Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia

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Killer-cell immunoglobulin-like receptor (*KIR*) genes form a diverse, immunogenetic system. Group *A* and *B KIR* haplotypes have distinctive centromeric (*Cen*) and telomeric (*Tel*) gene-content motifs. Aiming to develop a donor selection strategy to improve transplant outcome, we compared the contribution of these motifs to the clinical benefit conferred by *B* haplotype donors. We *KIR* genotyped donors from 1409 unrelated transplants for acute myelogenous leukemia (AML; n = 1086) and acute lymphoblastic leukemia (ALL; n = 323). Donor *KIR* genotype influenced transplantation outcome for AML but not ALL. Compared with *A* haplotype motifs, centromeric and telomeric *B* motifs both contributed to relapse protection and improved survival, but *Cen-B* homozygosity had the strongest independent effect. With *Cen-B/B* homozygous donors the cumulative incidence of relapse was 15.4% compared with 36.5% for *Cen-A/A* donors (relative risk of relapse 0.34; 95% confidence interval 0.2-0.57; *P* < .001). Overall, significantly reduced relapse was achieved with donors having 2 or more *B* gene-content motifs (relative risk 0.64; 95% confidence interval 0.48-0.86; *P* = .003) for both HLA-matched and mismatched transplants. *KIR* genotyping of several best HLA-matched potential unrelated donors should substantially increase the frequency of transplants by using grafts with favorable *KIR* gene content. Adopting this practice could result in superior disease-free survival for patients with AML. (*Blood*. 2010;116(14): 2411-2419)

Introduction

Acute myelogenous leukemia (AML) is the most common form of adult acute leukemia, with approximately 12 000 cases diagnosed annually in the United States.[1] For patients with high-risk or recurrent disease, hematopoietic cell transplantation (HCT) offers a potential cure.[2,3] Successful allogeneic HCT depends upon the elimination of leukemic cells by the combination of chemotherapy, radiotherapy, and T cell–mediated graft-versus-leukemia (GVL) reaction, with reconstitution of the patient's ablated hematopoietic system by donor stem cells. Although matching for human leukocyte antigen (HLA) class I and II alleles is the most important criterion for unrelated donor selection, consideration of other factors, such as donor sex, parity, cytomegalovirus serostatus, and age, also can improve transplant outcome.[4-6]

Natural killer (NK) cells were discovered for their capacity to kill cancer cells,[7] and later were shown to be an essential element of innate immunity.[8] Like killer CD8 T cells, the development and function of NK cells are controlled by NK-cell receptors that recognize HLA class I.[9] Among these ligand:receptor interactions, some are conserved, like that of HLA-E with the CD94:NKG2A NK-cell receptor,[10] but others are variable. Extreme in this regard are the polymorphic killer-cell immunoglobulin-like receptors (KIRs)[11,12] that recognize polymorphic epitopes of HLA-A, B, and C, called KIR ligands.[13] As a consequence of this genetic variation, donor-derived NK cells can mediate beneficial GVL reactions in HCT. Such beneficial NK-cell alloreactivity, which can be predicted from the differences in KIR ligands between donor and recipient based on their HLA class I type,[14] was first described for HLA haploidentical transplantation by the use of an extensively T cell–depleted graft[15] and later investigated in other transplantation settings.[16,17] These studies did not consider a role for *KIR* gene variability, which in its extent and functional importance approaches that of HLA class I.[18]

Because *HLA* and *KIR* segregate independently on different chromosomes, only a minority of HLA-matched transplants are *KIR* matched. Thus, 25% of HLA-matched siblings are *KIR* identical, and unrelated *HLA*-matched donors are rarely *KIR* identical.[19] This situation facilitated a retrospective analysis to examine the impact of donor and recipient *KIR* genotypes on transplant outcome. The simplest genetic distinction is the division of *KIR* haplotypes into groups *A* and *B* according to gene content.[12,20] *KIR A* haplotypes have simple, fixed gene content; *B* haplotypes have variable gene content and 1 or more of the *B*-specific genes: *KIR2DS1*, *2*, *3*, *5*, *KIR2DL2*, and *KIR2DL5*. When this distinction is used, all individuals can be assigned to the *A/A* genotype (homozygous for *A* haplotypes) or the *B/x* genotype (having 1 or 2 *B* haplotypes). Previously, we showed that the

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Table 1. Demographics of the transplanted leukemia patients

Variable	ALL	AML		
	Total (n = 323)	Total (n = 1086)	Donor with *KIR B*–content score 0 or 1 (n = 748)	Donor with *KIR B*–content score ≥ 2 (n = 338)
Median age, y (range)	18.5 (8-55)	38.9 (1-70)	39.0 (1-70)	38.6 (1-68)
Race				
White	261 (81)	757 (70)	512 (68)	245 (72)
Nonwhite	62 (19)	329 (30)	236 (32)	93 (28)
Karnofsky score				
90-100	232 (72)	666 (61)	460 (62)	206 (61)
10-80	91 (28)	329 (30)	227 (30)	102 (30)
Unknown		91 (8)	61 (8)	30 (9)
Disease status when transplanted*				
Early	80 (25)	290 (27)	200 (27)	90 (27)
Intermediate	159 (49)	349 (32)	232 (31)	117 (35)
Advanced	84 (26)	447 (41)	316 (42)	131 (39)
Disease risk by cytogenetics				
Low	0 (0)	120 (11)	75 (10)	45 (13)
Intermediate	133 (41)	471 (43)	341 (46)	130 (38)
High	68 (21)	232 (21)	164 (22)	68 (20)
Unknown	122 (38)	263 (24)	168 (22)	95 (28)
Donor:recipient match for HLA-A, B, C, DRB1, and DQB1				
10/10 allele matched	148 (46)	539 (50)	374 (50)	165 (49)
9/10	60 (19)	301 (28)	212 (28)	89 (26)
8/10	55 (17)	158 (15)	106 (14)	52 (15)
Less than 8/10	60 (19)	88 (8)	56 (8)	32 (10)
CMV serostatus				
Donor −/recipient −	127 (39)	331 (30)	233 (31)	98 (29)
Donor +/recipient −	80 (25)	144 (13)	92 (12)	52 (15)
Donor + or −/recipient +	111 (34)	578 (53)	398 (53)	180 (53)
Data missing	5 (2)	33 (3)	25 (3)	9 (3)
Graft type				
Bone marrow	301 (93)	641 (59)	429 (57)	212 (63)
Peripheral blood progenitor cells	22 (7)	445 (41)	319 (43)	126 (37)

Values are n (%) unless otherwise noted.

ALL indicates acute lymphoblastic leukemia; AML, acute myelogenous leukemia; and CMV, cytomegalovirus.

*Disease status was defined as early (first complete remission), intermediate (second or higher complete remission), and advanced (first relapse, second or higher relapse, primary induction failure).

outcome of unrelated donor transplantation for AML was significantly improved with *B/x* donors compared with *A/A* donors, whereas recipient *KIR* genotype had no effect.[21] Similar effects have been reported by other investigators in unrelated donor and sibling donor settings.[22,23] Because *KIR B* haplotypes are present in approximately two-thirds of unrelated registry donors, interventions that merely increase the probability of selecting *KIR B* donors are unlikely to affect survival because most donors already have this characteristic by chance. Further, the specific genetic mechanism for the protective effect of *B* haplotype donors, perhaps attributable to the presence or absence of individual or groups of inhibitory or activating KIR, remains unknown. This analysis was designed, from understanding of the organization of the *KIR* locus, to identify which particular *B*-specific genes improve the therapeutic effect of transplantation for AML. The results point to a clinically applicable donor selection strategy that could improve the success of transplantation.

Methods

Characteristics of the study cohort and clinical database

We studied 1409 patients who received myeloablative, T-cell–replete, unrelated donor (URD) transplantation as treatment for either AML (n = 1086) or ALL (n = 323). Transplants were facilitated by the National Marrow Donor Program (NMDP) between 1988 and 2006 (Table 1). For each donor and recipient, a DNA sample was obtained from the Research Sample Repository of the NMDP. Outcome data were obtained from the Center for International Blood and Marrow Transplant Research. Complete high-resolution HLA matching data at HLA-A, B, C, DRB1, and DQB1 were obtained from the NMDP retrospective typing program. Samples and clinical data were obtained after informed consent in accordance with the Declaration of Helsinki and approval from the NMDP and University of Minnesota Institutional Review Boards.

KIR genotyping and haplotype group assignment

The presence or absence of 15 *KIR* genes (*KIR3DL3*, *KIR2DS2*, *KIR2DL2*, *KIR2DL3*, *KIR2DL5A/B*, *KIR2DS3/2DS5*, *KIR2DP1*, *KIR2DL1*, *KIR3DP1*, *KIR2DL4*, *KIR3DL1*, *KIR3DS1*, *KIR2DS1*, *KIR2DS4*, and *KIR3DL2*) was determined by the use of high-throughput analysis of single-nucleotide polymorphisms by mass spectrometry as described[24] and applied.[21] Donors were assigned the *B/x* or *A/A* genotype as defined previously.[21,25] Genotypes for the centromeric (*Cen*) and telomeric (*Tel*) parts of the *KIR* locus were assigned according to the presence or absence of one or more *B* haplotype-defining *KIR* genes (Figure 1A). Thus, *Cen-A1* and *Tel-A1* are the centromeric and telomeric motifs, respectively, of the canonical *A KIR* haplotype; *Cen-B1* and *Cen-B2* are alternative centromeric motifs of common *B KIR* haplotypes, and *Tel-B1* is the common centromeric motif of *B* haplotypes.[26,27] For much of this analysis, *Cen-B1* and *Cen-B2* are

A

B

Centromeric (*2DS2, 2DL2, 2DL3*)		N (%)	
		AML	ALL
Cen-A/A	*2DL3* only	532 (49)	155 (48)
Cen-A/B	*2DL3* with *2DS2* and/or *2DL2*	439 (40)	140 (43)
Cen-B/B	*2DS2* and/or *2DL2*; no *2DL3*	115 (11)	28 (9)
Telomeric (*3DL1, 3DS1, 2DS1, 2DS4*)			
Tel-A/A	*3DL1* and *2DS4* only	659 (61)	185 (57)
Tel-A/B	*3DL1* and *2DS4* with *3DS1* and/or *2DS1*	382 (35)	119 (37)
Tel-B/B	lacking *3DL1* and/or *2DS4*	45 (4)	19 (6)

C

Donor *KIR* Genotype	*B* content score	*Cen*	*Tel*	N (%) AML	N (%) ALL
A/A	0	*A/A*	*A/A*	374 (34)	99 (31)
B/x	1	*A/A*	*A/B*	374 (34)	121 (37)
		A/B	*A/A*		
	2	*A/A*	*B/B*	254 (23)	78 (24)
		A/B	*A/B*		
		B/B	*A/A*		
	3	*A/B*	*B/B*	84 (8)	25 (8)
		B/B	*A/B*		
	4	*B/B*	*B/B*		

Figure 1. The *KIR* locus comprises centromeric (*Cen*) and telomeric (*Tel*) gene content motifs. (A) The organization of genes in the *KIR* locus. The centromeric and telomeric regions are separated by a unique recombination site (RS) sequence that can function to reassort the centromeric and telomeric gene motifs. The gene content of the common motifs is shown. The conserved framework genes are shaded gray, *B* haplotype genes are blue, and *A* haplotype genes are red. (B) Groups used for the *Cen* and *Tel* analysis on the basis of the content of the inhibitory (L-long) or activating (S-short) *KIR* genes, and their frequencies among donors in the AML and ALL cohorts. (C) *KIR B*–content score and the frequency of donors in each group. The *KIR B*–content score is calculated by adding the number of *Cen-B* and/or *Tel-B* motifs in each genotype.

grouped together as *Cen-B*, whereas *Cen-A1* is shortened to *Cen-A* and *Tel-A1* to *Tel-A* (Figure 1B).

We defined the *KIR B*–content score for each donor's *KIR* genotype as the number of centromeric and telomeric gene-content motifs containing *B* haplotype–defining genes. Permissible values for the *KIR B*–content score are 0, 1, 2, 3, and 4 (Figure 1C). A calculator for classification of the donor *KIR B* status (best, better, neutral) may be found at http://www.ebi.ac.uk/ipd/kir/.

Statistical analysis

Six measures of transplant outcome were considered: overall survival (OS) and disease-free survival (DFS), relapse, treatment-related mortality (TRM), and the incidence of both acute graft versus host disease (GVHD; grade II-IV) and chronic GVHD. OS and DFS were evaluated by the use of Kaplan-Meier curves; other outcomes were evaluated by the use of the cumulative incidence function. Unadjusted comparisons between *KIR* genotypes were made with the use of the log rank test on either the hazard rates for OS and DFS or the crude hazard rates for relapse, TRM, and chronic GVHD. For acute GVHD at 3 months, we used the pseudo-observation approach,[28] which reduces to a logistic regression model when there is no censoring. In this transplant cohort the completeness of follow-up at 3 years was more than 99%, and 90% of the events had occurred.

Cox proportional hazards models[29] were used to adjust for important clinical factors, including *KIR* genotype, HLA match, time from diagnosis to transplant, disease status at time of transplant, cytogenetic risk group (good, intermediate, poor), graft source, patient age, race, sex match, and Karnofsky performance score (KPS). Forward stepwise regression modeling was used to determine which factors required adjustment in each model on the basis of a significance level of 5%. All models included the *A/B KIR* genotype. Models for DFS and relapse in AML also included disease status, KPS, time from diagnosis to HCT, and, for DFS, high-resolution HLA matching and age required adjustment. Models for DFS and relapse in ALL included disease status, KPS, and graft source, and the DFS model required adjustment for age. Proportional hazards were checked in a time-dependent covariate model and graphically. No significant interactions were detected between the donor *KIR* genotype groups and any covariates for the outcomes of interest.

Results

Characteristics of the transplant recipients and donors studied

We studied 1409 myeloablative, T cell–replete URD transplants given to AML (n = 1086) or ALL (n = 323) patients performed between 1988 and 2006 (Table 1). The transplant recipients included patients with early, intermediate, and advanced disease. Approximately one-half of the donor-recipient pairs (AML: 50%, ALL: 46%) were 10/10 HLA-allele matched at HLA-A, B, C, DRB1, and DQB1, and one-half had some HLA mismatch. Transplant donors were typed for presence and absence of individual *KIR* genes (Figure 1A). From the genotypes we determined whether each donor was of *A/A* or *B/x* genotype. For the *B/x* donors we further determined whether their *B* haplotype genes were in the centromeric or telomeric part of the *KIR* locus, or in both (Figure 1B). From these data we calculated the *KIR B*–content score for each donor, which gives the total number of centromeric and telomeric motifs containing *B* haplotype genes (Figure 1C). There was no significant difference in the frequencies of *KIR* genes, haplotypes, or motifs between either the cohorts of ALL or AML transplant donors or with the white population, to which 72% of the donors belong.

Centromeric *KIR* genes of donor *B* haplotypes reduce relapse and improve survival of transplanted AML patients

By using multivariate models we analyzed the effect of donor *KIR* genotype on critical clinical outcomes after URD HCT for AML. As we reported previously,[21] a protective effect of donor *B/x* genotype was observed for the cohort of AML patients (DFS: donor *KIR B/x* vs *A/A*: relative risk of relapse [RR] 0.85; 95% confidence interval [95% CI] 0.73-0.99; $P = .04$) but not for the ALL patients. This result indicates that one or more of the *KIR* genes or alleles restricted to group *B* haplotypes is associated with the protective

Table 2. Multivariate analysis of relapse and disease-free survival

	AML patient transplantations						
		Relapse			Disease-free survival		
	n	RR of relapse	95% CI	*P*	RR of relapse or death	95% CI	*P*
Donor *KIR*: *A/A* and *B/x* genotypes							
Donor *KIR* genotype							
KIR-A/A	362	1.00			1.00		
KIR-B/x	696	0.72	0.58-0.89	.003	0.85	0.73-0.99	.04
Donor *KIR*: centromeric and telomeric genotypes							
Donor *Cen* genotype							
Cen-A/A	516	1.00			1.00		
Cen-A/B	427	0.87	0.70-1.09	.20	0.94	0.81-1.10	.45
Cen-B/B	115	0.34	0.20-0.57	< .001	0.72	0.55-0.93	.01
Cen-B/A vs *Cen-B/*B				< .001			.04
Donor *Tel* genotype							
Tel-A/A	643	1.00			1.00		
Tel-A/B	371	0.70	0.56-0.89	.003	0.87	0.74-1.02	.08
Tel-B/B	44	0.52	0.26-1.06	.07	0.82	0.55-1.20	.32
Tel-A/B vs *Tel-B/B*				.45			.77
Donor *KIR B*–content score							
Donor *KIR B* motifs							
0	364	1.00			1.00		
1	366	0.93	0.73-1.19	.56	0.94	0.79-1.11	.45
2	242	0.54	0.39-0.74	< .001	0.77	0.63-0.94	.01
3 or 4	86	0.44	0.27-0.73	< .001	0.76	0.56-1.01	.06
Donor *KIR*: *B*–content group							
KIR B = 0 or 1	730	1.00			1.00		
B ≥ 2 (*Cen-A/x, Tel-B/x)*	213	0.64	0.48-0.86	.003	0.84	0.70-1.01	.07
B ≥ 2 (C*en-B/B, Tel-x/x)*	115	0.33	0.20-0.55	< .001	0.70	0.55-0.90	.007
Donor *KIR* genotype and HLA match							
HLA-matched							
B = 0 or 1	374	1.00			1.00		
B ≥ 2	165	0.52	0.36-0.75	< .001	0.80	0.62-1.02	.07
HLA-mismatched							
B = 0 or 1	374	1.00			1.00		
B ≥ 2	173	0.52	0.35-0.75	< .001	0.78	0.63-0.97	.03
Disease status and HLA match							
Disease status							
Early	286	1.00			1.00		
Intermediate	341	1.51	1.01-2.26	.044	1.47	1.13-1.90	.005
Advanced	431	2.99	2.25-3.98	< .001	2.38	1.94-2.92	< .001
HLA match							
10/10	539	1.00			1.0		
Less than 10/10	547	0.94	0.88-1.17	.57	1.30	1.12-1.51	< .001

AML indicates acute myelogenous leukemia; 95% CI, 95% confidence interval; HLA, human leukocyte antigen; and RR, relative risk.

effect in AML. The logical first step to identify the protective gene was to address the inherent heterogeneity within the group of transplant donors having the *B/x* genotype.

Three conserved, framework regions divide the *KIR* locus into similarly sized centromeric and telomeric segments that differ in gene content.[20,27] As shown in Figure 1A, the *A* haplotype has invariant gene-content, comprising a centromeric *A* motif (*Cen-A*) and a telomeric *A* motif (*Tel-A*). In contrast, *B* haplotypes are of 3 distinct types: 1 combining a centromeric *B* motif (*Cen-B*) with a telomeric *B* motif (*Tel-B*); 1 combining *Cen-B* with *Tel-A*; and 1 combining *Cen-A* with *Tel-B*. These differences allowed us to compare the role of the centromeric and telomeric segments in the protective effect of donor *B/x* genotype in AML HCT (Table 2).

The analysis showed that the *B* haplotype genes of the centromeric region had a stronger effect in improving the outcome of transplantation than those of the telomeric region. For AML, donors having *B* genes in the centromeric region of both *KIR* haplotypes (*Cen-B/B* genotype) were associated with a striking decrease in the incidence of relapse compared with either *Cen-A*/*A* or *Cen-A*/*B* donors (*Cen-B/B* vs *Cen-A/A*: RR 0.34; 95% CI 0.2-0.57; $P < .001$); with absolute relapse rates of only 15.4% (*Cen-B/B*) versus 36.5% (*Cen-A/A*; Figure 2A; Table 2). The advantage of a *Cen-B/B* donor also was seen in improved OS (data not shown) and DFS (*Cen-B/B* vs *Cen-A/A*: RR of relapse or death 0.72; 95% CI 0.55-0.93; $P = .01$; Figure 2B). A partial contribution of *B* haplotype genes in the telomeric regions is also suggested by the reduced relapse associated with donors having *Tel-A/B* or *Tel-B/B* genotype, compared with *Tel-A/A* (Figure 3; Table 2). However, the effect did not result in significantly increased DFS (Table 2). Donor *KIR* genotype did not have any significant effect on rates of TRM, grade II-IV or III-IV acute GVHD or chronic GVHD (data not shown). No protection was observed for ALL patients, for whom relapse (*Cen-B/B* vs *Cen-A/A*: RR 0.97; 95% CI 0.43-2.16; $P < .94$; Figure 2C), DFS (*Cen-B/B* vs *Cen-A/A*: RR 0.99; 95% CI 0.61-1.62; $P < .97$; Figure 2D), and all other outcomes of transplantation (data not shown) were unaffected by the *KIR* genes of the transplant donor for ALL. These negative

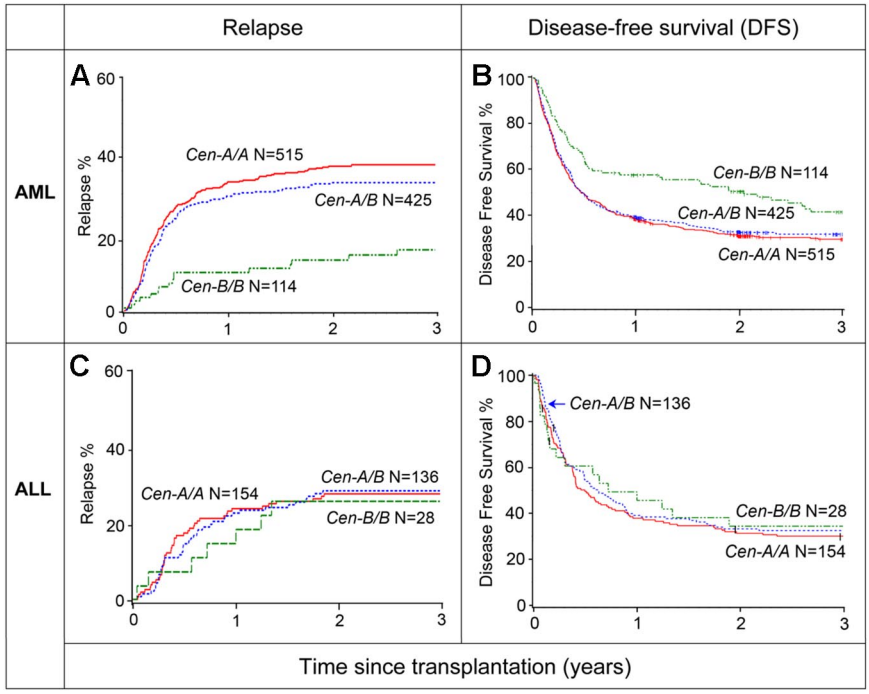

Figure 2. Specific reduction in relapse and improvement in DFS from donors with *Cen-B/B* after transplantation for AML but not ALL. Donors were assigned *Cen-A/A*, *Cen-A/B*, and *Cen-B/B* genotypes. Top, the incidence of relapse (A) and probability of DFS (B) for AML patients. Bottom, the incidence of relapse (C) and probability of DFS (D) are shown for ALL patients on the basis of their donor *Cen* genotype group.

results with ALL emphasize the specificity and importance of the AML effect.

Donors having *KIR B*–content scores of 2 or greater protect against relapse and improve DFS

We next examined how the number of *KIR B* gene motifs, irrespective of their centromeric or telomeric origin, influenced relapse and DFS (Table 2). Although there was little difference in outcome when donors had a *KIR B*–content score (the total number of *B*-associated domains) of 0 or 1, donors with a score of 2 or greater provided significantly better relapse protection. Within this *KIR B*–content score group (ie, > 2), the reduction in relapse (Figure 4A) and the increase of DFS (Figure 4B) were greater when the donor was homozygous for *Cen-B* (relapse: RR 0.33; 95% CI 0.20-0.55; $P < .001$ and DFS: RR 0.70; 95% CI 0.55-0.90; $P = .007$), further demonstrating the major role played by centromeric region *B* genes. However, not all of the favorable effect is determined by *Cen-B* homozygosity because donors having a *KIR B*–content score of 2 or greater and at least 1 copy of *Cen-A* also achieved significant protection against relapse (RR 0.64; 95% CI 0.48-0.86; $P = .003$). As shown in Figure 4A, the cumulative incidence curves for relapse for these donors lie between and are clearly separated from the curves for donors with a *KIR B*–content score of 0 or 1 and *Cen-B* homozygous donors. This led to improved DFS (Figure 4B). A similar beneficial effect of donor *KIR B*–content scores 2 or greater was observed for OS (Figure 5E-F). Further subsetting the *Cen-B/B* donors on the basis of gene content into *Cen B1/B1* and *Cen B2/x* groups revealed similar levels of relapse protection, with 10.8% versus 18.5% relapse rates, respectively. This finding suggests that relapse protection is associated with *2DL2/2DS2* (present in both groups) rather than the presence of *2DL5* and/or *2DS3/5* (only in *B2*; Figure 1A). Furthermore, compared with donors with *Cen-A/A*, *Tel-A/A*, a decreased relative risk of relapse is observed in both the *Cen-B/B*, *Tel-A/A* (0.23, $P = .001$, n = 52) and *Cen-A/A*, *Tel-B/B* (0.43, $P =$ n.s., n = 14) donor groups, suggesting that telomeric *KIR B* genes also contribute to the overall relapse protection and survival benefit associated with *KIR B/x* donors.

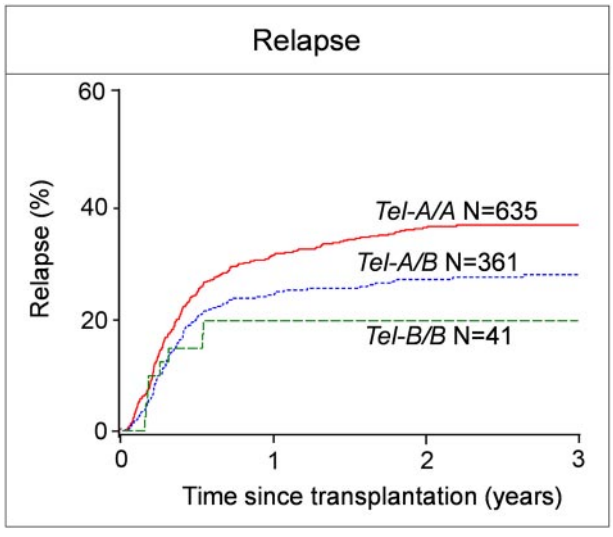

Figure 3. Donors with *Tel-B/B* contribute to reduction in relapse after transplantation for AML. Donors were assigned *Tel-A/A*, *Tel-A/B*, and *Tel-B/B* genotypes. The incidence of relapse is shown for the respective groups.

Donor selection for favorable *KIR-B* gene motifs improves outcomes in HLA-matched or HLA-mismatched URD HCT for AML

Our study shows that the outcome of allogeneic transplantation for AML is influenced by the donor's *KIR* genotype in a manner that appears insensitive to the HLA type of either donor or recipient.

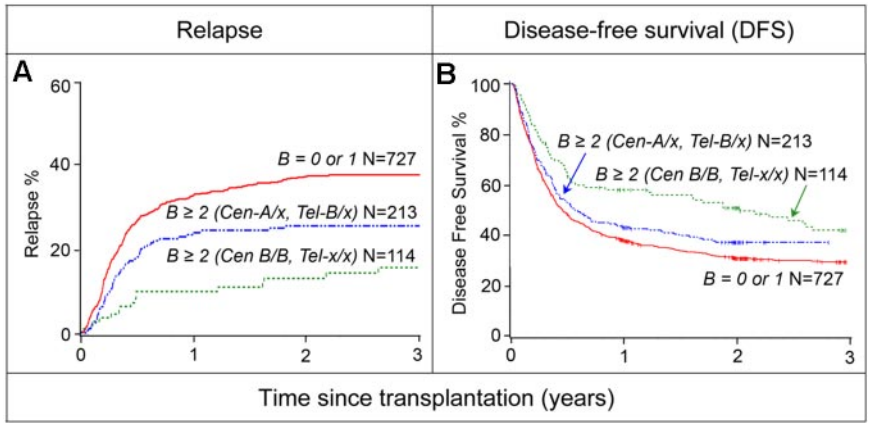

Figure 4. Protection against relapse and improved DFS on the basis of donor *KIR B*–content groups in AML. The AML cohort was evaluated for relapse (A) and DFS (B) on the basis of donor *KIR B* content by use of the indicated groups. On the basis of this analysis, donor *KIR B*–content groups are divided as: (1) "best" with a *KIR B*–content score of more than 2 where the *KIR* haplotype is *Cen-B/B, Tel-x/x* (defined as *2DL3* absent, *2DS2* and/or *2DL2* present); (2) "better" with a *KIR B*–content score of more than 2 and the *KIR* haplotype is *Cen-A/x, Tel-B/x* (defined as *2DL3* present, *2DS2* and/or *2DL2* present, *3DS1* and/or *2DS1* present or *2DL3* present, *2DS2* and/or *2DL2* absent, and *3DS1* and/or *2DS1* present); or (3) "neutral" with a *KIR B*–content score of 0 or 1 (defined as *2DL3* present, *2DS2* and/or *2DL2* absent, *3DL1* and *2DS4* present).

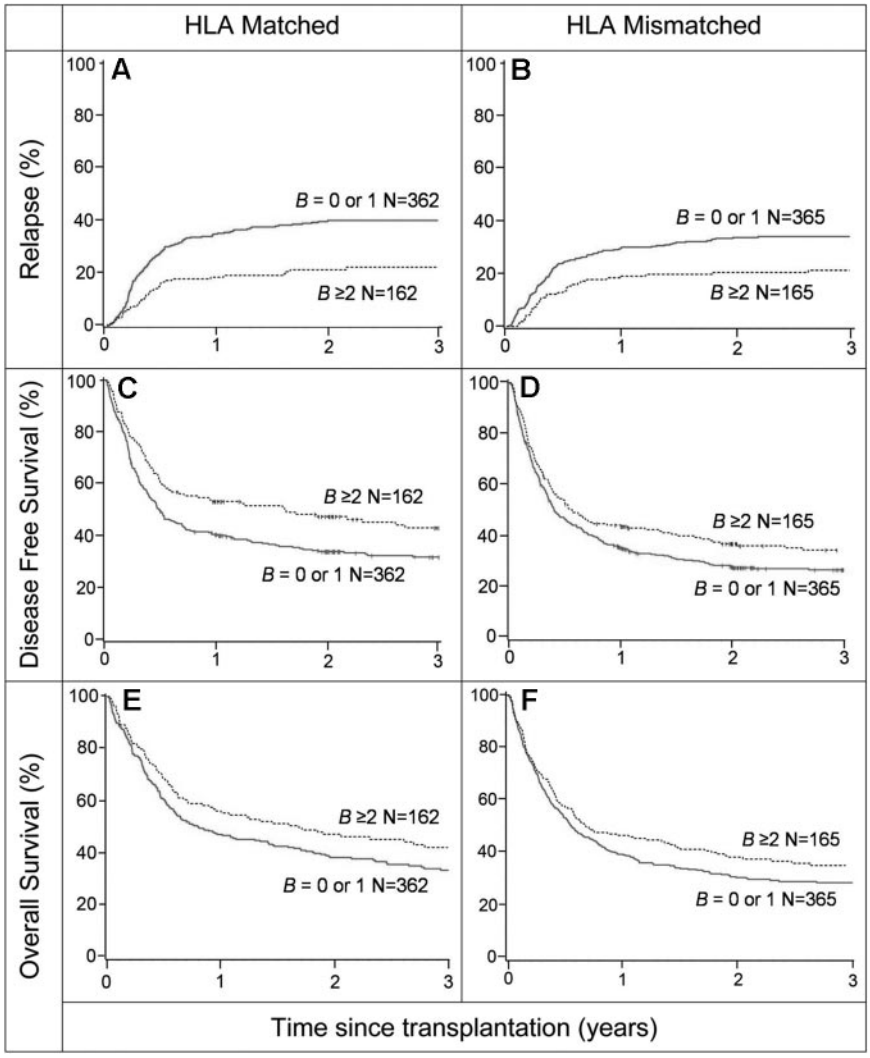

Figure 5. Selection of donors with *KIR B*–content scores of 2 of greater has significant potential to improve the outcome of HLA-matched and HLA-mismatched HCT. For patients transplanted for AML, the incidence of relapse (A-B), the probability of DFS (C-D) and the probability of overall survival (E-F) are shown. Panels A, C, and E show the data from HLA-matched transplants, and panels B, D, and F show the data from HLA-mismatched transplants. Comparison is made between donors with a *KIR B*–content score of 0 or 1 (374 matched and 374 mismatched) and donors with a *KIR B*–content score of 2 or greater (162 matched and 165 mismatched).

Benefit accrues with increasing number of *B* haplotype genes, with the centromeric genes exerting a greater effect than the telomeric genes. These results imply that the success of transplantation for AML could be improved by selecting donors on the basis of their *KIR* type as well as their HLA match by aiming to obtain the best HLA-matched donors that have *KIR B*–content score 2 or greater.

To evaluate the potential impact of such donor selection on URD HCT, we compared the outcome for transplants where the donor *KIR B*–content score was 0 or 1 with transplants in which the donor *KIR B*–content score was 2 or greater (Figure 5). This retrospective analysis also compared the effects of donor *KIR B* content for transplants that were fully HLA matched and transplants that were HLA mismatched. Significant and similar reductions in relapse (Figure 5A-B), along with improved DFS (Figure 5C-D) and OS (Figure 5E-F) were conferred by a donor *KIR B*–content score of 2 or greater for HLA-matched (Figure 5A,C,E) and mismatched (Figure 5B,D,F) transplants. Selecting for transplant donors with a *KIR B*–content score of 2 or greater has potential to reduce the risk of relapse by 50% and the relative risk of relapse or death by 20% (Table 2).

Discussion

For patients undergoing URD transplantation, the preferred donor is matched at the allele level for HLA class I and II.[4,5] Even then, the risk of relapse is high (21%-43% for early or advanced AML), and DFS rates of 13% to 38% are the norm.[30] Here, we investigated the contribution of a second immunogenetic system, the *KIR* gene family,[18] on the outcome of transplantation for AML. Of the 2 *KIR* haplotype groups, donor *B* haplotypes yield significantly superior protection against relapse and improved DFS compared with donor *A* haplotypes. Clinical benefit increases with the number of *B*-specific gene content motifs, particularly with homozygosity for those in the centromeric part of the *KIR* locus (*Cen-B/B*). Similar benefits were observed for patients receiving fully HLA-matched or partly mismatched grafts. Emphasizing the specificity of the effect, donor *KIR* genotype was not correlated with the outcome of transplantation for ALL.

The clinical benefit conferred by *Cen-B/B* could be caused by presence of *KIR2DS2* and *KIR2DL2*, absence of *KIR2DL3*, or by the combination of these 2 factors. A possible advantage of the activating KIR2DS2, which has no detectable avidity for HLA class I,[31,32] is that it may recognize a different type of ligand present on AML but not ALL cells. A possible advantage to KIR2DL2 is that it binds both the C1 and C2 epitopes of HLA-C with greater avidity than KIR2DL3.[33] Because NK-cell education through interaction between MHC class I ligands and cognate inhibitory receptors determines the strength of NK-cell responsiveness,[34,35] the stronger avidity of KIR2DL2 than KIR2DL3 could educate NK cells that are more effective at eliminating residual AML cells.

The fact that *Cen-B* heterozygosity has a beneficial effect much less than one-half of that achieved by *Cen-B* homozygosity (Figure 2A-B) raises the possibility that a negative effect caused by the *KIR2DL3 c*omponent of *Cen-A* is the causative mechanism rather than a positive effect caused by the *KIR2DS2* and/or *KIR2L2* of *Cen-B/B*. In the setting of acute hepatitis C virus infection, 2 copies of *Cen-A* were necessary to achieve a strong improvement in the response to infection.[36] Here, we find the opposite effect, namely that complete absence of *Cen-A* is required for strong improvement in the response to AML.

We should also consider the possibility that the clinical benefit associated with *Cen-B/B* homozygosity is not necessarily attributable to *KIR2DS2*, *KIR2DL2*, or *KIR2DL3*. The strong linkage disequilibrium between *KIR2DS2* and *KIR2DL2*[12] extends into the 3′ exons of the highly polymorphic framework gene, *KIR3DL3* for which ligands and functions are unknown.[37,38] Consequently, genetic analysis alone is unlikely to distinguish the contribution of individual *Cen-B* genes. Functional studies will be required to define the mechanism by which NK cells reconstituting from the transplanted hematopoietic stem cells of *Cen-B/B* donors protect against relapse in AML.

NK cells are the first lymphocyte population to expand after HCT and engraftment with alloreactive NK cells has many potential benefits, including (1) decreased rates of GVHD,[39] (2) decreased rates of graft rejection mediated by NK lysis of host T cells, (3) decreased relapse,[40] (4) improved engraftment mediated by NK-cell release of hematopoietic cytokines,[41] and (5) enhanced immune reconstitution and decreased infectious complications mediated by NK-cell antiviral activity. After haploidentical transplantation for AML, beneficial alloreactive NK cells that reduce relapse are generated when the HLA class I type of the donor includes a KIR ligand that the recipient lacks.[15] This observation stimulated many studies that examined the effect of HLA mismatches that involved KIR ligands in transplant outcome.[16,17,42,43] Additional reports demonstrate an association between KIR ligand mismatch and favorable clinical outcomes in myeloid malignancies, especially when T cells are depleted in vivo with antithymocyte globulin,[16] whereas others have found no benefit.[44] Beneficial effects from KIR-ligand mismatch have not been seen in the T cell–replete setting.[45,46]

Here, in contrast, we have focused on donor *KIR* gene variability, demonstrating a clinically significant influence on the success of transplantation for AML. Previously we have shown that the use of donors with *KIR B* haplotypes, who express more activating KIR, was associated with significant improvements in overall and relapse-free survival after T cell–replete, unrelated donor HCT for AML, with more than a 30% better relative risk in both these end points.[21] The benefit of donor *KIR B* haplotypes also has been observed for T cell–replete transplants from HLA-matched sibling donors,[47] an effect reported in other settings.[22,23] The authors of numerous other studies have reported varied effects of activating *KIR* on outcomes after various types of HCT, including increased rates of acute GVHD,[25,48,49] or protection against acute GVHD.[50] *KIR B* haplotypes are present in two-thirds of unrelated donors. Therefore, identification of the specific beneficial gene content is necessary to select which of those *KIR B* donors will provide the most relapse protection and to develop a donor selection strategy that translates into survival benefit.

Our results show how *KIR* genotyping could be used to supplement HLA typing to improve transplant outcome for AML by allowing the selection of donors with favorable *KIR* types. On the basis of gene content alone we can sort donors into 3 groups by their composition of their *KIR B* haplotype-defining genes. The donor *KIR B*–content groups are easily defined as (1) "best," (2) "better," or (3) "neutral" when the specific definitions applied in Figure 4 are used. On the basis of the *KIR* gene frequencies in this sample from the NMDP donor registry, we estimate that by *KIR* genotyping 3 of the best HLA-matched donors the ability to select donors with favorable *KIR B* genotypes (*KIR B*–content score ≥ 2) will be increased from the current, random rate of 31% to a rate of 67%. Similarly, the availability of *Cen-B/B* donors for selection would increase from 11% to 31%. This could be achieved by

adding *KIR* genotyping to the confirmatory *HLA* genotyping now performed on donor DNA samples. Inexpensive *KIR* genotyping methods are available in many HLA typing laboratories, and the necessary *KIR* typing could be completed using the sample sent for confirmatory typing. Thus, the addition of *KIR* genotyping would not cause any delays in the search time (formal search to transplant), which currently takes a median time of 12 weeks (95% CI 6-23 weeks). Overall, a 22% decrease in relapse is predicted by applying this donor selection strategy.

This analysis, determined by current understanding of the structure of the *KIR* gene locus, has identified donors with the *Cen-B* genes as conferring the most relapse protection and survival benefit. We have developed a simple algorithm on the basis of donor *KIR B* gene content that can be used today to identify unrelated donors who will provide the most protection against AML relapse in T cell–replete transplants. A prospective clinical trial selecting the best HLA-matched donors with favorable *KIR B* genotypes will begin next year.

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Authorship

Contribution: S.C., D.J.W., L.A.G., P.P., and J.S.M. designed this study, and analyzed data; J.P.K., T.W., and C.T.L. performed the biostatistical analysis for this study; S.S. and M.D.H. identified DNA samples, patient cohorts, and provided clinical data integration; S.G.E.M. and D.G. interpreted data and were involved in analysis; M.L. and E.T. performed all KIR genotyping and were involved in data analysis; and all authors wrote the manuscript.

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