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Following transplantation for AML, donor *KIR Cen B02* better protects against relapse than *KIR Cen B01*[1]

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Abstract

In the treatment of acute myelogenous leukemia (AML) with allogeneic hematopoietic cell transplantation (HCT), we previously demonstrated that there is a greater protection from relapse of leukemia when the HCT donor has either the *Cen B*/*B KIR* genotype or a genotype having two or more *KIR B* gene segments. In those earlier analyses, *KIR* genotyping could only be assessed at the low resolution of gene presence or absence. To give the analysis greater depth, we developed high resolution *KIR* sequence-based typing, which defines all the *KIR* alleles and distinguishes the expressed alleles from those that are not expressed. We now describe and analyze high resolution *KIR* genotypes for 890 donors of this human transplant cohort. *Cen B01* and *Cen B02* are the common *CenB* haplotypes, with *Cen B02* having evolved from *Cen B01* by deletion of the *KIR2DL5*, *2DS3/5*, *2DP1* and *2DL1* genes. We observed a consistent trend for *Cen B02* providing stronger protection against relapse than *Cen B01*. This correlation indicates that protection depends on the donor having inhibitory KIR2DL2 and/or activating KIR2DS2, and is

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enhanced by the donor lacking inhibitory KIR2DL1, 2DL3 and 3DL1. High resolution KIR typing has allowed us to compare the strength of the interactions between the recipient's HLA class I and the KIR expressed by the donor derived NK cells and T cells, but no clinically significant interactions were observed. The trend observed between donor *Cen B02* and reduced relapse of leukemia points to the value of studying ever larger transplant cohorts.

Introduction

Natural killer (NK) cell function is controlled by the interaction of many types of NK receptor with their ligands expressed on tissue cells (1). The most variable of these interactions are those between Killer-cell immunoglobulin-like receptors (KIR) and HLA class I ligands (2). Both the receptors and the ligands are encoded by gene families, some members of which are highly polymorphic (2). These gene families are located on different chromosomes and segregate independently, providing an additional level of variation distinguishing individuals (2). The impact of NK cell responses on clinical outcomes following hematopoietic cell transplantation (HCT) became a focus for investigation following the observation of improved survival in haploidentical transplants having a KIR ligand mismatch (3).

The initial study of Ruggeri et al (3) showed a reduction in relapse of leukemia and improved survival for AML patients receiving a haploidentical, T-cell depleted transplant from a family member, when the donor has a KIR ligand not present in the recipient. The proposed mechanism is that a subset of donor-derived NK cells kills the recipient's leukemia cells because the recipient cells lack an inhibitory ligand for KIR present in the donor. In that study, reduced graft-versus-host disease (GVHD) was also observed and proposed to be caused by donor NK cells killing recipient dendritic cells (4, 5). These results led to several investigations of the effect of NK cells and KIR ligand mismatch in other transplant settings (6–10). Emerging from such studies was the finding that NK cell effects were predominantly associated with transplantation treatment for AML and that they were influenced by various clinical factors including donor type (either related or unrelated), preparative regimen and graft characteristics including source and T-cell content (6–12).

Our previous investigations demonstrated a significant association of protection from relapse with a donor having *Cen B/B* and/or two or more *KIR B* segments in their *KIR* genotype (13–15) that was enhanced by the recipient having of a C1-bearing HLA-C allotype (14). At that time, further refinement of the association was not possible because high resolution *KIR* genotyping had yet to be developed. Greater understanding of the mechanism that provides protection from relapse could help facilitate the development of therapies that can harness the beneficial effects of NK cells in preventing relapse of leukemia. Developing NK cell therapies in place of, or in addition to, transplantation could reduce the reliance on donor selection, which restricts the pool of available donors based on *KIR* genotype (16).

In the study presented here, we performed high resolution *KIR* genotyping on a subset of 890 donors from our original retrospective cohort of 1532 (14). We successfully discriminated the two major forms of *Cen B,* as well as distinguishing the *KIR* alleles that specify functional proteins from those alleles that are not expressed. The *KIR* allele

data were also used to develop interaction scores that provide measures of the strength and diversity of the KIR:HLA interactions. These variables were used to test immunogenetic associations with clinical outcomes.

Materials and methods

Samples.

We studied 890 patients with AML, who comprise a subset of those analyzed previously (14). Between 1988 and 2009, these patients received myeloblative preparation for a URD HCT facilitated by the National Marrow Donor Program. DNA samples were obtained from the National Marrow Donor Program Research Sample Repository. Outcome data were obtained from the Center for International Blood and Marrow Transplant Research. The demographics of the cohort are shown in Figure 1. DNA samples and clinical data were obtained with informed consent and approval from the National Marrow Donor Program and University of Minnesota Institutional Review Boards.

KIR genotyping.

To prepare libraries for high throughput sequencing, genomic DNA was fragmented and enriched for those fragments originating from the *KIR* genomic region and the *HLA* class I genes, using a pool of oligonucleotide probes (17). Improvements to the library preparation were subsequently made (18). The captured fragments were subjected to paired-end sequencing using a MiSeq instrument and V3 sequencing chemistry (Illumina, San Diego, CA). *KIR* gene content and *KIR* allele identities were determined using the PING bioinformatics pipeline (17). *HLA-A, -B* and *-C* alleles were determined using the NGSengine 1.7.0 software (GenDx, Utrecht, the Netherlands) with the IPD-IMGT/HLA Database (19). Results were compared to the previous *KIR* and *HLA* genotyping of these samples (14).

Statistical analysis.

We tested the same clinical outcomes as in the previous analysis: overall survival, disease free survival, transplant related mortality, relapse, acute GVHD grades II-IV, acute GVHD grades III-IV and chronic GVHD. In the multivariable models we used, all clinical variables were tested first for the affirmation of the proportional hazard assumption. Factors violating this assumption were adjusted through stratification. A stepwise forward-backward selection procedure was then performed to determine the adjusted clinical variables (with a threshold of 0.05 for both entry and retention in the model). KIR variables were tested individually. There were 27 variables tested. Those grouped into four broad categories, refinements of previous analyses(13–15), specific KIR:HLA interactions (20), number of potential HLA:KIR interactions (21–23) and strength of the HLA:KIR interactions. The interaction score variables were tested as both continuous variables and as categorical variables discretized into tertiles. All other variables were tested as categorical variables. To adjust for multiple testing of 27 variables, a threshold of the overall $P<0.05/27=0.0018$ was used for determining statistical significance.

KIR:HLA interaction scores.

There were two different HLA:KIR interaction scoring models used to assess functional diversity. The first counted the number of interactions and the second used previously published HLA:KIR binding and expression data to calculate a score reflecting the strength and diversity of the interactions. In the case of homozygosity, the interaction was counted twice. As there were a number of mismatched transplants included in this cohort, analyses were performed for the combination of donor KIR with either donor or recipient HLA to determine if there were differential effects.

KIR and *HLA* genotypes were used for counting the number of possible interactions between the donor KIR and either donor or recipient HLA class I, as described previously (21–23). Binding partners have been described for the inhibitory KIR, 3DL1 (24, 25), 3DL2 (26, 27), and 2DL1, 2DL2, 2DL3 (28–30), and the activating KIR, 2DS1 (30), 2DS2 (31), 2DS4 (32) and 2DS5 (33). The interactions counted are shown in Supplemental Figure 1. Activating and inhibitory interactions were counted and tested separately. Due to the small numbers of individuals with potential activating KIR:HLA interactions, the test was also performed as the presence or absence of activating KIR:HLA interaction.

The allele level data were also used to calculate an interaction score based on the observed binding of KIR and HLA allotype pairs (30, 33–35). The complete scoring matrix is shown in Supplemental Figure 1. Only inhibitory interactions were scored. The centromeric score values come from published binding studies (30, 34) where they were reported as absolute binding values. Certain 2DL1 allotypes have been reported to have low cell surface expression (12, 36–38). The effect of altered expression was included by multiplying the binding values for those allotypes by 0.5 to account for the expression level difference. Telomeric interaction scores are based on the binding of 3DL1 and were developed using the binding data from Saunders et al (35). KIR3DL1 allotypes were assigned to one of four groups (K001, K004, K005, K015) based on similarity. These groups correspond to the non-expressed KIR3DL1 exemplified by 3DL1*004 (39, 40)(K004), the two deeply diverged lineages exemplified by 3DL1*005 and 3DL1*015 (41)(K005 and K015) and the interlineage recombinants exemplified by 3DL1*001 (41)(K001). These values were originally reported as percent of maximum and not as absolute binding values. We normalized them to the centromeric values so that the 100% score of Saunders was equivalent to the maximum centromeric value. KIR and/or HLA of the donor and/or recipient were given the value of the closest allele if they did not appear in the matrix. In homozygous individuals the interaction was counted twice, once for each allele. A total interaction score was computed by summing the centromeric and telomeric scores.

The interaction score reflected the aggregate strength of potential interactions and provides the possibility for a few strong interactions to score similarly to a larger number of weaker interactions. We also calculated average scores for the centromeric, telomeric and total interactions by dividing the interaction score by the number of interactions. This gives a value representing the overall average strength of the potential KIR:HLA interactions.

Results

High resolution KIR genotyping distinguishes CenB01 and CenB02.

The human *KIR* gene family maps to human chromosome 19q13.4 (42) and consists of distinctive centromeric (*Cen*) and telomeric (*Tel*) regions which are separated by a 13kb segment that lacks *KIR* genes. This region is rich in repetitive elements and is a hotspot for recombination (17, 42, 43) (Fig. 2A). Based upon *KIR* gene and allele content, the centromeric and telomeric regions are further distinguished by being part of a *KIR A* or *B* haplotype (17, 43, 44). The *Cen A* and *Tel A* regions are of fixed gene content, whereas the *B* regions vary in gene content (17, 43, 44). This feature is exemplified by the different *KIR* gene content of *CenB01* and *Cen B02*, the two most frequent forms of *Cen B*. Common to *Cen B01* and *Cen B02* are the *KIR3DL3*, *2DS2*, *2DL2/3* and *3DP1* genes. Where they differ is in the genomic segment containing the *KIR2DL5, 2DS3/5, 2DP1* and *2DL1 KIR* genes. This segment is present in *Cen B01*, but absent from *Cen B02* (Fig. 2A). In addition to the two frequent *Cen B* haplotypes, there is a variety of low frequency haplotypes that either lack one or more *KIR* genes or have one or more duplicated *KIR* genes (45–48).

Previous low resolution *KIR* genotyping analysis of this transplant cohort could not distinguish *Cen B01* from *Cen B02* (14) as in the absence of gene content or allelic information, presence of *Cen B02* could be masked by *Cen B01* and unambiguous assignment of *Cen B01* in the presence of *Tel B* was not possible. These two *Cen* haplotypes differ significantly in their *KIR* gene content, which is likely to result in different functional phenotypes. Shown on the left half of Figure 2B are pie charts showing the proportions of the different types of *Cen* and *Tel KIR* segments. The ‘Low resolution’ genotypes are those described in our original study (14), which did not distinguish *Cen B01* from *Cen B02*. The ‘High resolution’ *KIR* genotypes are those defined in this study, in which *Cen B01* and *Cen B02* are distinguished. The ‘Final’ *KIR* genotypes are the genotypes used for the statistical analysis. In this group, genotypes with duplications or deletions were assigned to one of the major genotype groups based on similarity (Supplemental Figure 2).

Of the 890 donors, 821 of them could be assigned genotypes that are combinations of the most frequent *KIR* haplotype segments shown in Figure 2A (Supplemental Figure 2). This gives rise to six centromeric genotypes and three telomeric genotypes. A minority subset of 69 donors (7.7% of the cohort) have *KIR* genotypes that are not combinations of the most frequent *KIR* haplotypes. These donors have at least one *KIR* haplotype that differs from a frequent haplotype by duplication or deletion of one or more *KIR* genes. For the analysis, each of these donors was included in one of the frequent genotype groups, based on their gene and allele content (Supplemental Figure 2). For the centromeric genotypes *Cen A/A* is defined as having only *3DL3–2DL3–2DP1–2DL1–3DP1* in the centromeric interval, regardless of gene copy number. *Cen A/B02* is defined by the addition of *2DS2* and/or *2DL2* to the *Cen A/A* genotype. *Cen A/B01* is defined by having *2DL5* and *2DS3/5*, as well as *2DS2* and/or *2DL2.* Distinguishing *Cen B01/B01, Cen B01/B02* and *Cen B02/B02* is the presence of 2 copies of *2DL1* in *Cen B01/B01*, one copy of *2DL1* in *Cen B01/B02,* and absence of *2DL1* in *Cen B02/B02*. In addition, *Cen B02/B02* is characterized by the lack of *2DL5–2DS3/5*. For the telomeric genotypes, *Tel A/A* is defined as having only

2DL4–3DL1–2DS4–3DL2 in the telomeric interval, regardless of gene copy number. *Tel B/B* is defined by presence of *3DS1, 2DL5, 2DS3/5* and/or *2DS1* in the telomeric interval, combined with absence of *3DL1* and *2DS4.* All other combinations of *KIR* are considered to be *Tel A/B*. A complete list of all of the duplication and deletion haplotypes and their assignments is given in Supplemental Figure 2.

The number of occurrences for individual *KIR* alleles is shown in Fig. 2C (allele and phenotype frequencies are shown in Supplemental Figure 3) and highlights the allelic variability in the *KIR* genes. The donor cohort is comprised primarily of individuals of European ancestry (Fig. 1) and the common alleles and allele frequencies are consistent with those observed in other European populations (http://allelefrequencies.net/) (49). The number of common alleles (>5% frequency) varies between the genes with some having a single dominant allele (e.g. *2DL5*, *2DS3* or *2DS5*) and others having several, with the largest number observed for *3DL1/S1* (eight alleles present at frequencies greater than 5%). It is expected that the allele frequencies and common alleles will vary depending on the population of origin. For example, one of the common *2DL3* alleles in this cohort, *2DL3*002*, is present at a frequency of 22%. In comparison it is rare in an African population with a reported frequency of less than 1% (23).

This extensive variability also produces high levels of heterozygosity for some of the *KIR* genes. Together these features result in numerous subgroups, each comprising a small number of individuals, when individual *KIR* alleles are considered separately. Even with our cohort of 890 transplants the numbers were too small for a robust analysis, when individual alleles were assessed. We therefore used the allelic information to develop interaction scores, which were used to test hypotheses that different strengths of interaction, or diversity of interactions, correlated with differences in transplant outcome. We also performed an analysis of functional presence/absence of *3DL1/S1* and of *2DS4*. In Europeans, these two *KIR* genes have a high frequency of non-expressed alleles (Fig. 2D). In our previous analysis (13) of association of presence/absence of individual genes with outcome these non-expressed alleles were considered to be ‘present’. In this revised analysis, the non-expressed alleles are considered to be absent. We found no association for the presence or absence of *3DL1/S1* or *2DS4* with any of the transplant outcomes tested (Fig. 3A).

Confirmation that Cen genotype and B-segment count both correlate with relapse protection.

We tested 27 variables in our statistical model (Fig. 3A and Supplemental Figure 4). They are of four broad categories; refinement of previous tests, tests of specific interactions, tests of the diversity of interactions and tests of overall interaction strength. As the transplant cohort contained samples from both HLA matched and HLA mismatched samples, we tested the combination of donor KIR and donor HLA as well as the combination of donor KIR and recipient HLA for all the interaction variables.

Boudreau *et al* found that combinations of KIR3DL1 and HLA-B that either interact weakly, or do not interact at all, correlate with reduced incidence of relapse following allogeneic hematopoietic cell transplant for AML (20). In that study, most transplant donors and recipients were HLA matched, whereas in our cohort, more transplants were mismatched

and the extent of their mismatch was generally greater. For this reason, we tested the combination of the donor KIR with both the donor and recipient HLA (Fig. 3B). In neither test did we find a correlation with any of the endpoints tested. These results are consistent with those of Schetelig *et al* (50), who saw no correlation of *3DL1:HLA-B* combinations with relapse or overall survival. The discordant findings of these three investigations could reflect differences in the transplants studied. Our study cohort comprises transplants primarily derived from bone marrow and having a higher overall degree of HLA mismatch (Fig. 1). The cohort of Boudreau *et al* was split between bone marrow (55%) and PBSC (45%) transplants and were either 9/10 (44%) or 10/10 (56%) HLA matched (20). The cohort of Schetelig *et al* comprised transplants that were 96% PBSC and having 9/10 (21%) or 10/10 (78 %) HLA match (50). These differences could result in distinct immune environments following transplant in which specific NK receptor:ligand pairs have a dominant effect. Further analysis of larger and contemporary cohorts will be needed to examine these effects.

In our previous study we correlated protection from relapse with *Cen B* genotype and *B*-segment count (14). Those results were replicated in the current analysis (Fig. 3A and Supplemental Fig. 4). We hypothesized that this correlation could be due either to the strength and/or the diversity of KIR:HLA interactions. In testing this hypothesis we used two systems for scoring the interactions. The first measured the diversity of KIR:HLA interactions by determining the number of potential KIR:HLA interactions between the allotypes encoded by the donor *KIR* and donor, or recipient, HLA class I allotypes. Each transplant was scored by counting potential interactions between donor KIR and both donor and recipient HLA class I (Supplemental Fig. 1). Interactions involving inhibitory KIR and activating KIR were scored separately. The distribution of the scores is shown in Fig. 3C. Because of the low number of activating KIR interactions, mainly due to the high frequency of non-functional *2DS4* alleles, the activating interaction score was also tested as presence/absence of activating KIR:HLA interaction without further stratification. None of these variables showed statistically significant association with relapse (Fig. 3A and Supplemental Fig. 4), nor any other endpoint (not shown).

The second type of interaction score we used was based on experimentally determined strengths of KIR-HLA class I interactions (30, 33–35). This analysis examined only the inhibitory KIR: KIR2DL1, 2DL2, 2DL3 and 3DL1. This score was assessed for the allotypes encoded by centromeric *KIR2DL1*, *2DL2*, and *2DL3*, for allotypes encoded by telomeric *KIR3DL1* and for the combination of centromeric and telomeric allotypes (Fig. 3D). The scores were also adjusted to obtain an average interaction score (Fig. 3D). The combination of donor KIR with either donor or recipient HLA was tested. Neither score showed a correlation with relapse that is statistically significant (Fig. 3A).

Comparing the effect of CenB01 versus CenB02.

Protection from relapse was first observed for transplant donors who were either *Cen B/B* or had two or more *KIR B* gene segments. We further stratified the *Cen B* genotypes into *Cen B01* and *Cen B02* (Fig. 2A and B) and improved the assessment of *B* segment counts by determining the deletion and duplication events that alter the *B* segment count. Greater

protection against relapse was associated with *Cen B01/Bx* and *Cen B02/B02* compared to all other *Cen* genotypes (Fig. 4, left panels). This additional refinement produced no statistically significant difference between *Cen B01* and *Cen B02* (Fig. 4, left panels). However, in both the *Cen B02/B02* versus *Cen B01/*Bx and the *Cen A/*B02 vs *Cen A/*B01 groups there is a clear trend for *Cen B02* to confer greater protection against relapse (HR 0.77, CI 0.58–1.04, P=0.0892) (Fig. 4, left panels).

Protection from relapse was also seen for donors having two or more *B* segments (Fig. 4, right panels). Testing for interaction between *B* segment count, *Cen* genotype and adjusted covariates showed no significant interaction. Because of the low number of *Tel B/B* genotypes, the majority of two B-segment genotypes have a *Cen B* segment. This points to a mechanism requiring the presence of *2DL2* and/or *2DS2* in a genomic background that encodes fewer or weaker inhibitory KIR that interact with HLA.

Discussion

Our previous studies of HCT treatment for AML (13–15) demonstrated a significant protection from relapse that is associated with a donor having the *Cen B/B KIR* genotype and/or two or more *KIR B* gene segments. Providing the best protection were donors having the *Cen B/B* genotype and recipients having $C1^+$ HLA-C. This observation is consistent with a mechanism that requires a donor with one or two *Cen B* gene segments as there is no significant association with donors that are *Tel B/B* and have no *Cen B*. Further refinement of this model to consider allelic differences was not possible without the higher resolution of the genotyping data provided in this study.

Two overlapping hypotheses arise from this study. First, protection from relapse is associated with one or more of the specific genes or alleles found in the *Cen B* segment. Supporting this hypothesis, is relapse protection was strongly associated with the presence of two *Cen B* segments and the association is retained in the presence of two or more *B* segments. While this latter group includes individuals who are *Tel B/B*, this group is very small. It is therefore possible that this association is driven by individuals who have at least one *Cen B* segment and is combined with one or two *Tel B* segments. The second hypothesis is that the association with protection from relapse reflects an association with strength or diversity of KIR:HLA interactions, and that it is interactions of the products encoded by the *Cen B* segment that more strongly influence the interaction.

Allotypic diversity of the KIR and their HLA ligands produces a range of interaction strengths. We developed an interaction score that accounts for binding strength and the expression levels of 2DL1, 2DL2, 2DL3 and 3DL1/S1 with their known ligands. We calculated an interaction score that reflects the sum of all interactions (range 0–495) and an average interaction score (range 0–39) (Fig. 3D). The statistical analysis incorporated the scores as continuous variables, as well as when the values are divided into tertiles. Although no statistically significant correlations with transplant outcome were observed, there is a clear trend toward significance when the data are analyzed as tertiles. It is possible that the cohort size of 890 was too small to detect a significant association and that a larger,

more contemporary cohort with opportunity to adjust for critical clinical variables such as preparatory regimen and graft source could better inform this question.

The overall diversity of interactions was examined by counting the number of distinct KIR:HLA interactions that are predicted by the genotype data. We analyzed activating and inhibitory interactions separately. A large part of the cohort had no activating KIR:HLA interactions due to the high percentage (56%, Fig. 2D) of *2DS4* alleles that are not-expressed. No statistically significant associations were identified. This may reflect the small number of individuals in some of the numeric categories (Fig. 3C).

Boudreau et al (20) reported that protection from relapse is associated with combinations of KIR and HLA class I that predict weak or non-inhibitory interactions. We similarly divided our cohort using the criteria in their study and examined the donor *KIR* genotype paired with either the donor *HLA* genotype or the recipient *HLA* genotype separately. Despite having a similarly sized transplant cohort as Boudreau et al, we found no significant association with relapse for either combination. Although differences in preparatory regimen were included in our statistical model, it is possible that other clinical correlates are responsible for the differences observed in our study and that of Boudreau et al.

Two main *Cen B* structures (Fig. 2A) differ in *KIR* gene content. With only presence/absence data available for our previous analyses, we (13, 14) could not discriminate *Cen B01* from *Cen B02* for all individuals. With high resolution *KIR* genotyping we have now resolved the *Cen B* content for all individuals tested (Fig. 2B). Although not statistically significant, there is a trend to significance indicating that *Cen B02* gives more effective protection from relapse than *Cen B01*. This is most apparent in the comparison of *Cen A/B01* individuals with *Cen A/B02* individuals (Fig. 4). *Cen A/B01* and *Cen A/A* individuals are not distinguished whereas *Cen A/B02* individuals show an increase in protection from relapse.

The confirmed association of donors with 2 or more *B*-segments, particularly *Cen B* homozygous donors, with relapse protection, supports the interpretation that KIR2DL2 and/or KIR2DS2 are necessary for protection. Consistent with this interpretation is the absence of any association with the *Tel* genotype. In the 3 and 4 *B*-segment groups all individuals have at least one *Cen B* and most have two (Fig. 2B). Even the 2 *B*-segment group is biased toward individuals having at least one *Cen B* (96% total, 23% *B/B*, 73% *A/B*). The effect of 2DL2 and/or 2DS2 is enhanced when the contribution of 2DL1, 2DL3 and 3DL1 to NK cell inhibitory potential is decreased or absent. We could not subset our cohort further to determine if there was a predictable hierarchy of contribution for each of these covariates. Testing the degree of contribution from each of these will require analysis of a much larger transplant cohort.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

KIR Cen B is associated with protection from relapse following HSCT

KIR Cen B02 provides stronger protection against relapse

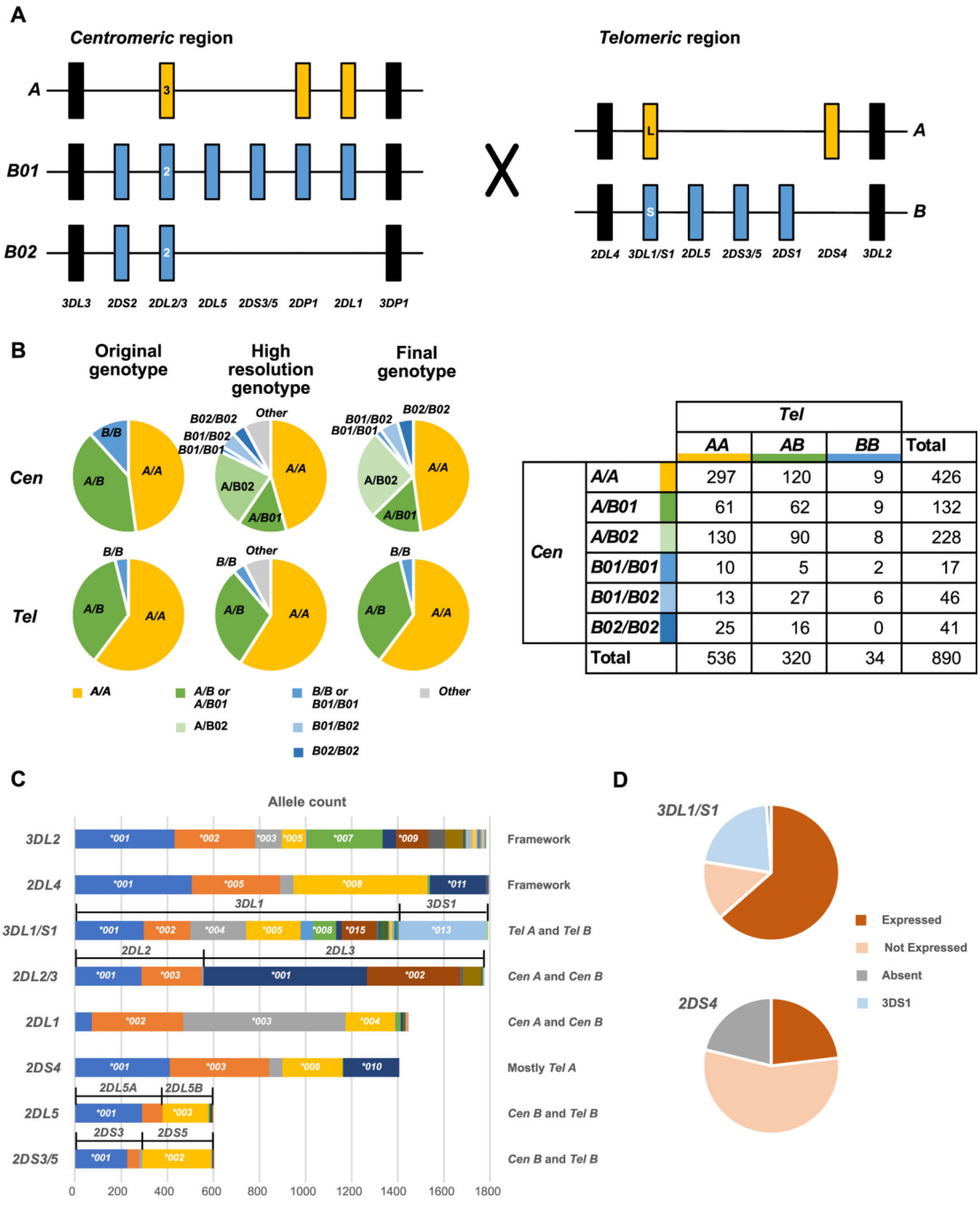
Protection from relapse associates with presence of less inhibitory KIR

Variables	Total N=890	A/A N=426	A/B01 N=132	A/B02 N=228	B/B* N=104	P Value
Donor age in years						*0.128* [A]
Mean ± SD	35.7 ± 8.8	36.1 ± 8.6	36.6 ± 9.1	34.7 ± 9.2	35.2 ± 7.7	
Median (q25 - q75)	35.4 (28.6 - 42.2)	35.9 (29.1 - 42.2)	36.4 (29.8 - 42.8)	33.1 (26.7 - 42.0)	34.6 (29.2 - 41.3)	
Donor race						*0.446* [C]
White	737 (85.3)	347 (84.0)	111 (85.4)	197 (88.3)	82 (83.7)	
African-American	28 (3.2)	11 (2.7)	6 (4.6)	6 (2.7)	5 (5.1)	
Asian	10 (1.2)	3 (0.7)	2 (1.5)	4 (1.8)	1 (1.0)	
Hispanic	43 (5.0)	22 (5.3)	6 (4.6)	8 (3.6)	7 (7.1)	
Other	46 (5.3)	30 (7.3)	5 (3.8)	8 (3.6)	3 (3.1)	
Missing	26	13	2	5	6	
Donor sex						*0.198* [C]
Male	550 (61.8)	262 (61.5)	79 (59.8)	152 (66.7)	57 (54.8)	
Female	340 (38.2)	164 (38.5)	53 (40.2)	76 (33.3)	47 (45.2)	
Donor age (categorical)						*0.527* [C]
0-19 years	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	
20-29 years	262 (29.4)	118 (27.7)	34 (25.8)	78 (34.2)	32 (30.8)	
30-39 years	344 (38.7)	165 (38.7)	53 (40.2)	83 (36.4)	43 (41.3)	
40 -49 years	229 (25.7)	115 (27.0)	35 (26.5)	52 (22.8)	27 (26.0)	
50 years and older	54 (6.1)	28 (6.6)	10 (7.6)	14 (6.1)	2 (1.9)	
Donor KIR haplotype						*<.001* [C]
AA	295 (33.1)	295 (69.2)	0 (0.0)	0 (0.0)	0 (0.0)	
B/x	595 (66.9)	131 (30.8)	132 (100)	228 (100)	104 (100)	
CMV match						*0.190* [C]
-/-	279 (31.3)	126 (29.6)	45 (34.1)	81 (35.5)	27 (26.0)	
-/+	281 (31.6)	145 (34.0)	35 (26.5)	66 (28.9)	35 (33.7)	
+/-	118 (13.3)	48 (11.3)	19 (14.4)	30 (13.2)	21 (20.2)	
+/+	184 (20.7)	90 (21.1)	30 (22.7)	43 (18.9)	21 (20.2)	
Unknown	28 (3.1)	17 (4.0)	3 (2.3)	8 (3.5)	0 (0.0)	
Recipient-donor sex match						*0.463* [C]
M/M	318 (35.7)	146 (34.3)	49 (37.1)	88 (38.6)	35 (33.7)	
M/F	232 (26.1)	116 (27.2)	30 (22.7)	64 (28.1)	22 (21.2)	
F/M	164 (18.4)	78 (18.3)	30 (22.7)	32 (14.0)	24 (23.1)	
F/F	176 (19.8)	86 (20.2)	23 (17.4)	44 (19.3)	23 (22.1)	
HLA matching						*0.571* [C]
<=8/10	209 (23.5)	103 (24.2)	27 (20.5)	52 (22.8)	27 (26.0)	
9/10	241 (27.1)	115 (27.0)	40 (30.3)	66 (28.9)	20 (19.2)	
10/10	440 (49.4)	208 (48.8)	65 (49.2)	110 (48.2)	57 (54.8)	
HLA mismatch						*0.384* [C]
matched	440 (49.4)	208 (48.8)	65 (49.2)	110 (48.2)	57 (54.8)	
Class I mismatch only	310 (34.8)	151 (35.4)	48 (35.4)	81 (35.5)	30 (28.8)	
Class II mismatch only	54 (6.1)	22 (5.2)	5 (3.8)	21 (9.2)	6 (5.8)	
both Class I and II mismatched	86 (9.7)	45 (10.6)	14 (10.6)	16 (7.0)	11 (10.6)	
GVHD prophylaxis						*0.183* [C]
Tacrolimus ± MTX ± Other	354 (39.8)	181 (42.5)	46 (34.9)	85 (37.3)	42 (40.4)	
Cyclosporine + MTX ± Other	478 (53.7)	218 (51.2)	71 (53.8)	133 (58.3)	56 (53.8)	
CSA ± Other (No MTX)	40 (4.5)	17 (4.0)	13 (9.8)	4 (1.8)	6 (5.8)	
MMF ± Other	3 (0.3)	1 (0.2)	0 (0.0)	2 (0.9)	0 (0.0)	
MTX ± Other (No CSA)	6 (0.7)	3 (0.7)	1 (0.8)	2 (0.9)	0 (0.0)	
Other	9 (1.0)	6 (1.4)	1 (0.8)	2 (0.9)	0 (0.0)	
Conditioning regimen						*0.423* [C]
Cy + TBI >500 cGY single or >800cGY fractionated	610 (68.5)	283 (66.4)	95 (72.0)	166 (72.8)	66 (63.5)	
Bu + Cy	255 (28.7)	132 (31.0)	32 (24.2)	56 (24.6)	35 (33.7)	
Other	25 (2.8)	11 (2.6)	5 (3.8)	6 (2.6)	3 (2.9)	

Variables	Total N=890	A/A N=426	A/B01 N=132	A/B02 N=228	B/B* N=104	P Value
Recipient age in years						*0.539* [A]
Mean ± SD	34.7 ± 15.2	35.2 ± 15.4	33.1 ± 15.1	34.4 ± 15.5	35.3 ± 14.0	
Median (q25 - q75)	37.5 (23.5 - 46.7)	38.5 (23.7 - 47.0)	34.5 (22.6 - 44.7)	37.2 (22.3 - 46.7)	35.9 (24.0 - 45.3)	
Recipient race						*0.665* [C]
White	808 (91.6)	388 (91.9)	117 (88.6)	211 (93.4)	92 (90.2)	
African-American	35 (4.0)	15 (3.6)	6 (4.5)	9 (4.0)	5 (4.9)	
Asian	15 (1.7)	7 (1.7)	3 (2.3)	3 (1.3)	2 (2.0)	
Hispanic	18 (2.0)	10 (2.4)	3 (2.3)	2 (0.9)	3 (2.9)	
Other	6 (0.7)	2 (0.5)	3 (2.3)	1 (0.4)	0 (0.0)	
Missing	8	4	0	2	2	
Recipient sex						*0.451* [C]
Male	482 (54.2)	224 (52.6)	79 (59.8)	120 (52.6)	59 (56.7)	
Female	408 (45.8)	202 (47.4)	53 (40.2)	108 (47.4)	45 (43.3)	
Recipient age (categorical)						*0.854* [C]
0-9 years	68 (7.6)	32 (7.5)	9 (6.8)	21 (9.2)	6 (5.8)	
10-19 years	98 (11.0)	48 (11.3)	16 (12.1)	25 (11.0)	9 (8.7)	
20-29 years	160 (18.0)	68 (16.0)	30 (22.7)	42 (18.4)	20 (19.2)	
30-39 years	170 (19.1)	84 (19.7)	24 (18.2)	37 (16.2)	25 (24.0)	
40 -49 years	251 (28.2)	124 (29.1)	33 (25.0)	69 (30.3)	25 (24.0)	
50 years and older	143 (16.1)	70 (16.4)	20 (15.2)	34 (14.9)	19 (18.3)	
Graft type						*0.210* [C]
Bone marrow	563 (63.3)	260 (61.0)	94 (71.2)	143 (62.7)	66 (63.5)	
PBSC	327 (36.7)	166 (39.0)	38 (28.8)	85 (37.3)	38 (36.5)	
Karnofsky score						*0.588* [C]
90-100	268 (30.1)	133 (31.2)	43 (32.6)	63 (27.6)	29 (27.9)	
Oct-80	547 (61.5)	259 (60.8)	74 (56.1)	145 (63.6)	69 (66.3)	
Missing	75 (8.4)	34 (8.0)	15 (11.4)	20 (8.8)	6 (5.8)	
Cytogenetic status						*0.246* [C]
Favorable	97 (10.9)	40 (9.4)	11 (8.3)	30 (13.2)	16 (15.4)	
Intermediate	381 (42.8)	191 (44.8)	62 (47.0)	94 (41.2)	34 (32.7)	
Unfavorable	187 (21.0)	94 (22.1)	27 (20.5)	46 (20.2)	20 (19.2)	
Missing	225 (25.3)	101 (23.7)	32 (24.2)	58 (25.4)	34 (32.7)	
Year of transplant						*0.252* [C]
2004-2006	277 (31.1)	151 (35.4)	34 (25.8)	64 (28.1)	28 (26.9)	
2000-2003	303 (34.0)	134 (31.5)	46 (34.8)	89 (39.0)	34 (32.7)	
1996-1999	174 (19.6)	75 (17.6)	32 (24.2)	42 (18.4)	25 (24.0)	
1988-1995	136 (15.3)	66 (15.5)	20 (15.2)	33 (14.5)	17 (16.3)	
Time from diagnosis to transplant						*0.138* [C]
< 6 month	278 (31.2)	151 (35.4)	39 (29.5)	61 (26.8)	27 (26.0)	
6-12 month	249 (28.0)	114 (26.8)	42 (31.8)	67 (29.4)	26 (25.0)	
>= 12 month	363 (40.8)	161 (37.8)	51 (38.6)	100 (43.9)	51 (49.0)	
TBI use						*0.165* [C]
No	269 (30.3)	139 (32.7)	35 (26.5)	59 (25.9)	36 (34.6)	
Yes	620 (69.7)	286 (67.3)	97 (73.5)	169 (74.1)	68 (65.4)	
Missing	1	1	0	0	0	
ATG/ alemtuzumab use						*0.602* [C]
ATG + alemtuzumab	4 (0.4)	1 (0.2)	1 (0.8)	1 (0.4)	1 (1.0)	
ATG Alone	85 (9.6)	48 (11.3)	12 (9.1)	14 (6.1)	11 (10.6)	
alemtuzumab Alone	17 (1.9)	9 (2.1)	2 (1.5)	3 (1.3)	3 (2.9)	
No ATG or alemtuzumab	784 (88.1)	368 (86.4)	117 (88.6)	210 (92.1)	89 (85.6)	

Fig. 1. Demographic characteristics of the transplant cohort.

Shown are the characteristics of the transplant cohort. Subgroups correspond to *KIR Cen* genotypes with *B/B* the sum of *B01/B01, B01/B02* and *B02/B02*. Values shown are N (%). P-value calculation type is indicated by the superscript, [A] ANOVA F-test, [C] Chi-square.



		Tel			
		AA	AB	BB	Total
Cen	A/A	297	120	9	426
	A/B01	61	62	9	132
	A/B02	130	90	8	228
	B01/B01	10	5	2	17
	B01/B02	13	27	6	46
	B02/B02	25	16	0	41
	Total	536	320	34	890

Fig. 2. Allele level *KIR* genotyping resolves haplotypes and alleles encoding functional differences.

Panel A) Schematic representation of different centromeric (*Cen*) and telomeric (*Tel*) *KIR* haplotype structures. *KIR A* haplotype specific alleles/genes are in gold, *KIR B* haplotype specific alleles/genes are in blue, framework genes are in black. The large X indicates the central repetitive region which facilitates recombination between the *Cen* and *Tel* segments. Panel B) Genotypes were assigned as combinations of one of the major structural groups shown in Panel A. (Left) Pie charts showing the proportion of each genotype identified in the original genotyping (left), full genotyping (center) and final genotyping (right). In the

full genotyping ~7% of genotypes differed from those formed by the major structural groups shown in Panel A by either deletion or duplication of genes. These were assigned to one of the major structural groups (Supplemental Figure 2) for the final genotyping. (Right) Table of *Cen*/*Tel* combinations present in the dataset. Panel C) Number of alleles present in the dataset. The greater number of *2DL4* alleles compared to *3DL2* is due to the duplication genotypes that contain additional *2DL4* genes. To the right of the graph, the location of genes in the major structural types (Panel A) are indicated. Panel D) For *3DL1/S1* and *2DS4*, the percent of expressed, non-expressed and absent alleles are shown.

A

Variable tested	Categories	Notes	Overall p-value (relapse)
Refinements			
Cen genotype	A/A, A/B01, A/B02, B01/ 01, B01/B02, B02/B02		0.0009 #
	A/A, A/B01, A/B02, B01/x, B02/B02	combined B01/B01 and B01/B02 due to low number of B01/B01	0.0003 #
Tel genotype	A/A, A/B, B/B		0.1005
B segment count	0, 1, 2, 3, 4		0.0015 #
	0, 1, 2, 3+4	combined 3 and 4 due to low numbers	0.0001 #
3DL1 expressed	expressed, not-expressed		0.7134
2DS4 expressed	expressed, not-expressed		0.7647
Specific Interactions			
3DL1:Bw4(recipient)	non-interacting, weak interaction, strong interaction	scored as in Boudreau, et al (20); used recipient HLA	0.6655
3DL1:Bw4(donor)	non-interacting, weak interaction, strong interaction	scored as in Boudreau, et al (20); used donor HLA	0.6564
Number of donor KIR and recipient HLA interactions			
Inhibitory interactions	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	scored as in (21-23)	0.6154
Activating interactions	0, 1, 2, 3, 4, 5, 6	scored as in (21-23)	0.6701
	present, absent	activating interactions >0 combined due to low numbers	0.5442
Number of donor KIR and donor HLA interactions			
Inhibitory interactions	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	scored as in (21-23)	0.171
Activating interactions	0, 1, 2, 3, 4, 5, 6	scored as in (21-23)	0.2218
	present, absent	positive combined due to low numbers	0.2554
Interaction scores for donor KIR and recipient HLA			
Centromeric	continuous range from 0 to 226	sum of interactions	0.4526
Avg. Centromeric	continuous range from 0 to 72	average interaction	0.2547
Telomeric	continuous range from 0 to 319	sum of interactions	0.6839
Avg. Telomeric	continuous range from 0 to 77	average interaction	0.3365
Total	continuous range from 16 to 495	sum of interactions	0.5445
Avg. Total	continuous range from 2.22 to 49	average interaction	0.7497
Interaction scores for donor KIR and donor HLA			
Centromeric	continuous range from 2 to 232	sum of interactions	0.5967
Avg. Centromeric	continuous range from 0.33 to 72	average interaction	0.6677
Telomeric	continuous range from 0 to 319	sum of interactions	0.7427
Avg. Telomeric	continuous range from 0 to 77	average interaction	0.5458
Total	continuous range from 14 to 495	sum of interactions	0.8315
Avg. Total	continuous range from 1.29 to 49	average interaction	0.375

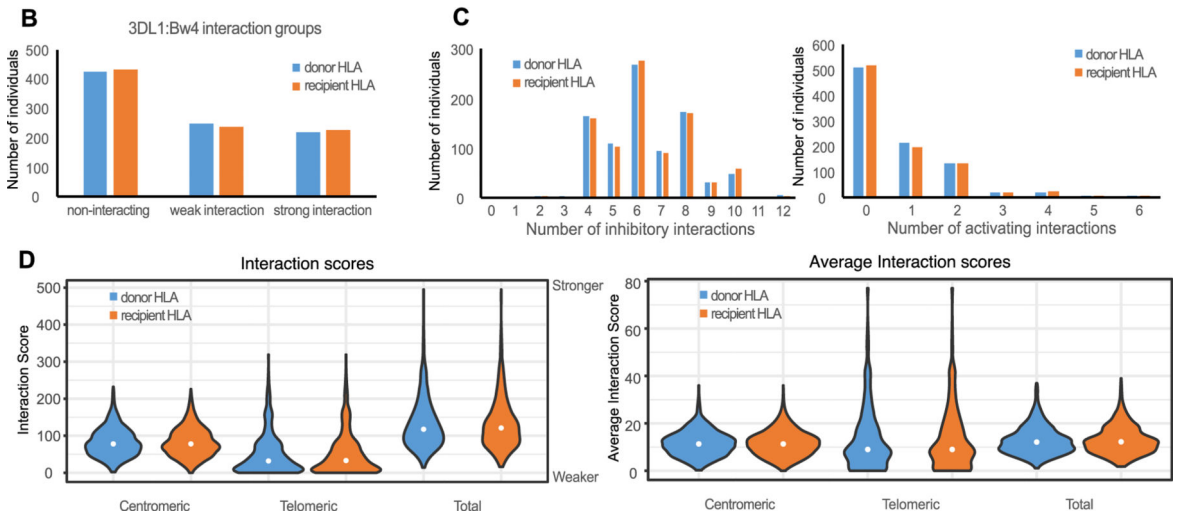
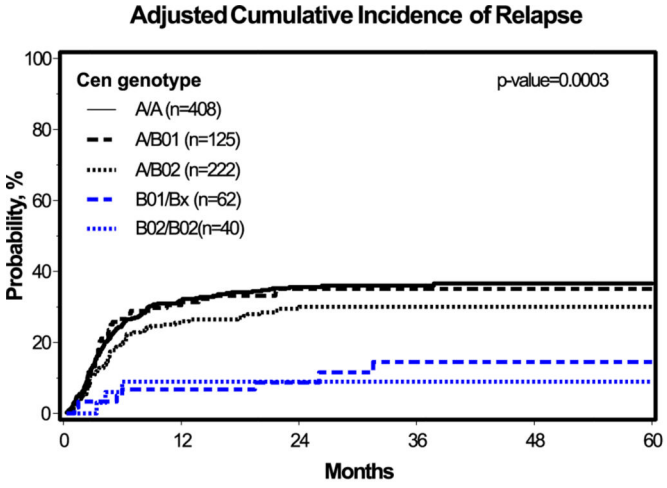
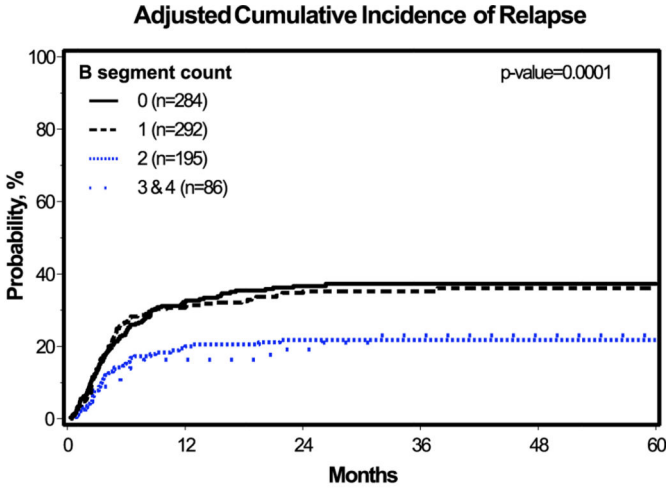


Fig. 3. *Cen B/B* and a *B* segment count of two or more correlate with protection from relapse.

Panel A) Individual variables tested in the statistical model are shown for each category investigated. The overall p-values for the analysis of association with relapse are shown in the rightmost column; those with statistical significance are indicated by a #. Complete results are in Supplemental Figure 4. Panel B) Distribution of the classes of 3DL1:Bw4 interaction (20) considering donor 3DL1 and either donor (blue) or recipient (orange) HLA-B. Panel C) Distribution of the number of inhibitory and/or activating interactions (21–23) considering donor KIR with either donor (blue) or recipient (orange) HLA class I. Panel D)

Distribution of interaction scores (centromeric, telomeric and total) and average interaction scores (centromeric, telomeric and total) considering donor KIR with either donor (blue) or recipient (orange) HLA class I.





Category	Number	Hazard Ratio (HR)	HR Low	HR High	P-value
Cen genotype					0.0003
A/A	408	1			
A/B01	125	1	0.72	1.41	0.9807
A/B02	222	0.77	0.58	1.04	0.0892
B01/Bx	62	0.24	0.11	0.52	0.0003
B02/B02	40	0.23	0.07	0.73	0.0122

Category	Number	Hazard Ratio (HR)	HR Low	HR High	P-value
B segment count					0.0001
0	284	1			
1	292	1.01	0.77	1.33	0.946
2	195	0.53	0.37	0.77	0.0007
3 & 4	86	0.46	0.27	0.77	0.0029

Fig. 4. Cumulative incidence of leukemia relapse for *Cen* genotype and *B* segment count.

Shown is the graph of adjusted cumulative incidence (top) and stratified hazard ratios and p-values (bottom). Tests for interaction showed no interaction between the variables. In the *Cen* genotype model, *B01/B01*(n=17) and *B01/B02* (n=45) were combined into *B01/Bx* because of the low frequency of *B01*. Similarly, the *B*-segment counts of 3 (n=71) and 4 (n=15) were combined, because of the low number of donors having four *B* segments. For the centromeric genotype there is a trend toward significance for the *A/B02* genotype compared to either the *A/A* or *A/B01* genotypes.