DL2+*). To assess the quantitative contribution of *KIR2DS2/L2* to the favorable relapse-free survival benefit using B/x donors we constructed a multivariate Cox model adjusting for the same covariates and comparing A/A donors to B/x donors who either have or lack *KIR2DS2*. Transplantations from either KIR B/x genotype group (*KIR2DS2+* or *KIR2DS2-*) was associated with similarly significant improvements in relapse-free survival (*2DS2+* vs. A/A: RR 0.70 [95% CI: 0.55-0.89], p = 0.003; *2DS2-* vs. A/A: RR 0.69 [95% CI: 0.49-0.97], p = 0.03). Thus presence of *KIR2DS2* is not uniquely responsible for the survival benefit associated with B KIR haplotypes (Figure 4). Using regression modeling to assess the impact of increasing numbers of activating or inhibitory KIR genes, we found no additive effect. Only the presence of at least one (vs. none) KIR B haplotype-defining gene was associated with significant improvement in outcome. Neither did we observe any interaction between the donor KIR genotype (A/A or B/x) and the KIR-ligand status of the recipient. The protective effect of B/x donors was similar in recipients having different combinations of the HLA-C (C1/C1, C2/C2 and C1/C2) and HLA-B (Bw4/x vs. Bw6/Bw6) epitopes that bind to KIR. In these recipient subsets we detected no significant interaction between donor KIR and recipient HLA KIR-ligands using the same model for relapse-free survival (HLA-C: p = 0.8; HLA-B: p = 0.5). Similarly, no differences were observed in acute GVHD, chronic GVHD or overall survival based on KIR-ligand status (all p = NS). In summary, transplantation from a KIR B/x donor was independently associated with improved relapse-free survival.

To assess how KIR B/x donors improve transplant outcome, we analyzed the cumulative incidence estimates and multivariate competing risks models for differences in risk of relapse, TRM, acute and chronic GVHD in transplantations from donors with KIR A/A vs. B/x genotypes (Figure 5). All multivariate competing risks models adjusted for HLA match group, disease status, age, KPS, and time from diagnosis to transplant. Transplantations from KIR B/x donors were associated with modest though not significantly decreased risks for both relapse and TRM. No interaction was detected between CMV serostatus and the effect of a donor B haplotype on any outcomes. A donor KIR B haplotype did not alter the incidence of grade II-IV acute GVHD (adjusted RR 1.10 [95% CI: 0.76-1.59], p = .61), but was associated with increased 3 year cumulative incidence of chronic GVHD [B/x: 39% (95% CI: 34-45) vs. A/A: 28% [95% CI: 20-35] p=.02). Multivariate models confirmed the significant association of donor B/x with increased RR of chronic GVHD (B/x vs. A/A RR 1.51 [95% CI: 1.01-2.18], p = .03).

Discussion

Previous studies on the effects of KIR genotype in HCT involved small cohorts of patients treated for different diseases and reported conflicting results. Here we report our analysis of a large homogeneous cohort of AML patients receiving myeloablative conditioning followed by a T-cell replete unrelated donor grafts. Moreover, we used careful matching criteria to select clinically comparable cohorts of donor:recipient pairs, and we controlled for important clinical covariates using multivariate models.

In this multicenter study, we found that transplantation from a donor having one or two KIR B haplotypes was associated with significant improvements in overall and relapse-free survival, with more than 30% better relative risk in both these endpoints for the KIR B/x donor group. The clinical benefit associated with donor KIR B haplotypes was evident across the entire KIR-ligand matched cohort, and

was not influenced by the recipients' KIR genotype, the extent of HLA match, or the recipients' disease status at the time of transplant. In contrast, the only group showing no benefit from a B/x donor was the small subset of transplants in which there was both HLA and KIR-ligand mismatch. The HLA-C group mismatches that impart KIR-ligand mismatches are known to adversely affect clinical outcomes[17]. Consequently it is possible that the clinical advantage conferred by a KIR B/x donor is insufficient to overcome the disadvantage arising from a KIR-ligand (HLA-C) mismatch.

While our results suggest that donor *2DL2* and *2DS2* may independently be advantageous, the analysis (not adjusted for multiple comparisons) was not definitive because of its limited power. Importantly, the clinical benefit of a B/x donor did not depend on the presence of either *KIR2DL2* or *KIR2DS2*, or any other particular B haplotype-defining KIR gene; nor was there an association with increasing number of activating KIR genes. Ongoing analyses of a larger cohort will address the effect of individual KIR genes. In conclusion, our results indicate that all donors with one or two KIR B haplotypes have the potential to provide an improved clinical benefit in HCT for AML and should be selected over an otherwise equivalent donor who lacks a KIR B haplotype.

The benefit of donor KIR B haplotypes has also been observed for T-cell replete transplants from HLA matched sibling donors. There the improved survival and reduced transplant related mortality were attributed to lower rates of cytomegalovirus reactivation[22], an effect reported in other settings[23] and paralleled in the mouse model of cytomegalovirus infection[24,25]. Independent evidence that human activating KIR in resist infection is the reduced progression to AIDS in HIV infected patients having *KIR3DS1* and a cognate HLA Bw4 ligand [26]. Beneficial and detrimental clinical effects on outcomes after HCT have been associated with activating KIR [9-11,27]. Increased acute GVHD was associated with higher numbers of donor activating KIR in HLA-matched URD transplants.

KIR2DS3, in particular, was strongly associated with GVHD. In our study donor KIR B/x genotype (but not individual KIR genes) was associated with higher rates of chronic, but not acute GVHD. Activating KIR are similarly associated with the chronic inflammation that characterizes autoimmune diseases[7,28].

In haploidentical, T-cell deplete (TCD) HCT, donors mismatched for KIR-ligands were associated with NK-cell alloreactivity and improved survival [3,29]. In URD HCT for myeloid malignancies, mismatching for KIR ligands can confer clinical benefit but only for T-cell depleted grafts[2], not T-cell replete grafts[1,30]. This difference is attributed to T-cells in the graft that interfere with NK cell development and KIR reconstitution after URD HCT[31]. Here we observed no benefit associated with mismatch of KIR ligands, consistent with the previous studies of T-cell replete URD HCT[32,33].

Although the underlying mechanism remains uncertain, this multicenter analysis demonstrates a significant and substantial (>10%) survival benefit for AML patients receiving grafts from unrelated donors having one or two KIR B haplotypes. In our cohort approximately two thirds of the donors had at least one KIR B haplotype, indicating that KIR genotyping of donors to preferentially select for the presence of a B haplotype is a feasible proposition. If implemented, such typing could yield a KIR B haplotype donor option for a majority of the patients who have several high-resolution HLA-matched donors. While a sophisticated and high through-put method based upon mass spectrometry was used in this analytical study, simpler and less expensive methods could be used in clinical immunogenetics laboratories to reliably distinguish A/A and B/x donors[34]. The significant survival benefit observed in this multicenter study of HCT for AML provides compelling evidence that KIR B haplotype donors should be used preferentially in HLA matched or HLA mismatched unrelated donor transplantation.

TABLES

Table 1: Patient Demographics

	Donor KIR Genotype		
	Genotype A/A n = 131 (29%)	Genotype B/x n = 317 (71%)	p-value*
Age (years)			0.57
Median (range)	34.1 (1.8-58.4)	33.3 (0.8-60.8)	
Disease Status			0.53
Early	25 (19.1%)	61 (19.2%)	
Intermediate	42 (32.1%)	118 (37.2%)	
Advanced	64 (48.9%)	138 (43.5%)	
Donor: Recipient HLA-allele matching at A, B, C, DRB1, and DQB1			0.01
10/10 allele matched	51 (38.9%)	158 (49.8%)	
9/10	28 (21.4%)	67 (21.1%)	
8/10	39 (29.8%)	51 (16.1%)	
7/10	13 (9.9%)	41 (12.9%)	
HLA Match Group			0.10
HLA 10/10 allele matched	51 (38.9%)	158 (49.8%)	
HLA mismatched KIR-ligand matched	58 (44.3%)	111 (35.0%)	
HLA mismatched KIR-ligand mismatched	22 (16.8%)	48 (15.1%)	
Sex match (Donor/Recipient)			0.33
Male/Male	40 (30.5%)	117 (36.9%)	
Male/Female	32 (24.4%)	85 (26.8%)	
Female/Male	24 (18.3%)	52 (16.4%)	
Female/Female	35 (26.7%)	63 (19.9%)	
CMV serostatus			0.62
Donor - / recipient -	40 (30.5%)	106 (33.4%)	
Donor + / recipient -	17 (13.0%)	47 (14.8%)	
Donor any / recipient +	73 (55.7%)	160 (50.5%)	
Missing	1 (0.8%)	4 (1.3%)	
Graft type			0.89
Bone Marrow	116 (88.5%)	281 (88.6%)	
Peripheral blood progenitor cells	15 (11.5%)	36 (11.4%)	
Karnofsky Score			0.08
90-100	77 (58.8%)	196 (61.8%)	
10-80	52 (39.7%)	103 (32.5%)	
Unknown	2 (1.5%)	18 (5.7%)	
Time from diagnosis to transplant			0.46
Median years (range)	1.1 (0.0, 4.0)	1.2 (0.0, 8.2)	
Race			0.91
Caucasian	117 (89.3%)	286 (90.2%)	
Non-Caucasian	14 (10.7%)	31 (10.8%)	

*p-values from Welch's t-test or a Chi-square test.

Table 2: Relapse-free Survival after URD HCT: Multivariate Analysis

All samples	RR of Relapse or Death	95% CI	p-value
KIR Genotype			
B/x (compared to A/A)	0.70	(0.55-0.88)	0.002
Transplant Group			
HLA mismatched / KIR-ligand match (compared to 10/10 HLA matched)	1.21	(0.95-1.54)	0.13
HLA mismatched / KIR-ligand mismatch (compared to10/10 HLA matched)	1.65	(1.22-2.23)	0.001
Disease Status			
Intermediate (compared to Early)	1.41	(0.99-2.02)	0.06
Advanced (compared to Early)	2.17	(1.58-2.99)	<0.0001
Age (categorical)			0.03
Karnofsky Performance Status			0.01
Time from diagnosis to transplant (per year)	0.88	(0.77-1.02)	0.08
Non-Caucasian	1.34	(0.95-1.88)	0.10

Table 3: Effect of Individual KIR Genes on Relapse-Free Survival and Overall Survival after URD HCT

KIR Gene	RR for Relapse-free Survival	95% CI	p-value	KIR Gene	RR of Overall Survival	95% CI	p-value
2DL1	1.33	(0.71, 2.49)	0.38	*2DL1*	1.22	(0.65, 2.29)	0.54
2DL2	0.80	(0.65, 0.98)	0.034	*2DL2*	0.82	(0.67, 1.02)	0.07
2DL3	1.54	(1.06, 2.23)	0.022	*2DL3*	1.42	(0.98, 2.06)	0.06
2DL5	0.80	(0.65, 0.98)	0.033	*2DL5*	0.83	(0.67, 1.02)	0.08
2DS1	0.91	(0.74, 1.12)	0.37	*2DS1*	0.94	(0.76, 1.16)	0.54
2DS2	0.75	(0.61, 0.93)	0.008	*2DS2*	0.77	(0.63, 0.95)	0.02
2DS3	0.87	(0.69, 1.09)	0.22	*2DS3*	0.88	(0.70, 1.11)	0.29
2DS4	1.06	(0.61, 1.84)	0.84	*2DS4*	1.03	(0.59, 1.79)	0.93
2DS5	0.85	(0.68, 1.07)	0.16	*2DS5*	0.88	(0.70, 1.10)	0.24
3DL1	1.05	(0.62, 1.80)	0.85	*3DL1*	1.01	(0.59, 1.73)	0.96
3DS1	0.88	(0.71, 1.09)	0.23	*3DS1*	0.91	(0.73, 1.12)	0.37

Shown are the relative risks (RR), 95% confidence intervals (CI) and p-values for the effect in donors of the presence (vs. absence) of each KIR gene on relapse-free survival (RFS) and overall survival (OS). The only KIR locus that is significant at a level of 0.05 for both RFS and OS is *2DS2* (present in 79% of the population). Although the analysis was not adjusted for multiple comparisons, similar results were obtained for *KIR2DL2*, which is in high linkage disequilibrium with *KIR2DS2*. *KIR2DL5,* the other inhibitory KIR associated with a better RR of RFS, was present in 52% of the cohort. The inhibitory *KIR2DL3*, which was associated with a significantly worse relative risk of RFS, is present in 90% of donors in this cohort.

FIGURES

Figure 1: Transplantation using donors with a KIR B haplotype improves relapse-free survival (RFS) irrespective of the recipient KIR haplotype status.
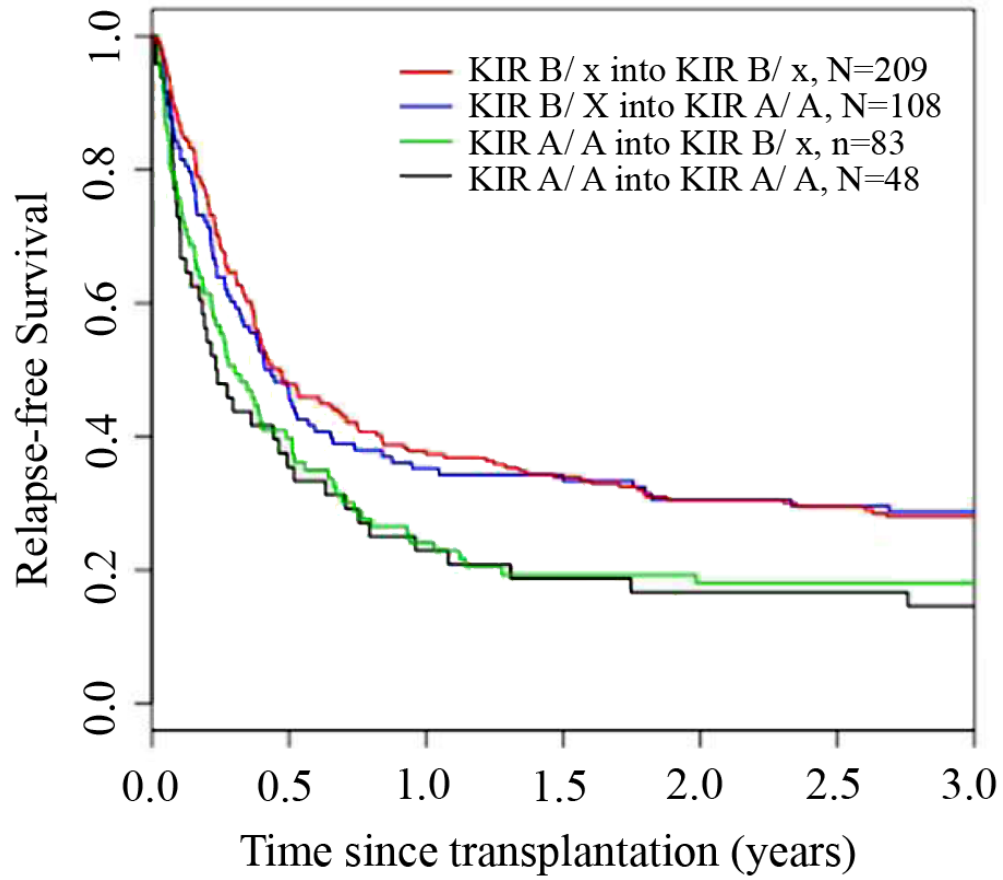
Figure 2: Transplantation using donors with KIR B haplotypes improves overall survival.
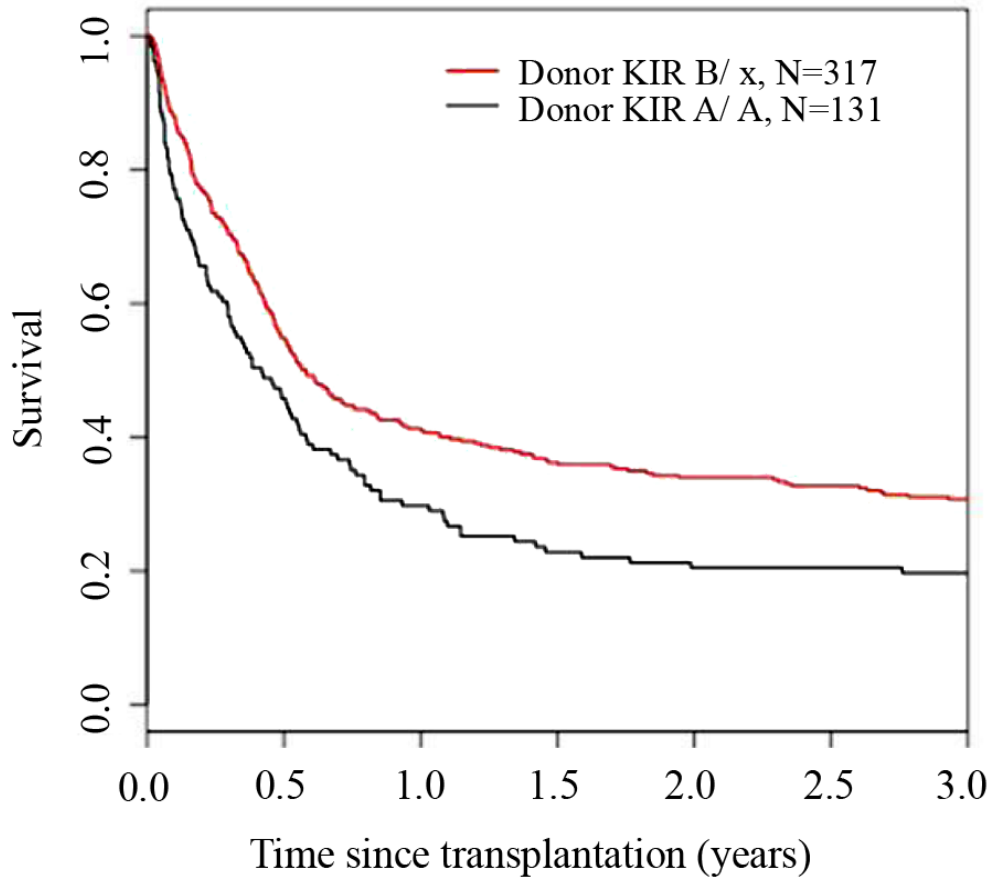
Figure 3: Relapse-free survival benefit from KIR B haplotype donors after HLA-matched and HLA-matched/KIR ligand-matched transplantation.
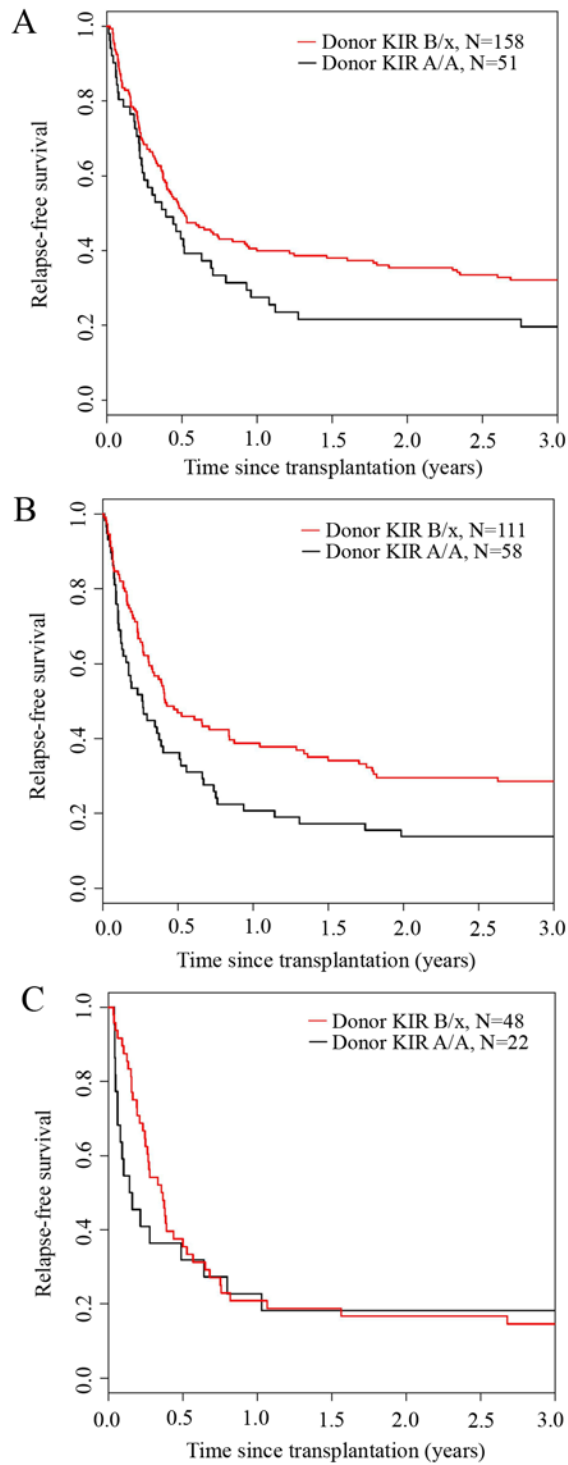
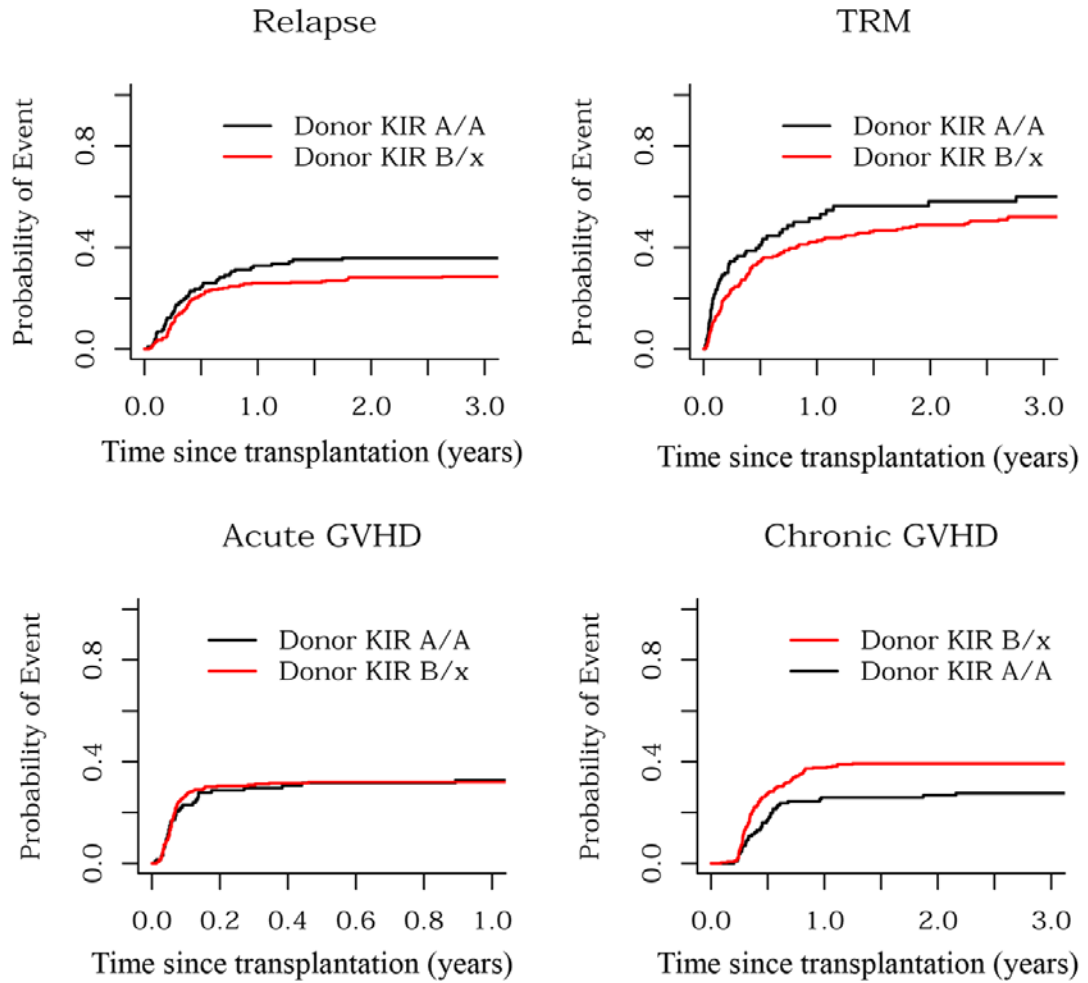**Figure 4:** The survival benefit of a donor KIR B haplotype is not dependent on the presence of KIR2DS2.

Figure 5: Transplantation using donors with KIR B haplotypes leads to less relapse and TRM, and equivalent acute GVHD, but increased chronic GVHD.

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APPENDIX A

(Reprint of published manuscript)

Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia

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Survival for patients with acute myeloid leukemia (AML) is limited by treatment-related mortality (TRM) and relapse after unrelated donor (URD) hematopoietic cell transplantation (HCT). Natural killer (NK)–cell alloreactivity, determined by donor killer-cell immunoglobulin-like receptors (KIRs) and recipient HLA, correlates with successful HCT for AML. Hypothesizing that donor KIR genotype (A/A: 2 A KIR haplotypes; B/x: at least 1 B haplotype) would affect outcomes, we genotyped donors and recipients from 209 HLA-matched and 239 mismatched T-replete URD transplantations for AML. Three-year overall survival was significantly higher after transplantation from a KIR B/x donor (31% [95% CI: 26-36] vs 20% [95% CI: 13-27]; P = .007). Multivariate analysis demonstrated a 30% improvement in the relative risk of relapse-free survival with B/x donors compared with A/A donors (RR: 0.70 [95% CI: 0.55-0.88]; P = .002). B/x donors were associated with a higher incidence of chronic graft-versus-host disease (GVHD; RR: 1.51 [95% CI: 1.01-2.18]; P = .03), but not of acute GVHD, relapse, or TRM. This analysis demonstrates that unrelated donors with KIR B haplotypes confer significant survival benefit to patients undergoing T-replete HCT for AML. KIR genotyping of prospective donors, in addition to HLA typing, should be performed to identify HLA-matched donors with B KIR haplotypes. (Blood. 2009;113:726-732)

Introduction

Allogeneic hematopoietic cell transplantation (HCT) using related or unrelated donors (URDs) is standard treatment for many patients with hematologic malignancies who are unlikely to be cured by chemotherapy alone. Although the most important variable for donor selection is the match for HLA class I and class II, other relevant factors include donor sex, parity, cytomegalovirus (CMV) serostatus, and age.[1] Alloreactive donor-derived natural killer (NK) cells have been correlated with improved survival after HCT for acute myelogenous leukemia, and are thought to promote engraftment, reduce graft-versus-host disease (GVHD), and decrease leukemic relapse.[2,3] NK-cell function is determined by the net effect of signaling through several receptor families, including activating and inhibitory killer-cell immunoglobulin-like receptors (KIRs). Although the ligands for activating KIRs remain elusive, the interactions between inhibitory KIRs on the donor-derived NK cells and HLA class I molecules on the recipient's healthy and leukemic cells determine NK-cell alloreactivity. All HLA-C allotypes have either the C1 epitope, the ligand for *KIR2DL2/3*, or the C2 epitope, the ligand for *KIR2DL1*. Analogously, all HLA-B allotypes have either the Bw4 or Bw6 epitope, but only the Bw4 epitope is a ligand for KIRs: its cognate inhibitory receptor being *KIR3DL1*.[4] In healthy individuals, interactions of inhibitory KIRs with cognate HLA class I prevent NK cells from attacking healthy cells.[5] In the setting of allogeneic transplantation, donor NK cells attack the allogeneic cells if the recipient HLA class I ligands do not sufficiently engage their inhibitory receptors.

The KIR genes on chromosome 19 segregate independently of HLA, with the important consequence for unrelated allogeneic transplantation that matching for HLA does not match for KIRs. Diverse KIR haplotypes can be simplified into 2 biologically distinct groups, A and B. Group A haplotypes have a fixed number of genes that encode inhibitory receptors with the exception of *2DS4*, whereas group B haplotypes have variable gene content including additional activating receptor genes. These KIR haplotypes have been associated with reproductive success, responses to viral infections such as HIV and hepatitis C virus (HCV), susceptibility to autoimmune disease, and outcomes of HCT.[6-8] All individuals can be categorized as having 1 of 2 KIR genotypes: A/A, which is homozygous for group A KIR haplotypes, or B/x, which contains either 1 (A/B heterozygotes) or 2 (B/B homozygotes) group B haplotypes. Previous HCT studies have reported varied and sometimes inconsistent associations between KIR haplotype and clinical outcome.[9-16] The results were confounded by the small size and heterogeneity of the cohorts studied. In aggregate, these results suggested the hypothesis that combining KIR and HLA genotyping could help selection of transplant donors and improve the outcome of transplantation. To test this hypothesis, we analyzed the effects of KIR genotype in a homogeneous cohort of 448 AML patients who received transplants from unrelated donors.

Methods

Population

Collection of DNA samples from 448 donor/recipient pairs from URD HCT performed to treat AML was facilitated by the National Marrow Donor Program (NMDP, Minneapolis, MN) Research Sample Repository. Outcome data were obtained from the Center for International Blood and

Table 1. Patient demographics

	Donor KIR genotype		
	Genotype A/A, n = 131 (29%)	Genotype B/x, n = 317 (71%)	P
Median age, y (range)	34.1 (1.8-58.4)	33.3 (0.8-60.8)	.57
Disease status (%)			.53
Early	25 (19.1)	61 (19.2)	
Intermediate	42 (32.1)	118 (37.2)	
Advanced	64 (48.9)	138 (43.5)	
Donor: recipient HLA-allele matching at A, B, C, DRB1, and DQB1 (%)			.01
10/10 allele matched	51 (38.9)	158 (49.8)	
9/10	28 (21.4)	67 (21.1)	
8/10	39 (29.8)	51 (16.1)	
7/10	13 (9.9)	41 (12.9)	
HLA match group (%)			.10
HLA 10/10 allele matched	51 (38.9)	158 (49.8)	
HLA mismatched KIR-ligand matched	58 (44.3)	111 (35.0)	
HLA mismatched KIR-ligand mismatched	22 (16.8)	48 (15.1)	
Sex match, donor/recipient (%)			.33
Male/male	40 (30.5)	117 (36.9)	
Male/female	32 (24.4)	85 (26.8)	
Female/male	24 (18.3)	52 (16.4)	
Female/female	35 (26.7)	63 (19.9)	
CMV serostatus (%)			.62
Donor −/recipient −	40 (30.5)	106 (33.4)	
Donor +/recipient −	17 (13.0)	47 (14.8)	
Donor any/recipient +	73 (55.7)	160 (50.5)	
Missing	1 (0.8)	4 (1.3)	
Graft type (%)			.89
Bone marrow	116 (88.5)	281 (88.6)	
Peripheral blood progenitor cells	15 (11.5)	36 (11.4)	
Karnofsky score (%)			.08
90 to 100	77 (58.8)	196 (61.8)	
10 to 80	52 (39.7)	103 (32.5)	
Unknown	2 (1.5)	18 (5.7)	
Median time from diagnosis to transplantation y (range)	1.1 (0.0-4.0)	1.2 (0.0-8.2)	.46
Race (%)			.91
White	117 (89.3)	286 (90.2)	
Nonwhite	14 (10.7)	31 (10.8)	

*P values from Welch t test or a χ^2 test.

Marrow Transplant Research (CIBMTR, Milwaukee, WI). Samples and clinical data were collected after informed consent and approval from the NMDP and University of Minnesota Institutional Review Boards were obtained in accordance with the Declaration of Helsinki. To decrease the cohort's clinical heterogeneity, samples from 3 groups (HLA matched/KIR-ligand matched, HLA mismatched/KIR-ligand matched, and HLA-mismatched/KIR-ligand mismatched) were selected using an algorithm that matched on important demographic and clinical variables (Table 1). KIR-ligand matching status was determined using high-resolution HLA-B and HLA-C genotypes,[17] following the algorithm available on the KIR-ligand calculator tool maintained by the HLA Informatics Group of the Anthony Nolan Research Institute (http://www.ebi.ac.uk/ipd/kir/).[18]

Procedures

KIR genotyping. The presence or absence of 16 KIR genes (*KIR2DL5A* and *KIR2DL5B* were considered together) was determined using a high-throughput SNP-based Sequenom MassARRAY system (Sequenom, San Diego, CA) and the matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) platform for the large scale KIR genotyping of DNA samples as previously validated.[19]

Haplotype assignment. Detection of at least 1 of the KIR B haplotype-defining loci (*KIR2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS5*, or *3DS1*) in a sample dictated that the genotype contains at least 1 B haplotype. Such samples

were assigned the genotype designation B/x. Samples lacking all KIR B loci were assigned the genotype A/A.

Statistical analysis

We considered 6 outcomes in the analysis: (survival, relapse-free survival [RFS], relapse, treatment-related mortality [TRM], and grades II-IV acute and chronic GVHD). Overall survival (OS) and RFS were summarized by Kaplan-Meier curves, whereas the other 4 events were summarized by cumulative incidence functions. OS and RFS were compared between KIR genotype groups using the log-rank test. Other outcomes were compared using Gray test.[20] The completeness of follow-up was more than 90% at 3 years after follow-up and 95% of events occurred by 3 years after HCT.

For OS and RFS, a Cox proportional hazards model was used to adjust for important clinical factors. All variables were tested for proportional hazards using a time-dependent covariate approach. The multivariate model accounted for HLA matching, and other covariates listed in Table 1 were examined using backward stepwise selection. Variables were removed when the multiple degree of freedom test P value exceeded .10. A second model that stratified on HLA type gave similar results.

For the other events (relapse, TRM, acute and chronic GVHD), the Cox model was replaced by the model of Fine and Gray for the subdistributional hazard based on the cumulative incidence function.[21] Relapse and death were considered as competing risks.
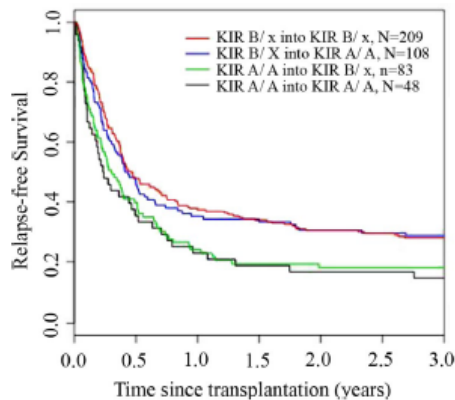
Figure 1. Transplantation using donors with a KIR B haplotype improves relapse-free survival (RFS) irrespective of the recipient KIR haplotype status. Donor (D) and recipient (R) DNA samples from 448 URD transplants were genotyped for 16 KIR loci. KIR gene content was used to identify haplotypes (A or B), from which KIR genotypes were assigned (KIR A/A or KIR B/x). The Kaplan-Meier curves demonstrate RFS for each donor/recipient genotype pairing (A/A into A/A, A/A into B/x, B/x into A/A, and B/x into B/x). RFS was significantly better after transplantation using KIR B/x donors (28% [95% CI: 23-33]; n = 317) compared with A/A donors (17% [11%-24%]; n = 131; P = .003). The recipient KIR genotype had no effect (P = .75).
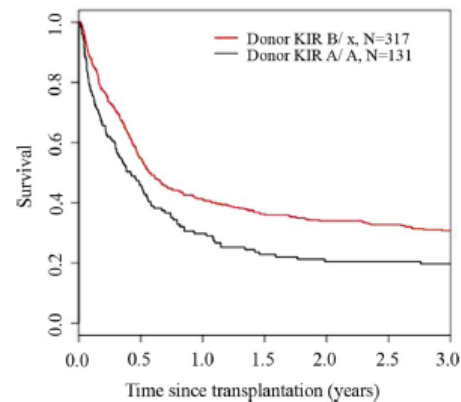

Figure 2. Transplantation using donors with KIR B haplotypes improves overall survival. The univariate Kaplan-Meier curves for overall survival (OS) are shown for patients receiving transplants from donors with (KIR B/x; n = 317) or without (KIR A/A; n = 131) KIR B haplotypes. Survival is significantly better if the donor had a KIR B haplotype (31% [95% CI: 26-36] vs 20% [95% CI: 13-27]; P = .007).

Results

The 448 AML patients underwent URD myeloablative, T cell–replete transplantations facilitated by the National Marrow Donor Program between 1988 and 2003. The cohort consisted of 3 HLA-matched groups (Table 1). Approximately half (47%) were 10/10 HLA-allele matched (and thus KIR-ligand matched) at HLA-A, -B, -C, -DRB1, and -DQB1. The HLA-mismatched group (53%) was further divided into KIR-ligand–mismatched (29%) and KIR ligand–matched (71%) groups. KIR genotyping for the presence of 16 KIR genes shows that the donors and recipients in this predominantly white population had similar and typical KIR frequencies. In this cohort, 29.2% of donors and 34.8% of recipients had the A/A KIR genotype; the remainder had the B/x genotype. There were no significant differences in KIR gene or haplotype frequencies between the 3 HLA match groups; nor did they vary for transplantations performed at different times during the period 1988 to 2002: in any given year approximately 66% of donors were B/x.

We analyzed the effect of donor and recipient KIR genotype on outcomes after URD HCT for AML. Whereas the recipient KIR haplotype content had no effect on relapse-free survival (P = .75), KIR B/x donors were associated with significantly better 3-year relapse-free survival (donor KIR B/x: 28% [95% CI: 23-33] vs donor KIR A/A: 17% [95% CI: 11-24]; P = .003) (Figure 1). In univariate analysis, 3-year overall survival was also significantly higher after transplantation using a B/x donor (31% [95% CI: 26-36] vs 20% [95% CI: 13-27]; P = .007) (Figure 2). Stratifying patients by HLA matching, we found that the 3-year relapse-free survival was higher with KIR B/x versus KIR A/A donors after 10/10 allele HLA-matched/KIR ligand–matched (32% vs 20%) and HLA-mismatched but KIR ligand–matched (29% vs 14%) transplantations, but not in the cohort that received HLA-mismatched/KIR ligand–mismatched transplants (15% vs 18%) (Figure 3).

To explore further the influence of donor KIR haplotypes, a multivariate Cox proportional hazards model was constructed

adjusting for statistically significant (P < .10) clinical and demographic factors including HLA match group, disease status (first or greater complete remission [CR] vs primary induction failure [PIF] or relapse), time from diagnosis to transplantation, age, race, and Karnofsky performance status (KPS). After adjustment, the benefit of transplantation using KIR B/x donors (compared with A/A) remained highly significant, with a favorable RR of 3-year relapse-free survival of 0.70 (95% CI: 0.55-0.88; P = .002). Graft type (peripheral blood progenitor cell [PBPC] vs marrow) was not a significant covariate and was not included in the multivariate model. There was no significant interaction between graft type and the donor KIR haplotype effect and the conclusions were unchanged in a subset analysis that excluded those patients (11% of total) who received PBPCs; RR with B/x donors of 3-year relapse-free survival of 0.71 (95% CI: 0.56-0.92; P = .008).

As expected, older and less fit patients being treated for intermediate and advanced leukemia or receiving grafts from HLA-mismatched donors had poorer relapse-free survival (Table 2). The multivariate analysis confirmed that donor KIR B/x genotype conferred a significant relapse-free survival benefit (RR: 0.69 [95% CI: 0.54-0.90], P = .005) for all KIR ligand–matched transplantations (HLA matched and HLA mismatched). We also analyzed the group with 1 or 2 HLA allele mismatches only (n = 185), excluding those with greater degrees of HLA mismatch. In this partially HLA-matched group, the 3-year estimate of relapse-free survival using KIR B/x genotype donors (26% [95% CI: 19-34]) was comparable with the 3-year relapse-free survival estimate for all (donor KIR A/A or B/x) 10/10 HLA-allele–matched transplantations (29% [95% CI: 23-35]). A multivariate Cox model comparing these groups showed no difference (RR: 1.07 [95% CI: 0.76-1.50]), suggesting that favorable donor KIR genotype can, at least in part, overcome the adverse effect of partial HLA mismatch.

After establishing that B/x haplotype donors conferred a survival advantage over A/A donors, we performed further analyses to explore the underlying mechanism. The influence of individual donor KIR genes on survival outcomes was examined (Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). The 2 KIRs showing independent effects on overall survival were *KIR2DL2* (P = .07) and *KIR2DS2* (P = .02), which are in strong linkage disequilibrium with each other. Of the 448 donors, 246 had both *KIR2DL2*
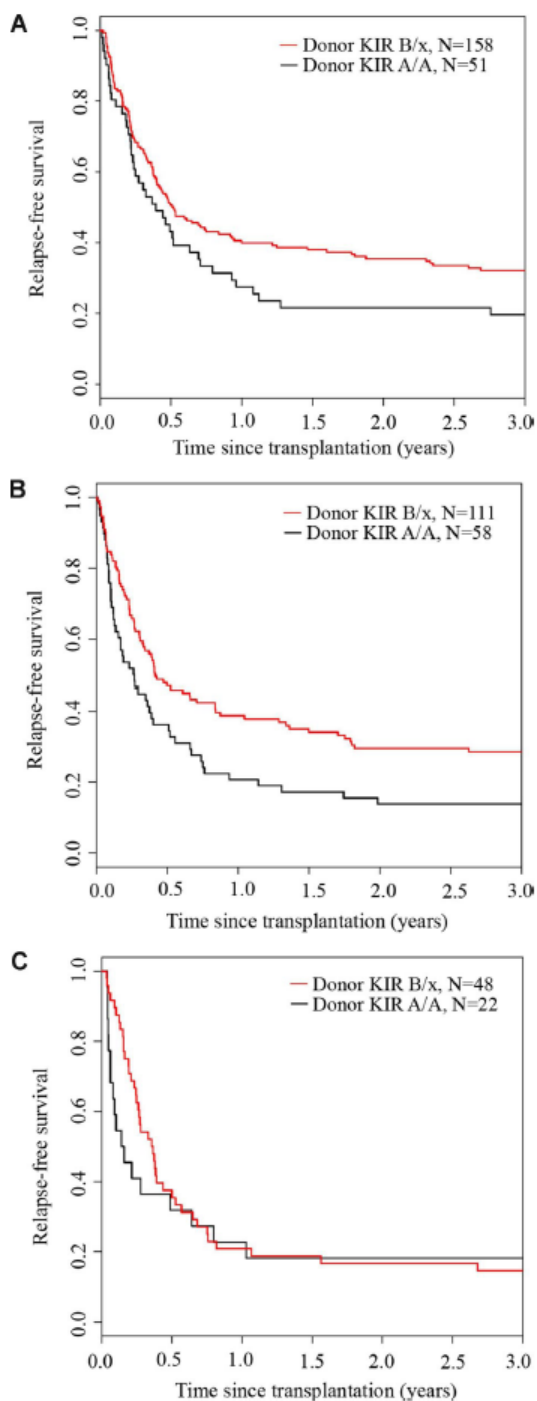
Figure 3. Relapse-free survival benefit from KIR B haplotype donors after HLA-matched and HLA-matched/KIR ligand–matched transplantation. The univariate Kaplan-Meier curves demonstrate the relapse-free survival (RFS) for patients receiving transplants from donors with or without KIR B haplotypes who were (A) 10/10 HLA matched, (B) HLA mismatched and KIR-ligand matched, or (C) HLA mismatched and KIR-ligand mismatched.

and *KIR2DS2*, 195 had neither, and only 7 had just 1 of them (6 *2DS2+/2DL2−* and 1 *2DS2−/2DL2+*). To assess the quantitative contribution of *KIR2DS2/L2* to the favorable relapse-free survival benefit using B/x donors, we constructed a multivariate Cox model adjusting for the same covariates and comparing A/A donors to B/x donors who either have or lack *KIR2DS2*. Transplantation using either KIR B/x genotype group (*KIR2DS2+* or *KIR2DS2−*) was associated with similarly significant improvements in relapse-free survival (*2DS2+* vs A/A: RR: 0.70 [95% CI: 0.55-0.89], $P = .003$; *2DS2−* vs A/A: RR: 0.69 [95% CI: 0.49-0.97], $P = .03$). Thus presence of *KIR2DS2* is not uniquely responsible for the survival benefit associated with B KIR haplotypes (Figure 4). Using regression modeling to assess the impact of increasing numbers of activating or inhibitory KIR genes, we found no additive effect. Only the presence of at least 1 (vs none) KIR B haplotype-defining gene was associated with significant improvement in outcome. Nor did we observe any interaction between the donor KIR genotype (A/A or B/x) and the KIR-ligand status of the recipient. The protective effect of B/x donors was similar in recipients having different combinations of the HLA-C (C1/C1, C2/C2, and C1/C2) and HLA-B (Bw4/x vs Bw6/Bw6) epitopes that bind to KIRs. In these recipient subsets, we detected no significant interaction between donor KIR and recipient HLA KIR ligands using the same model for relapse-free survival (HLA-C: $P = .8$; HLA-B: $P = .5$). Similarly, no differences were observed in acute GVHD, chronic GVHD, or overall survival based on KIR-ligand status (all $P = $ NS). In summary, transplantation using a KIR B/x donor was independently associated with improved relapse-free survival.

To assess how KIR B/x donors improve transplantation outcome, we analyzed the cumulative incidence estimates and multivariate competing risks models for differences in risk of relapse, TRM, and acute and chronic GVHD in transplantations using donors with KIR A/A versus B/x genotypes (Figure 5). All multivariate competing risks models were adjusted for HLA match group, disease status, age, KPS, and time from diagnosis to transplantation. Transplantations using KIR B/x donors were associated with modest though not significantly decreased risks for both relapse and TRM. No interaction was detected between CMV serostatus and the effect of a donor B haplotype on any outcomes. A donor KIR B haplotype did not alter the incidence of grades II to IV acute GVHD (adjusted RR: 1.10 [95% CI: 0.76-1.59], $P = .61$), but was associated with increased 3-year cumulative incidence of chronic GVHD (B/x: 39% [95% CI: 34-45] vs A/A: 28% [95% CI: 20-35], $P = .02$). Multivariate models confirmed the significant association of donor B/x with increased RR of chronic GVHD (B/x vs A/A RR: 1.51 [95% CI: 1.01-2.18], $P = .03$).

Discussion

Previous studies on the effects of KIR genotype in HCT involved small cohorts of patients treated for different diseases and reported conflicting results. Here we report our analysis of a large homogeneous cohort of AML patients receiving myeloablative conditioning followed by a T cell–replete unrelated donor grafts. Moreover, we used careful matching criteria to select clinically comparable cohorts of donor-recipient pairs, and we controlled for important clinical covariates using multivariate models.

In this multicenter study, we found that transplantation using a donor with 1 or 2 KIR B haplotypes was associated with significant improvements in overall and relapse-free survival, with more than 30% better relative risk in both these end points for the KIR B/x

Table 2. Relapse-free survival after URD HCT: multivariate analysis

All samples	RR of relapse or death	95% CI	P
KIR genotype			
B/x (compared with A/A)	0.70	0.55-0.88	.002
Transplant group			
HLA mismatched/KIR-ligand match (compared with 10/10 HLA matched)	1.21	0.95-1.54	.13
HLA mismatched/KIR-ligand mismatch (compared with10/10 HLA matched)	1.65	1.22-2.23	.001
Disease status			
Intermediate (compared with early)	1.41	0.99-2.02	.06
Advanced (compared with early)	2.17	1.58-2.99	<.001
Age (categoric)			.03
Karnofsky performance status			.01
Time from diagnosis to transplantation, per year	0.88	0.77-1.02	.08
Nonwhite	1.34	0.95-1.88	.10

donor group. The clinical benefit associated with donor KIR B haplotypes was evident across the entire KIR ligand–matched cohort, and was not influenced by the recipients' KIR genotype, the extent of HLA match, or the recipients' disease status at the time of transplantation. In contrast, the only group showing no benefit from a B/x donor was the small subset of transplants in which there was both HLA and KIR-ligand mismatch. The HLA-C group mismatches that impart KIR-ligand mismatches are known to adversely affect clinical outcomes.[17] Consequently, it is possible that the clinical advantage conferred by a KIR B/x donor is insufficient to overcome the disadvantage arising from a KIR-ligand (HLA-C) mismatch.

Although our results suggest that donor 2DL2 and 2DS2 may independently be advantageous, the analysis (not adjusted for multiple comparisons) was not definitive because of its limited power. Importantly, the clinical benefit of a B/x donor did not depend on the presence of either KIR2DL2 or KIR2DS2, or any other particular B haplotype–defining KIR gene; nor was there an association with increasing number of activating KIR genes. Ongoing analyses of a larger cohort will address the effect of individual KIR genes. In conclusion, our results indicate that all donors with 1 or 2 KIR B haplotypes have the potential to provide an improved clinical benefit in HCT for AML and should be selected over an otherwise equivalent donor who lacks a KIR B haplotype.
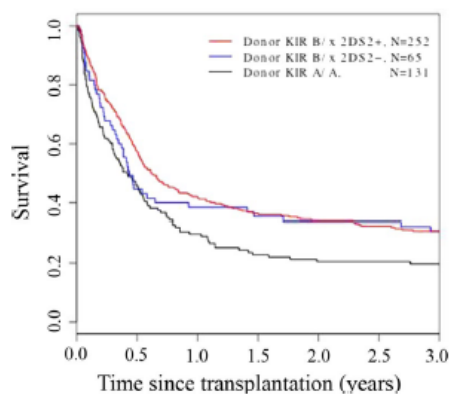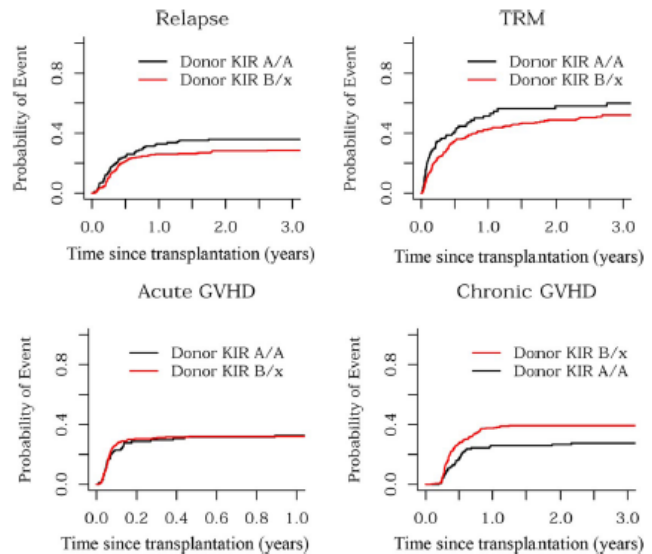

Figure 4. The survival benefit of a donor KIR B haplotype is not dependent on the presence of KIR2DS2. Donors with KIR B haplotypes were divided into 2 groups based on the presence (2DS2+, n = 252) or absence (2DS2−, n = 65) of KIR2DS2. Survival was significantly better for patients who received transplants from donors with either KIR B haplotype (2DS2+: 31% [95% CI: 25-37] or 2DS2−: 31% [95% CI: 20-42]) versus donors homozygous for the A haplotype (A/A: 20% [95% CI: 13-27]; P = .02).

The benefit of donor KIR B haplotypes has also been observed for T cell–replete transplantations using HLA-matched sibling donors. There the improved survival and reduced transplantation-related mortality were attributed to lower rates of cytomegalovirus reactivation,[22] an effect reported in other settings[23] and paralleled in the mouse model of cytomegalovirus infection.[24,25] Independent evidence for human activating KIRs in resisting infection is the reduced progression to AIDS in HIV-infected patients having KIR3DS1 and a cognate HLA Bw4 ligand.[26] Beneficial and detrimental clinical effects on outcomes after HCT have been associated with activating KIRs.[9-11,27] Increased acute GVHD was associated with higher numbers of donor activating KIRs in HLA-matched URD transplantations. KIR2DS3, in particular, was strongly associated with GVHD. In our study, donor KIR B/x genotype (but not individual KIR genes) was associated with higher rates of chronic, but not acute, GVHD. Activating KIRs are similarly associated with the chronic inflammation that characterizes autoimmune diseases.[7,28]

In haploidentical, T cell–depleted (TCD) HCT, donors mismatched for KIR ligands were associated with NK-cell alloreactivity and improved survival.[3,29] In URD HCT for myeloid malignancies, mismatching for KIR ligands can confer clinical benefit but only for T cell–depleted grafts,[2] not T cell–replete grafts.[1,30] This difference is attributed to T cells in the graft that interfere with NK-cell development and KIR reconstitution after URD HCT.[31] Here we observed no benefit associated with mismatch of KIR ligands, consistent with the previous studies of T cell–replete URD HCT.[32,33]

Although the underlying mechanism remains uncertain, this multicenter analysis demonstrates a significant and substantial (> 10%) survival benefit for AML patients receiving grafts from unrelated donors having 1 or 2 KIR B haplotypes. In our cohort, approximately two-thirds of the donors had at least 1 KIR B haplotype, indicating that KIR genotyping of donors to preferentially select for the presence of a B haplotype is a feasible proposition. If implemented, such typing could yield a KIR B haplotype donor option for a majority of the patients who have several high-resolution HLA-matched donors. Although a sophisticated and high-throughput method based upon mass spectrometry was used in this analytic study, simpler and less expensive methods could be used in clinical immunogenetics laboratories to reliably distinguish A/A and B/x donors.[34] The significant survival benefit observed in this multicenter study of HCT for AML provides compelling evidence that KIR B haplotype donors should be used preferentially in HLA-matched or HLA-mismatched unrelated donor transplantation.

Figure 5. Transplantation using donors with KIR B haplotypes leads to less relapse and TRM, and equivalent acute GVHD, but increased chronic GVHD. The cumulative incidences of relapse, TRM, acute GVHD, and chronic GVHD are shown for transplantation using donors with (KIR B/x) or without KIR B (KIR A/A) haplotypes. The incidence of chronic GVHD was higher using KIR B/x donors (39% [95% CI: 34-45]) than when using KIR A/A donors (28% [95% CI: 20-35], $P = .02$). Acute GHVD, relapse, and TRM were not significantly different between donor KIR haplotype groups ($P > .15$).

Acknowledgments

Authorship

Contribution: S.C. participated in the design and interpretation of the analysis and the writing of the paper; D.J.W., P.P., S.G.E.M., and J.S.M. planned, directed, and coordinated the research and revised the paper; E.T. participated in the design and interpretation of the analysis, led the team who performed genotyping of the DNA samples, and revised the paper; K.S. performed all of the genotyping, interpreted the data, and revised the paper; L.A.G. provided quality control data, helped interpret the genotyping data, and revised the paper; and T.L.B. performed the statistical analyses and helped write the paper with input from J.K. and C.T.L.

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