Title

Presence of donor-encoded centromeric KIR B content increases the risk of infectious mortality in recipients of myeloablative, T cell deplete, HLA-matched HCT to treat AML

Running title

Donor Cen-B increases NRM in matched adult MAC AML patients

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Abstract

The reported influence of donor Killer-cell Immunoglobulin-like Receptor (KIR) genes on the outcomes of haematopoietic cell transplantation (HCT) are contradictory, in part due to diversity of disease, donor sources, era and conditioning regimens within and between different studies. Here, we describe the results of a retrospective clinical analysis establishing the effect of donor KIR motifs on the outcomes of 119 HLA-matched, unrelated donor HCT for adult acute myeloid leukaemia (AML) using myeloablative conditioning (MAC) in a predominantly T cell deplete (TCD) cohort. We observed that HCT involving donors with at least one KIR B haplotype were more likely to result in non-relapse mortality (NRM) than HCT involving donors with two KIR A haplotypes ($p=0.019$). Upon separation of KIR haplotypes into their centromeric (Cen) and telomeric (Tel) motif structures, we demonstrated that the Cen-B motif was largely responsible for this effect ($p=0.001$). When the cause of NRM was investigated further, infection was the dominant cause of death ($p=0.006$). No evidence correlating donor KIR B haplotype with relapse risk was observed. The results from this analysis confirm previous findings in the unrelated, TCD, MAC transplant setting and imply a protective role for donor-encoded Cen-A motifs against infection in allogeneic HCT recipients.

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Author statement

None of the authors declare any conflicts of interest.

Introduction

Despite developments in the treatment of patients with haematological malignancies to specifically target diseased cells, achieving long term remission in adult acute myeloid leukaemia (AML) remains challenging and haematopoietic cell transplantation (HCT) continues as the mainstay of treatment for high risk patients[1]. Selection of volunteer unrelated donors (VUD) for allogeneic HCT is primarily based on HLA allele matching at the HLA-A, -B, -C, -DRB1 and -DQB1 loci, although many centres have also recently adopted a permissible matching model including the HLA-DPB1 locus[2-5]. However, even in recipients of well-matched grafts, five year overall survival (OS) remains <50%, with both relapse and death from transplant-related complications remaining significant problems[1, 6]. As such, investigation into secondary donor characteristics have been performed and confirmed the importance of non-HLA factors, particularly donor age and CMV matching, in reducing non-relapse mortality (NRM)[4, 7, 8].

In addition to these secondary donor characteristics, selection of donors for non-HLA genetic factors has also been explored as a method to improve HCT outcomes. The Killer-cell Immunoglobulin-like Receptors (KIR), predominantly expressed on the surface of natural killer (NK) cells, are amongst the most promising non-HLA candidate gene families. KIR form a family of activating and inhibitory receptors which, upon binding their cognate HLA ligand, may elicit, or inhibit, an immune response. The genes encoding these proteins can be grouped into two main haplotypes: KIR A haplotypes are conserved in gene content and encode only one activating KIR gene (KIR2DS4) in combination with multiple inhibitory genes (KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2 and KIR3DL3). By contrast, KIR B haplotypes have a more variable gene content and encode at least one of the alternative KIR genes[9]. In addition, KIR haplotypes may be further defined according to their centromeric (Cen) or telomeric (Tel) gene motifs[10].

The relevance of KIR-mediated immunity in HCT to treat AML was first discovered by investigating disparity between donor and recipient inhibitory KIR ligands, subsets of HLA class I molecules

encoding the HLA-C1, -C2 and -Bw4 motifs, in haploidentical T cell-depleted (TCD) transplantations[11]. Ruggeri *et al.* (2002)[12], demonstrated protection from disease relapse without concurrent increase in frequency of graft *versus* host disease (GVHD) in AML recipients whose grafts were derived from donors possessing KIR ligands that were not present in the recipient, often referred to as "missing self". As such, they proposed that graft *versus* leukaemia (GVL) alloreactivity could be mediated by donor NK cells when KIR ligand disparity was present. Importantly, this effect appeared to be limited to AML recipients as the same effect was not observed in acute lymphoblastic leukaemia (ALL) patients. Following this, several studies have confirmed this model in haploidentical and other HLA-mismatched allogeneic transplant settings[13, 14].

In addition to relapse and GVHD, infection remains a major contributor to the high mortality rates associated with HCT. In addition to *de novo* infections acquired during the extended periods of immunosuppression, viral reactivation is also a common cause of morbidity and mortality. In the UK, frequent use of TCD as GVHD prophylaxis, often utilising alemtuzumab, may exacerbate this issue[15]. NK cells are the first lymphocyte subset to reconstitute following HCT and are known to target virally-infected cells. However, NK cell reactivity resulting from KIR-ligand mismatching has, in contrast to its findings in relapse, been proposed to increase patients' susceptibility to infection-related mortality[16, 17].

Although mismatches between donor and recipient KIR ligands are not possible in HLA-matched transplants, KIR-mediated alloreactivity may still exist, as donor NK cells may express inhibitory KIR specific for ligands that are not encoded by either the patient or donor. This represents a "missing ligand" condition that has been shown to increase the risk of acute GVHD (aGVHD) but decrease the risk of relapse, ultimately increasing OS and disease-free survival (DFS)[18-23]. In addition, there are KIR molecules whose ligands are yet to be defined which may also permit KIR-mediated alloreactivity.

The most recent KIR-mediated alloreactivity model has been proposed based on findings from a large cohort of T cell replete, myeloablative conditioning (MAC) transplants. Using this model, a scale of alloreactivity is established based on the activating KIR content of the graft, reflected by the donor's KIR haplotypes. This has shown that OS can be increased by selecting donors who encode at least one copy of the KIR B haplotype (KIR Bx)[24]. Upon further investigation, it was discovered that Cen-B motifs were predominantly associated with this outcome, and their presence correlated with a significant reduction in relapse and improved DFS, particularly in HLA-C mismatched transplants where the recipient encodes the HLA-C1 ligand[10, 25]. However, when a similar comparison investigating Cen motifs was performed in a large cohort of transplants utilising reduced intensity conditioning (RIC) regimens, no significant difference was observed[18, 20].

The effect of KIR genotype polymorphism on HCT outcomes is therefore controversial and appears highly dependent on a variety of transplant characteristics. To reduce heterogeneity within the cohort, this study focusses only on the outcomes of a specific group of HCT recipients: TCD, HLA-matched, adult, myeloablative transplants to treat AML. Thereafter, we have investigated the influence of donor KIR genotypes on the outcomes of HCT within this UK cohort.

Materials and Methods

Study cohort

One hundred and nineteen HCT recipients and their respective VUDs were included in this study. All transplants took place between December 1996 and June 2011. Transplant inclusion criteria were as follows: i) UK-based adult transplanted to treat AML, ii) MAC regimen, iii) stem cells provided from an Anthony Nolan VUD and iv) complete allele-level HLA matching for HLA-A, -B, -C, -DRB1 and –DQB1, as described previously[26]. Clinical outcomes data were obtained in collaboration with the British Society of Blood and Marrow Transplantation. Ethical approval was obtained from the National Research Ethics Service (www.nres.nhs.uk, application number: MREC 01/8/31). The

project was approved by Anthony Nolan medical and scientific committees. Informed consent was obtained from all participants prior to donation of blood or buccal cell samples for genetic analysis.

DNA extraction

Genomic DNA was extracted from whole blood or buccal swab samples. When extracted from blood, DNA was obtained either from salting-out[27] or paramagnetic bead-based DNA purification (Promega, Madison, WI, USA). When extracted from buccal swabs, DNA was obtained using Gentra Puregene Buccal Cell Kit (QIAGEN, Hilden, Germany).

KIR genotyping

Briefly, presence or absence of 16 individual KIR genes was analysed using a polymerase chain reaction sequence-specific priming (PCR-SSP) approach described previously[28]. No distinction was made between the presence of KIR2DL5A or KIR2DL5B. The presence of at least one KIR B haplotype-specific locus indicated that the genotype contained at least one B haplotype. Such samples were depicted as KIR Bx. All samples that lacked the presence of all KIR B loci were assigned the AA genotype designation (KIR AA). Centromeric (Cen) and telomeric (Tel) gene motifs were assigned as described previously[10]. HLA-C1, -C2 and -Bw4 epitope ligands for KIR molecules were inferred from previous HLA typing.

Statistical analysis

Survival and DFS probability curves were calculated by the method of Kaplan-Meier[29]. Groups were compared using the log-rank test, whilst multivariate analysis was performed by Cox regression[30]. Several analyses incurred competing risks. The competing risk in relapse analysis was non-relapse mortality (NRM), whilst relapse was the competing risk in NRM analysis. When comparing the risk of infectious mortality between different groups, relapse or death due to any other cause were the competing risks. For these competing risk analyses, univariate probabilities were calculated using the cumulative incidence function[31]. Multivariate competing risk analysis was performed using the method by Fine and Gray[32]. A forward stepwise selection of covariates for multivariate analysis was

performed using p≤0.05 inclusion criteria. Statistical significance was denoted at p≤0.05, whilst statistical trend was signified by p≤0.1. All univariate and multivariate analyses were performed using 'R' software (version 3.4.2).

Results

Patient and donor characteristics

Donor and recipient demographics and HCT conditions are given in Table 1. Of the 84 donors encoding at least one KIR B haplotype, 65 encoded at least one Cen-B motif (Cen-Bx). The remaining 54 donors (45%) encoded only Cen-A haplotype motifs (Cen-AA). When comparing the Cen-AA and Cen-Bx donor groups, the only statistically significant difference was between donor-recipient gender matching, by which gender-matched transplants were more likely to utilise Cen-Bx donors. As donor KIR genotyping was not performed prior to donor selection, this criterion was not knowingly selected. No other significant differences in clinical or prognostic factors were observed between those transplants using donors encoding Cen-AA or Cen-Bx.

The overall probabilities of survival (38.6%) and relapse (34.5%) were assessed at the five year timepoint, whilst NRM (23.0%) was assessed one year post-transplant. When assessing the impact of the clinical variables on these outcomes of HCT, several factors demonstrated trends and borderline significance with detrimental outcomes. Older recipients (>40 years) had decreased OS at five years post-transplant (p=0.049), as did recipients with a history of previous autografts (p=0.028).

Presence of donor KIR B haplotypes increase incidence of non-relapse mortality

Univariate analysis of the effect of donor KIR haplotypes on the outcomes of HCT associated the presence of donor-encoded KIR B haplotype with an increase in the incidence of NRM after one year post-transplant (KIR AA: 9%, 95% confidence interval [CI]=2.9-26.1 *vs* KIR Bx: 29%, CI=20.6-40.6; p=0.019; Figure 1A, Table 2). This increase in NRM was associated with statistical trends towards decreased OS (KIR AA: 49%, CI=34.5-69.4 *vs* KIR Bx: 34%, CI=25.4-46.6; p=0.06) and DFS (KIR

AA: 46%, CI=32.2-66.9 *vs* KIR Bx: 31%, CI=22.5-43.4; p=0.087) at five years post-transplant. Interestingly, despite most previous analyses implicating KIR-mediated differences in relapse risk, no statistically significant differences were observed in this dataset (Table 2).

Following the observation that the presence of donor KIR B haplotypes was associated with increased NRM probability, donor genotypes were stratified by their Cen and Tel motif patterns. Outcomes in patients receiving HCT from donors encoding the Tel-Bx motif were not associated with any difference when compared to Tel-AA donor transplants (Table 2). Presence of the Cen-B motif within donors, however, was associated with a significant increase in the probability of NRM at one year post-transplant (Cen-AA: 9%, CI=4.0-21.7 *vs* Cen-Bx: 34%, CI=24.4-48.4; p=0.001, Figure 1B). This observation correlated with significantly improved five year OS (Cen-AA: 48%, CI=35.7-63.7 *vs* Cen-Bx: 31%, CI=21.6-45.1; p=0.024) and DFS (Cen-AA: 45%, CI=32.9-60.5 *vs* Cen-Bx: 29%, CI=19.3-42.6; p=0.045, Table 2). In a multivariate regression analysis, the significant difference between outcomes of Cen-AA and Cen-Bx donor transplants was preserved (OS: Cen-Bx hazard ratio [HR]=1.9, CI=1.2-3.1, p=0.01; NRM: Cen-Bx HR=4.2, CI=1.6-11.0, p=0.004, Table 3).

When compared to the Cen-AA motif structure, the impact of each additional Cen-B motif was also assessed. This revealed a dose effect, whereby the more copies of donor-encoded Cen-B motif, the higher the risk of NRM at one year post-transplant (Cen-AA: 9%, CI=4.0-21.7 *vs* Cen-AB: 33%, CI=22.0-48.5 *vs* Cen-BB: 42%, CI=20.5-84.8; p=0.005, Figure 2A). This corresponded with significant differences in OS (Cen-AA: 48%, CI=35.7-63.7 *vs* Cen-AB: 37%, CI=25.7-52.7 *vs* Cen-BB: 8%, CI=1.3-54.4; p=0.01, Figure 2B) and DFS (Cen-AA: 45%, CI=32.9-60.5 *vs* Cen-AB: 34%, CI=22.9-49.8 *vs* Cen-BB: 8%, CI=1.3-54.4; p=0.031, Table 2) at five years post-transplant.

Cause-of-death analysis implicates donor Cen-B with impaired viral protection

To further investigate how donor-encoded centromeric motif structure affects NRM risk, the 27 transplants resulting in NRM were stratified by cause-of-death. Infection was recorded as a cause-of-death in 19 recipients, whilst GVHD was implicated in only five (cause-of-death in one recipient

included both GVHD and infection). One transplant resulted in NRM without infection or GVHD, and data was missing for three further transplants. Accordingly, a competing risk analysis assessing the risk of death by infection at one year between transplants utilising Cen-AA and Cen-Bx donors was performed and revealed a strong protective effect of donor-encoded Cen-AA (Cen-AA: 6%, CI=1.8-17.0 *vs* Cen-Bx: 25%, CI=15.8-38.4; p=0.006). This withstood multivariate analysis as the only remaining statistically significant factor (Cen-Bx: HR=5.5, CI=1.5-20.3, p=0.011, Table 3). Of the 15 instances where data on the type of infection was available, 13 cases (87%) involved viral infection.

Discussion

The relevance of matching between donor and recipient HLA types has been well-documented and is a key determinant of HCT success[3, 4]. However, the KIR genotype of the donor, encoding receptors for these hyperpolymorphic HLA, is not routinely considered in VUD selection. Previous studies in T cell replete MAC cohorts have implicated donor-encoded Cen-B haplotype motif presence with a beneficial reduction in relapse risk, leading to improved OS and DFS[10, 25]. By contrast, the results obtained in this predominantly TCD cohort fail to indicate any beneficial reduction in AML relapse associated with donor-encoded Cen-B motifs, and instead implicate these motifs with increased NRM risk, leading to decreased OS and DFS.

Although our findings contradict these apparently similar studies, the different T cell content between the grafts may be responsible for the conflicting outcomes. These data may support an orchestrated role for NK cell interaction with T cells[33], interpreted as innate NK cells playing a coordinating role for early T cell reconstitution after transplant. This NK cell-T cell interaction is likely to be common to all HCT, but the effects may be more apparent after TCD where T cell function is impaired or delayed. In addition, our findings concur with the study by Kröger *et al.* (2006)[17], whereby a higher number of different activating KIRs encoded by the donor corresponded with increased NRM in a MAC, TCD cohort. Furthermore, another study investigating the effect of TCD on KIR-mediated

immunity following HCT also observed elevated NRM as a result of increased infection-related mortality, theorising the observation as a result of increased targeting of antigen-presenting dendritic cells by activated NK cells[16, 34].

When the cause of death was investigated in the study presented here, infection, particularly viral infection, was strongly associated with increased mortality in Cen-Bx donor transplants, whereas a greater level of protection against infection-related mortality was offered by Cen-AA donors. This, again, contrasts with studies in T cell replete transplants where increasing numbers of activating KIR, and particularly KIR2DS2 (restricted to the Cen-B motif), were demonstrated to aid control of human cytomegalovirus (CMV) reactivation[35]. Viruses, such as CMV, display a range of functions aimed to modulate NK cell reactivity, including the upregulation of expression of the inhibitory ligand, HLA-E[36], as well as sequestration of activating ligands such as major histocompatibility complex class I polypeptide-related sequence B (MICB)[37]. However, viral downregulation of HLA class I antigen expression, as a means of evading T cell-mediated immunity, can also stimulate NK cell activation via the recognition of "missing-self"[38, 39]. Licensed NK cells, which are more functional owing to expression of at least one inhibitory receptor for a host-encoded HLA class I molecule, recognize the lack of inhibition and mount an immune response.

The strong avidity offered by alleles of KIR2DL2/3 commonly located on the Cen-B haplotype motif has been shown to correspond with functionally stronger licensing than KIR2DL2/3 alleles which tend to reside on the Cen-A motif[40, 41]. This increased level of licensing, when tested in cells lines that fail to express any HLA class I on the cell surface, is capable of stimulating an increased response. However, complete absence of HLA class I expression is unlikely to be environmentally plausible during viral infection. As such, presence of high avidity Cen-B KIR2DL2/3 alleles in combination with downregulated HLA-C may actually offer a greater level of inhibition than the equivalent interaction between Cen-A KIR2DL2/3 alleles and downregulated HLA-C. The increased inhibition would require a greater activating signal to supersede it, resulting in decreased NK cell reactivity. In addition, the delayed reconstitution of KIR2DL1 following HCT may place additional burden on

KIR2DL2/3 licensed NK cell immunity[42]. Differential NK cell inhibition via KIR2DL2/3 has also been proposed as a theory to explain the observation that increasing copies of KIR2DL3-HLA-C1 (typically weak avidity interactions) results in improved resolution of hepatitis C virus infection[43, 44]. Additionally, evidence that NK cell education via activating KIRs (such as those which define the Cen-B motif) renders NK cells hyporesponsive may also indicate improved NK cell reactivity associated with the Cen-A haplotype motif[45].

Several limitations to the study mean that the results must be approached with some caution. Although care was taken to maximise cohort homogeneity, the retrospective, multicentre aspect of this study introduces the caveat of variable transplant protocols and presented difficulties in collecting complete clinical follow-up data. In addition, the era of transplants ranged considerably, from 1996 to 2011. Amongst other factors, significant evolution of antiviral and antifungal agents has occurred over this time period. Furthermore, the relatively small sample size and event incidence may be underpowered to resolve some compound variables. The KIR locus itself introduces a range of complexities not accounted for in this study. For example, the highly polymorphic nature of each KIR gene introduces variety in the expression and functionality of each locus. The implementation of high resolution, allelic-level KIR typing is warranted to resolve these issues in the future[46]. Finally, the scope of this analysis has been limited to only investigate the KIR-mediated aspect of immunity, ignoring other NK cell receptor-ligand signalling pathways and alloreactivity mediated by T and B cells. Future, well-defined prospective studies using uniform transplant conditions may help to clarify the effects of the combinations of donor KIR and recipient ligands on HCT outcomes.

In summary, we have demonstrated that donor-encoded KIR genes can affect the NRM risk following VUD HCT. Specifically, the presence of donor-encoded Cen-B haplotype motifs conveys a significant risk of infectious mortality, which in turn equates to a significant reduction in OS. Multivariate analysis adjusting for other transplant characteristics suggested that donor KIR centromeric genotype was the only significant determinant for NRM risk. However, these findings may only be applicable to cases of HLA-matched, unrelated donor, MAC, TCD transplants to treat

adult AML, as differing HCT scenarios have repeatedly generated contradictory findings, including observations in our own TCD, RIC cohort (unpublished data). This highlights the important differences between transplant scenarios and suggests that, when selecting donors based on KIR genotype information, it is unlikely that a 'one-size-fits-all' donor KIR genotype exists. Instead, these findings support the selection of VUDs based on KIR genotype, but only when considered in parallel with other transplant factors.

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Table 1 – Recipient and donor demographics

Variable	Donor KIR Cen-AA	%	Donor KIR Cen-BX	%	P-value
Donor age, years					
Median (Range)	34 (20-49)		35 (19-60)		0.88
≤30	17	31.5	22	33.8	0.94
>30	37	68.5	43	66.2	
Recipient age, years					
Median (Range)	34 (18-64)		37 (18-67)		0.17
≤40	40	74.1	45	69.2	0.71
>40	14	25.9	20	30.8	
Donor sex					
Female	10	18.5	7	10.8	0.35
Male	44	81.5	58	89.2	
Recipient sex					
Female	22	40.7	24	36.9	0.81
Male	32	59.3	41	63.1	
Recipient-donor sex matching					
Matched	26	48.1	44	67.7	*0.049*
Mismatched	28	51.9	21	32.3	
Recipient-donor CMV					
Matched	43	79.6	48	73.8	0.57
Mismatched	10	18.5	16	24.6	
Missing	1	1.9	1	1.5	
Donor positive, Recipient positive	9	16.7	6	9.2	0.32
Donor positive, Recipient negative	0	0.0	4	6.2	
Donor negative, Recipient positive	10	18.5	12	18.5	
Donor negative, Recipient negative	34	63.0	42	64.6	
Missing	1	1.9	1	1.5	
Transplant era					
1996-1999	9	16.7	6	9.2	0.69
2000-2003	19	35.2	25	38.5	
2004-2007	17	31.5	22	33.8	
2008-2011	9	16.7	12	18.5	
T cell deplete					
Yes	43	79.6	54	83.1	0.41
No	4	7.4	2	3.1	
Missing	7	13.0	9	13.8	
Disease risk – EBMT score					
Good	19	35.2	32	49.2	0.20
Intermediate/Poor	34	63.0	33	50.8	
Missing	1	1.9	0	0.0	
Stem cell source					
BM	26	48.1	28	43.1	0.71
PBSC	28	51.9	37	56.9	
Previous autografts					
0	50	92.6	62	95.4	0.70
≥1	4	7.4	3	4.6	

CMV = Cytomegalovirus, BM = bone marrow, PBSC = peripheral blood stem cells.

Categorical variables were compared by Chi-squared test (or Fisher's Exact test when n≤5 for any subgroup). Continuous variables were compared by Mann-Whitney test. Statistically significant p-values are denoted in *italics*.

Table 2 – Univariate analyses of recipient and donor factors on OS, relapse, DFS and NRM

Variable	Valid cases (n)	5 year OS		5 year relapse[§]		5 year DFS[§]		1 year NRM[§]	
		%	P-value	%	P-value	%	P-value	%	P-value
Donor age, years									
<30	39	42.2	0.67	24.2	0.12	42.9	0.37	28.6	0.36
>30	80	37.2		39.2		32.6		20.2	
Recipient age, years									
<40	85	42.6	*0.049*	34.3	0.79	38.4	0.083	19.2	0.097
>40	34	28.5		35.3		29.1		32.4	
Donor sex									
Female	17	35.9	0.99	43.7	0.66	26.9	0.53	29.4	0.49
Male	102	38.8		33.1		37.3		21.9	
Recipient sex									
Female	46	39.0	0.97	37.9	0.47	32.5	0.59	19.8	0.51
Male	73	38.3		32.3		37.9		25.0	
Recipient-donor sex matching									
Matched	70	41.4	0.41	35.4	0.86	38.0	0.54	21.7	0.69
Mismatched	49	34.6		33.3		32.6		24.7	
Recipient-donor CMV matching									
Matched	91	40.8	0.17	32.8	0.33	38.2	0.14	21.1	0.52
Mismatched	26	29.4		43.5		25.4		26.9	
Transplant era									
1996-1999	15	60.0	0.45	28.6	*0.049*	50.0	0.60	21.4	0.11
2000-2003	44	34.1		50.0		31.8		13.6	
2004-2007	39	35.6		20.5		33.1		35.9	
2008-2011[†]	21	38.6		31.2		40.7		19.9	
T cell deplete									
Yes	97	37.5	0.28	34.0	0.46	34.9	0.22	24.1	0.63
No	6	66.7		16.7		66.7		16.7	
Disease risk – EBMT score									
Good	51	36.7	0.89	26.7	0.12	31.2	0.72	28.0	0.30
Intermediate/Poor	67	39.3		40.8		38.1		19.6	
Stem cell source									
BM	54	46.0	0.13	37.7	0.59	39.5	0.49	18.9	0.41
PBSC	65	31.88		31.6		32.1		26.4	
Previous autografts									
0	112	40.1	*0.028*	34.0	0.62	37.2	0.063	21.7	0.18
≥1	7	14.3		42.9		14.3		42.9	
Donor KIR genotype									
KIR AA	35	48.9	0.060	38.7	0.60	46.5	0.087	8.7	*0.019*
KIR BX	84	34.4		32.8		31.3		28.9	
Donor Tel motif pattern									
Tel-AA	74	36.2	0.42	33.6	0.77	34.2	0.47	27.6	0.13
Tel-BX	45	42.3		36.1		38.2		15.6	
Donor Cen motif pattern									
Cen-AA	54	47.7	*0.024*	38.0	0.45	44.6	*0.045*	9.3	*0.001*
Cen-BX	65	31.2		31.5		28.6		34.4	
Cen-AA	54	47.7		38.0		44.6		9.3	
Cen-AB	53	36.8	*0.010*	31.2	0.75	33.7	*0.031*	32.7	*0.005*
Cen-BB	12	8.3		33.3		8.3		41.7	

[§] NRM/DFS/Relapse data missing for one transplant.

[†] Estimated incidence of OS, relapse and DFS at latest clinical follow-up (4 years) reported.

Statistically significant results (≤0.05) are italicized. OS = Overall survival, NRM = Non-relapse mortality, CMV = Cytomegalovirus, BM = bone marrow, PBSC = peripheral blood stem cells

Table 3 – Multivariate analysis of OS, NRM and death by infection

Variable	5 year OS		1 year NRM†		1 year death by infection†‡	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Recipient age, years						
<40	1.00	-	1.00	-	1.00	-
>40	1.91 (1.15-3.16)	*0.012*	1.81 (0.82-4.01)	0.15	2.28 (0.91-5.69)	0.078
Transplant era						
1996-1999					1.00	-
2000-2003					1.15 (0.15-8.99)	0.89
2004-2007					5.27 (0.84-32.9)	0.075
2008-2011					0.74 (0.05-9.93)	0.82
Previous autografts						
0	1.00	-	1.00	-		
≥1	3.05 (1.30-7.15)	*0.010*	2.45 (0.55-10.92)	0.24		
Donor Cen motif pattern						
Cen-AA	1.00	-	1.00	-	1.00	-
Cen-BX	1.90 (1.17-3.10)	*0.010*	4.16 (1.58-11.00)	*0.004*	5.50 (1.49-20.32)	*0.011*

Statistically significant results (≤0.05) are italicized. OS = Overall survival, NRM = Non-relapse mortality

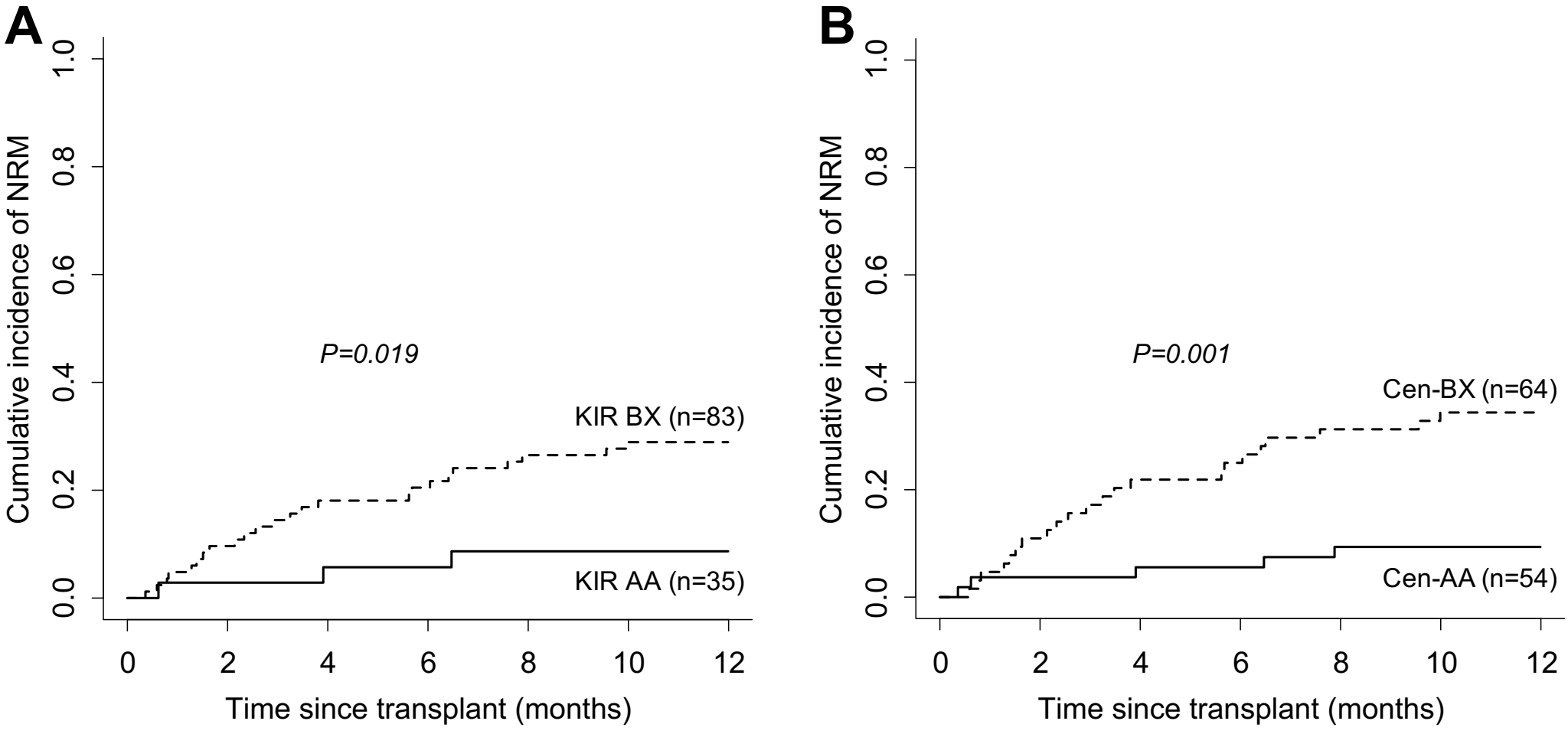
† NRM data missing for one transplant.

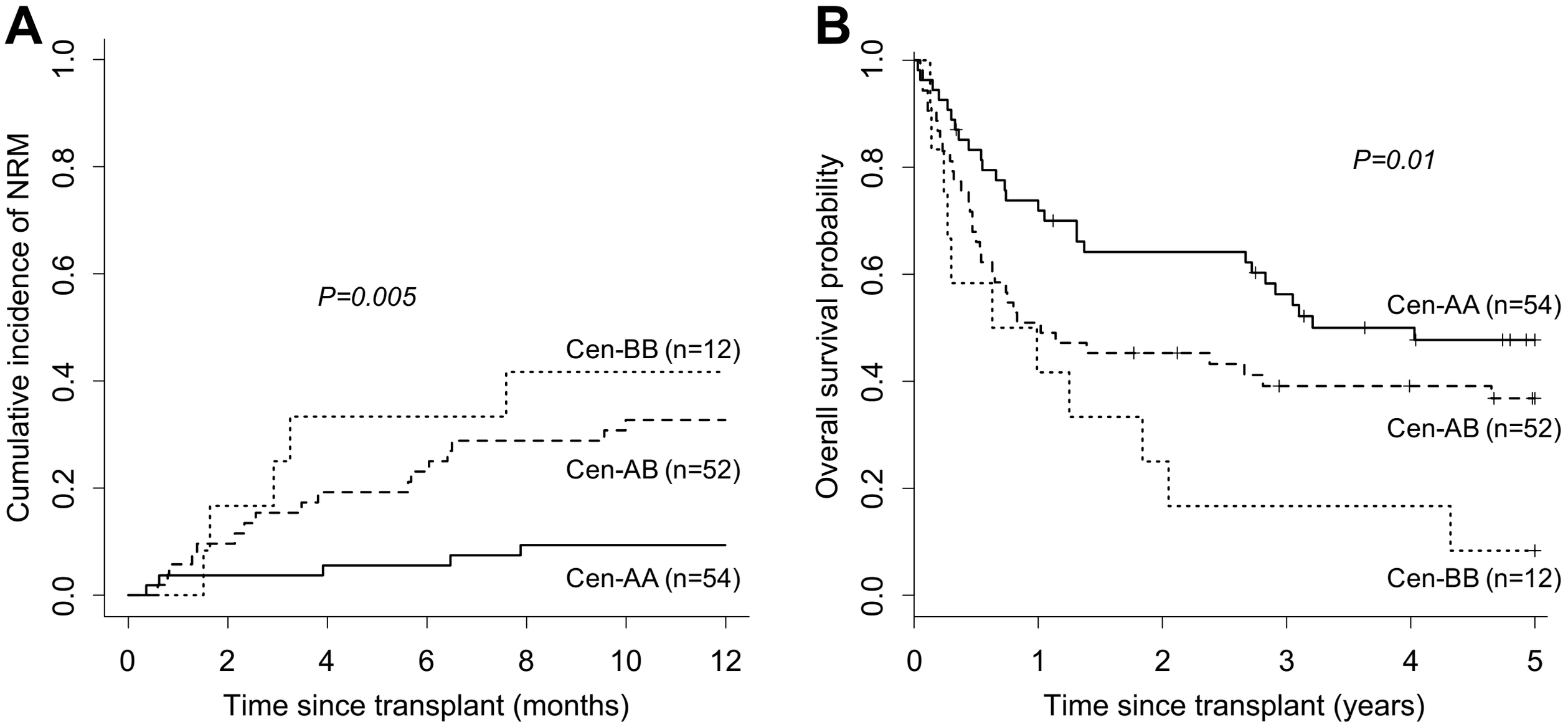
‡ Cause-of-death data missing for three transplants.

Figure legends

Figure 1: Donor KIR B genotype increases NRM. A) Univariate probability of NRM at one year post-transplant for groups based on the presence of at least one donor-encoded KIR B haplotype. This demonstrates that a significant increase in NRM is associated with donors encoding the KIR BX haplotype structure. B) When the haplotype structure is refined according to centromeric motif structure, donor-encoded Cen-B appears culpable for the increase in NRM.

Figure 2: Effect of donor Cen-B is dose-dependent. A) Univariate probability of NRM at one year post-transplant for groups based on donor-encoded Cen-B motif copy number. With each additional Cen-B motif, risk of NRM increases. B) When OS is assessed with the same grouping strategy, the detrimental effect of donor Cen-B is also evident.


A
Cumulative incidence of NRM
0.0
0.2
0.4
0.6
0.8
1.0
P=0.019
KIR BX (n=83)
KIR AA (n=35)
0
2
4
6
8
10
12
Time since transplant (months)
B
Cumulative incidence of NRM
0.0
0.2
0.4
0.6
0.8
1.0
P=0.001
Cen-BX (n=64)
Cen-AA (n=54)
0
2
4
6
8
10
12
Time since transplant (months)


A
Cumulative incidence of NRM
0.0
0.2
0.4
0.6
0.8
1.0
P=0.005
Cen-BB (n=12)
Cen-AB (n=52)
Cen-AA (n=54)
0
2
4
6
8
10
12
Time since transplant (months)
B
Overall survival probability
0.0
0.2
0.4
0.6
0.8
1.0
P=0.01
Cen-AA (n=54)
Cen-AB (n=52)
Cen-BB (n=12)
0
1
2
3
4
5
Time since transplant (years)