

1 **Title**

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

4

5 **Running title**

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

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24

1 **Abstract**

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

16

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20

21 **Author statement**

22 None of the authors declare any conflicts of interest.

23

1 **Introduction**

2 Despite developments in the treatment of patients with haematological malignancies to specifically
3 target diseased cells, achieving long term remission in adult acute myeloid leukaemia (AML) remains
4 challenging and haematopoietic cell transplantation (HCT) continues as the mainstay of treatment for
5 high risk patients¹. Selection of volunteer unrelated donors (VUD) for allogeneic HCT is primarily
6 based on HLA allele matching at the HLA-A, -B, -C, -DRB1 and -DQB1 loci, although many centres
7 have also recently adopted a permissible matching model including the HLA-DPB1 locus²⁻⁵.
8 However, even in recipients of well-matched grafts, five year overall survival (OS) remains <50%,
9 with both relapse and death from transplant-related complications remaining significant problems^{1, 6}.
10 As such, investigation into secondary donor characteristics have been performed and confirmed the
11 importance of non-HLA factors, particularly donor age and CMV matching, in reducing non-relapse
12 mortality (NRM)^{4, 7, 8}.

13
14 In addition to these secondary donor characteristics, selection of donors for non-HLA genetic factors
15 has also been explored as a method to improve HCT outcomes. The Killer-cell Immunoglobulin-like
16 Receptors (KIR), predominantly expressed on the surface of natural killer (NK) cells, are amongst
17 the most promising non-HLA candidate gene families. KIR form a family of activating and inhibitory
18 receptors which, upon binding their cognate HLA ligand, may elicit, or inhibit, an immune response.
19 The genes encoding these proteins can be grouped into two main haplotypes: KIR A haplotypes are
20 conserved in gene content and encode only one activating KIR gene (KIR2DS4) in combination with
21 multiple inhibitory genes (KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DL2 and KIR3DL3). By
22 contrast, KIR B haplotypes have a more variable gene content and encode at least one of the
23 alternative KIR genes⁹. In addition, KIR haplotypes may be further defined according to their
24 centromeric (Cen) or telomeric (Tel) gene motifs¹⁰.

25
26 The relevance of KIR-mediated immunity in HCT to treat AML was first discovered by investigating
27 disparity between donor and recipient inhibitory KIR ligands, subsets of HLA class I molecules

1 encoding the HLA-C1, -C2 and -Bw4 motifs, in haploidentical T cell-depleted (TCD)
2 transplantations¹¹. Ruggeri *et al.* (2002)¹², demonstrated protection from disease relapse without
3 concurrent increase in frequency of graft *versus* host disease (GVHD) in AML recipients whose grafts
4 were derived from donors possessing KIR ligands that were not present in the recipient, often referred
5 to as “missing self”. As such, they proposed that graft *versus* leukaemia (GVL) alloreactivity could be
6 mediated by donor NK cells when KIR ligand disparity was present. Importantly, this effect appeared
7 to be limited to AML recipients as the same effect was not observed in acute lymphoblastic leukaemia
8 (ALL) patients. Following this, several studies have confirmed this model in haploidentical and other
9 HLA-mismatched allogeneic transplant settings^{13, 14}.

10

11 In addition to relapse and GVHD, infection remains a major contributor to the high mortality rates
12 associated with HCT. In addition to *de novo* infections acquired during the extended periods of
13 immunosuppression, viral reactivation is also a common cause of morbidity and mortality. In the UK,
14 frequent use of TCD as GVHD prophylaxis, often utilising alemtuzumab, may exacerbate this issue¹⁵.
15 NK cells are the first lymphocyte subset to reconstitute following HCT and are known to target
16 virally-infected cells. However, NK cell reactivity resulting from KIR-ligand mismatching has, in
17 contrast to its findings in relapse, been proposed to increase patients’ susceptibility to
18 infection-related mortality^{16, 17}.

19

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

27

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

11

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

17

18 **Materials and Methods**

19 *Study cohort*

20 One hundred and nineteen HCT recipients and their respective VUDs were included in this study. All
21 transplants took place between December 1996 and June 2011. Transplant inclusion criteria were as
22 follows: i) UK-based adult transplanted to treat AML, ii) MAC regimen, iii) stem cells provided from
23 an Anthony Nolan VUD and iv) complete allele-level HLA matching for HLA-A, -B, -C, -DRB1 and
24 -DQB1, as described previously²⁶. Clinical outcomes data were obtained in collaboration with the
25 British Society of Blood and Marrow Transplantation. Ethical approval was obtained from the
26 National Research Ethics Service (www.nres.nhs.uk, application number: MREC 01/8/31). The

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

3 4 *DNA extraction*

5 Genomic DNA was extracted from whole blood or buccal swab samples. When extracted from blood,
6 DNA was obtained either from salting-out²⁷ or paramagnetic bead-based DNA purification (Promega,
7 Madison, WI, USA). When extracted from buccal swabs, DNA was obtained using Gentra Puregene
8 Buccal Cell Kit (QIAGEN, Hilden, Germany).

9 10 *KIR genotyping*

11 Briefly, presence or absence of 16 individual KIR genes was analysed using a polymerase chain
12 reaction sequence-specific priming (PCR-SSP) approach described previously²⁸. No distinction was
13 made between the presence of KIR2DL5A or KIR2DL5B. The presence of at least one KIR B
14 haplotype-specific locus indicated that the genotype contained at least one B haplotype. Such samples
15 were depicted as KIR Bx. All samples that lacked the presence of all KIR B loci were assigned the
16 AA genotype designation (KIR AA). Centromeric (Cen) and telomeric (Tel) gene motifs were
17 assigned as described previously¹⁰. HLA-C1, -C2 and -Bw4 epitope ligands for KIR molecules were
18 inferred from previous HLA typing.

19 20 *Statistical analysis*

21 Survival and DFS probability curves were calculated by the method of Kaplan-Meier²⁹. Groups were
22 compared using the log-rank test, whilst multivariate analysis was performed by Cox regression³⁰.
23 Several analyses incurred competing risks. The competing risk in relapse analysis was non-relapse
24 mortality (NRM), whilst relapse was the competing risk in NRM analysis. When comparing the risk
25 of infectious mortality between different groups, relapse or death due to any other cause were the
26 competing risks. For these competing risk analyses, univariate probabilities were calculated using the
27 cumulative incidence function³¹. Multivariate competing risk analysis was performed using the
28 method by Fine and Gray³². A forward stepwise selection of covariates for multivariate analysis was

1 performed using $p \leq 0.05$ inclusion criteria. Statistical significance was denoted at $p \leq 0.05$, whilst
2 statistical trend was signified by $p \leq 0.1$. All univariate and multivariate analyses were performed using
3 ‘R’ software (version 3.4.2).

4 5 **Results**

6 *Patient and donor characteristics*

7 Donor and recipient demographics and HCT conditions are given in Table 1. Of the 84 donors
8 encoding at least one KIR B haplotype, 65 encoded at least one Cen-B motif (Cen-Bx). The remaining
9 54 donors (45%) encoded only Cen-A haplotype motifs (Cen-AA). When comparing the Cen-AA and
10 Cen-Bx donor groups, the only statistically significant difference was between donor-recipient gender
11 matching, by which gender-matched transplants were more likely to utilise Cen-Bx donors. As donor
12 KIR genotyping was not performed prior to donor selection, this criterion was not knowingly selected.
13 No other significant differences in clinical or prognostic factors were observed between those
14 transplants using donors encoding Cen-AA or Cen-Bx.

15
16 The overall probabilities of survival (38.6%) and relapse (34.5%) were assessed at the five year
17 timepoint, whilst NRM (23.0%) was assessed one year post-transplant. When assessing the impact of
18 the clinical variables on these outcomes of HCT, several factors demonstrated trends and borderline
19 significance with detrimental outcomes. Older recipients (>40 years) had decreased OS at five years
20 post-transplant ($p=0.049$), as did recipients with a history of previous autografts ($p=0.028$).

21 22 *Presence of donor KIR B haplotypes increase incidence of non-relapse mortality*

23 Univariate analysis of the effect of donor KIR haplotypes on the outcomes of HCT associated the
24 presence of donor-encoded KIR B haplotype with an increase in the incidence of NRM after one year
25 post-transplant (KIR AA: 9%, 95% confidence interval [CI]=2.9-26.1 vs KIR Bx: 29%, CI=20.6-40.6;
26 $p=0.019$; Figure 1A, Table 2). This increase in NRM was associated with statistical trends towards
27 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

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

4
5 Following the observation that the presence of donor KIR B haplotypes was associated with increased
6 NRM probability, donor genotypes were stratified by their Cen and Tel motif patterns. Outcomes in
7 patients receiving HCT from donors encoding the Tel-Bx motif were not associated with any
8 difference when compared to Tel-AA donor transplants (Table 2). Presence of the Cen-B motif within
9 donors, however, was associated with a significant increase in the probability of NRM at one year
10 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
11 observation correlated with significantly improved five year OS (Cen-AA: 48%, CI=35.7-63.7 vs
12 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%,
13 CI=19.3-42.6; p=0.045, Table 2). In a multivariate regression analysis, the significant difference
14 between outcomes of Cen-AA and Cen-Bx donor transplants was preserved (OS: Cen-Bx hazard ratio
15 [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).

16
17 When compared to the Cen-AA motif structure, the impact of each additional Cen-B motif was also
18 assessed. This revealed a dose effect, whereby the more copies of donor-encoded Cen-B motif, the
19 higher the risk of NRM at one year post-transplant (Cen-AA: 9%, CI=4.0-21.7 vs Cen-AB: 33%,
20 CI=22.0-48.5 vs Cen-BB: 42%, CI=20.5-84.8; p=0.005, Figure 2A). This corresponded with
21 significant differences in OS (Cen-AA: 48%, CI=35.7-63.7 vs Cen-AB: 37%, CI=25.7-52.7 vs
22 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:
23 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.

24 25 *Cause-of-death analysis implicates donor Cen-B with impaired viral protection*

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

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

9

10 **Discussion**

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

19

20 Although our findings contradict these apparently similar studies, the different T cell content between
21 the grafts may be responsible for the conflicting outcomes. These data may support an orchestrated
22 role for NK cell interaction with T cells³³, interpreted as innate NK cells playing a coordinating role
23 for early T cell reconstitution after transplant. This NK cell-T cell interaction is likely to be common
24 to all HCT, but the effects may be more apparent after TCD where T cell function is impaired or
25 delayed. In addition, our findings concur with the study by Kröger *et al.* (2006)¹⁷, whereby a higher
26 number of different activating KIRs encoded by the donor corresponded with increased NRM in a
27 MAC, TCD cohort. Furthermore, another study investigating the effect of TCD on KIR-mediated

1 immunity following HCT also observed elevated NRM as a result of increased infection-related
2 mortality, theorising the observation as a result of increased targeting of antigen-presenting dendritic
3 cells by activated NK cells^{16, 34}.

4
5 When the cause of death was investigated in the study presented here, infection, particularly viral
6 infection, was strongly associated with increased mortality in Cen-Bx donor transplants, whereas a
7 greater level of protection against infection-related mortality was offered by Cen-AA donors. This,
8 again, contrasts with studies in T cell replete transplants where increasing numbers of activating KIR,
9 and particularly KIR2DS2 (restricted to the Cen-B motif), were demonstrated to aid control of human
10 cytomegalovirus (CMV) reactivation³⁵. Viruses, such as CMV, display a range of functions aimed to
11 modulate NK cell reactivity, including the upregulation of expression of the inhibitory ligand,
12 HLA-E³⁶, as well as sequestration of activating ligands such as major histocompatibility complex
13 class I polypeptide-related sequence B (MICB)³⁷. However, viral downregulation of HLA class I
14 antigen expression, as a means of evading T cell-mediated immunity, can also stimulate NK cell
15 activation via the recognition of “missing-self”^{38, 39}. Licensed NK cells, which are more functional
16 owing to expression of at least one inhibitory receptor for a host-encoded HLA class I molecule,
17 recognize the lack of inhibition and mount an immune response.

18
19 The strong avidity offered by alleles of KIR2DL2/3 commonly located on the Cen-B haplotype motif
20 has been shown to correspond with functionally stronger licensing than KIR2DL2/3 alleles which
21 tend to reside on the Cen-A motif^{40, 41}. This increased level of licensing, when tested in cells lines that
22 fail to express any HLA class I on the cell surface, is capable of stimulating an increased response.
23 However, complete absence of HLA class I expression is unlikely to be environmentally plausible
24 during viral infection. As such, presence of high avidity Cen-B KIR2DL2/3 alleles in combination
25 with downregulated HLA-C may actually offer a greater level of inhibition than the equivalent
26 interaction between Cen-A KIR2DL2/3 alleles and downregulated HLA-C. The increased inhibition
27 would require a greater activating signal to supersede it, resulting in decreased NK cell reactivity. In
28 addition, the delayed reconstitution of KIR2DL1 following HCT may place additional burden on

1 KIR2DL2/3 licensed NK cell immunity⁴². Differential NK cell inhibition via KIR2DL2/3 has also
2 been proposed as a theory to explain the observation that increasing copies of KIR2DL3-HLA-C1
3 (typically weak avidity interactions) results in improved resolution of hepatitis C virus infection^{43, 44}.
4 Additionally, evidence that NK cell education via activating KIRs (such as those which define the
5 Cen-B motif) renders NK cells hyporesponsive may also indicate improved NK cell reactivity
6 associated with the Cen-A haplotype motif⁴⁵.

7
8 Several limitations to the study mean that the results must be approached with some caution.
9 Although care was taken to maximise cohort homogeneity, the retrospective, multicentre aspect of
10 this study introduces the caveat of variable transplant protocols and presented difficulties in collecting
11 complete clinical follow-up data. In addition, the era of transplants ranged considerably, from 1996 to
12 2011. Amongst other factors, significant evolution of antiviral and antifungal agents has occurred
13 over this time period. Furthermore, the relatively small sample size and event incidence may be
14 underpowered to resolve some compound variables. The KIR locus itself introduces a range of
15 complexities not accounted for in this study. For example, the highly polymorphic nature of each KIR
16 gene introduces variety in the expression and functionality of each locus. The implementation of high
17 resolution, allelic-level KIR typing is warranted to resolve these issues in the future⁴⁶. Finally, the
18 scope of this analysis has been limited to only investigate the KIR-mediated aspect of immunity,
19 ignoring other NK cell receptor-ligand signalling pathways and alloreactivity mediated by T and B
20 cells. Future, well-defined prospective studies using uniform transplant conditions may help to clarify
21 the effects of the combinations of donor KIR and recipient ligands on HCT outcomes.

22
23 In summary, we have demonstrated that donor-encoded KIR genes can affect the NRM risk following
24 VUD HCT. Specifically, the presence of donor-encoded Cen-B haplotype motifs conveys a
25 significant risk of infectious mortality, which in turn equates to a significant reduction in OS.
26 Multivariate analysis adjusting for other transplant characteristics suggested that donor KIR
27 centromeric genotype was the only significant determinant for NRM risk. However, these findings
28 may only be applicable to cases of HLA-matched, unrelated donor, MAC, TCD transplants to treat

1 adult AML, as differing HCT scenarios have repeatedly generated contradictory findings, including
2 observations in our own TCD, RIC cohort (unpublished data). This highlights the important
3 differences between transplant scenarios and suggests that, when selecting donors based on KIR
4 genotype information, it is unlikely that a 'one-size-fits-all' donor KIR genotype exists. Instead, these
5 findings support the selection of VUDs based on KIR genotype, but only when considered in parallel
6 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	<i>0.049</i>
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	0.010	38.0	0.75	44.6	0.031	9.3	0.005	
Cen-AB	53	36.8		31.2		33.7		32.7		
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)	<i>0.012</i>	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)	<i>0.010</i>	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)	<i>0.010</i>	4.16 (1.58-11.00)	<i>0.004</i>	5.50 (1.49-20.32)	<i>0.011</i>

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.



