Activating KIR Haplotype Influences Clinical Outcome Following HLA-Matched Sibling Hematopoietic Stem Cell Transplantation.

Susan L Heatley [1,2+], Charles G Mullighan [3], Kathleen Doherty [2,#], Silke Danner [2,4], Geraldine M O'Connor[1~], Uwe Hahn [4], Jeff Szer [1,5], Anthony Schwarer [6,^], Kenneth Bradstock [7], Lucy C Sullivan [1], Peter G Bardy [2,4 *] & Andrew G Brooks [1*].

* Joint senior and corresponding authors

1. University of Melbourne, Melbourne, Vic, Australia
2. Australian Red Cross Blood Service, Adelaide, SA, Australia
3. St Jude Children's Research Hospital, Memphis, TN, USA
4. Royal Adelaide and Queen Elizabeth Hospitals, SA Pathology, Adelaide, SA, Australia
5. Royal Melbourne Hospital, Melbourne, Vic, Australia
6. Alfred Hospital, Melbourne, Vic, Australia
7. Westmead Hospital, Sydney, NSW, Australia

Current address: [+]Cancer Theme, South Australian Health & Medical Research Institute, Adelaide, Australia, [#] University of Tasmania, Hobart, Australia, ^Eastern Health Monash University Clinical School, Melbourne, Australia, [~] University of Chester, Chester CH1 4BJ, UK.

Corresponding authors:
Professor Andrew Brooks
Head, Department of Microbiology and Immunology
Peter Doherty Institute for Infection and Immunity
University of Melbourne
729 Elizabeth Street, Melbourne, Victoria, 3000
Australia

Tel: +61 3 8344 9925
E: agbrooks@unimelb.edu.au

A/Professor Peter Bardy
Clinical Haematologist
Royal Adelaide Hospital
Frome Road, Adelaide, South Australia, 5000
Australia

Tel: +61 8 8222 4000
E: peter.bardy@sa.gov.au

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Abstract

Natural killer cells are thought to influence the outcome of hematopoietic stem cell transplant (HSCT), impacting on relapse, overall survival, graft versus host disease and the control of infection, in part through the complex interplay

between the large and genetically diverse killer immunoglobulin-like receptor (KIR) family and their ligands. This study examined the relationship between KIR gene content and clinical outcomes including the control of opportunistic infections such as cytomegalovirus in the setting of human leucocyte antigen (HLA)-matched sibling HSCT in an Australian cohort. The presence of the KIR B haplotype which contain more activating receptors in the donor, in particular centromeric B haplotype genes (Cen-B), was associated with improved overall survival of patients with acute myeloid leukemia (AML) undergoing sibling HSCT and receiving myeloablative conditioning. Donor Cen-B haplotype was also associated with reduced acute graft versus host disease grades II-IV whereas donor telomeric-B haplotype was associated with decreased incidence of CMV reactivation. In contrast, we were not able to demonstrate a reduced rate of relapse when the donor had KIR Cen-B, however relapse with a donor Cen-A haplotype was a competing risk factor to poor overall survival. Here we show that the presence of donor activating KIR led to improved outcome for the patient, potentially through reduced relapse rates and decreased incidence of acute GvHD translating to improved overall survival.

Introduction

Natural killer (NK) cells play a crucial a role in the control and elimination of tumours. Consistent with a role in anti-tumour immunity, studies have shown that antibody-mediated depletion of NK cells can result in increased tumour

burdens, typically in the context of transplantation.[1-3] The activation of NK cells is controlled by a balance of activating and inhibitory signals that are transduced by an array of cell surface receptors many of which interact with Major Histocompatibility Class I (MHC-I) molecules.[4]

In humans, NK cell surveillance of the expression of classical HLA class I proteins is largely mediated by killer cell immunoglobulin-like receptors (KIR), a large family of polymorphic receptors. The gene complex encoding KIR is found on chromosome 19q13.4 within the Leukocyte Receptor Complex consisting of 14 genes and 2 pseudogenes. At a population level, this complex is highly diverse with considerable variability in the gene content of KIR haplotypes as well as significant polymorphism within individual KIR genes.[5,6]

The presence or absence of clusters of particular KIR genes has been used to define two broad haplotypes termed A and B. The A haplotypes have a relatively well-defined set of KIR genes including KIR2DL1, -2DL3, -3DL1 and -2DS4 of which only one, KIR2DS4 is an activating receptor. In contrast B haplotypes are far more varied in terms of gene content but typically possess more genes encoding activating receptors than A haplotypes. B haplotype genes include KIR2DS1, -2DS2, -2DS3, -2DS5, -2DL2, -2DL5 and -3DS1, which may be in various combinations due to reciprocal recombination events.[4,7,8]

In an attempt to understand how KIR/HLA interactions impact on the control of infection or cancer, numerous studies have correlated allotypic variation of either HLA class I or KIR with clinical outcomes, most notably perhaps in the setting of haematological malignancy. Ruggeri *et al* first observed that mismatching of KIR ligands between donor and recipient was associated with improved outcomes in patients receiving haploidentical hematopoietic stem cell transplantation (HSCT) for acute myeloid leukemia (AML).[3,9] These data suggested that the lack of KIR ligands in transplant recipients resulted in reduced rates of relapse and graft versus host disease (GvHD). Further studies examining recipient and donor KIR haplotype showed evidence of protection against severe GvHD and improved survival in HLA-matched sibling transplants and unrelated HSCT when the donor had the B haplotype or presence of activating KIR.[10-13] Activating KIR and KIR B haplotypes have also been implicated in the role of NK cell mediated control of cytomegalovirus (CMV) reactivation in HSCT, a major cause of morbidity.[14,15]

While two broad types of haplotype have been identified, in an effort to identify the genes associated with clinical outcomes, a number of studies have focussed on centromeric (Cen) or telomeric (Tel) clusters of genes belonging to either the A or B haplotype. This further defines the KIR repertoire, as inhibitory receptors that recognise HLA-C C1 and C2 (based upon on the amino acid present at positions 77 and 80) are located in the centromeric region whereas those that recognise Bw4 and A3/11 are within the telomeric region.[11,16] Indeed using this approach patients receiving transplants from donors possessing clusters of centromeric genes from B

haplotypes (Cen-B) have improved outcomes following unrelated HSCT than individuals that lack this cluster.[11,17,18] Furthermore, renal transplant recipients that possess the telomeric cluster of the B genes (Tel-B) have been reported to maintain better control of CMV replication.[19]

Many previous studies have been retrospective reports of myeloablative transplants. Non-myeloablative preparative regimens are often used for older patients and it is expected that the early balance between recipient and donor immunity will be markedly different from that in the myeloablative setting. In the study presented here, we have examined the relationship between KIR haplotype and the outcome of T-replete HLA-matched sibling HSCT in an Australian cohort. We found improved survival, less aGVHD and decreased reactivation of CMV of patients when their donor had a B haplotype. This cohort was collected prospectively and includes patients that received myeloablative and reduced intensity conditioning.

Methods

Patient Demographics

A total of 152 donor and recipient siblings were recruited from the Royal Adelaide Hospital, Adelaide, the Royal Melbourne and The Alfred Hospitals, Melbourne and Westmead Hospital in Sydney over the period 2002 - 2007. The study was approved by the human institutional review boards of all participating institutions. All donors and recipients provided informed consent to the study.

KIR Genotyping

DNA was extracted from 10 ml whole blood EDTA anticoagulated peripheral blood samples, taken prior to the commencement of therapy, using a modified salting out method.[20] KIR genotyping was performed by PCR using multiplex sequence specific primers as previously described.[21] KIR haplotypes were classified as the A haplotype carrying the KIR2DL1, -2DL3, -3DL1 and -2DS4 genes, all other combinations were denoted as the B haplotype. Combinations of KIR haplotype were assigned according to donor and then recipient haplotype (D-R), where Bx included BB and BA. Donor centromeric and telomeric haplotypes were assigned as previously described.[11,22] Briefly, KIR2DS2, -2DL1, -2DL2 and -2DL3 were considered centromeric and KIR3DL1/S1, -2DS5, -2DS1 and -2DS4 were considered telomeric. Combinations of these gave the haplotypes as centromere (Cen) or telomere (Tel) AA, BA or BB. As only 4 donors had the Cen BB haplotype and 10 with Tel BB, the BA and BB haplotypes were combined and analysed as Bx.

Clinical data was reviewed to determine day of death, relapse, CMV reactivation, GvHD or last known outpatient appointment. Data was analysed by GraphPad Prism 6 using Kaplan-Meier survival curves and compared using the Log Rank (Mantel-Cox) test. The result was considered significant if $P<0.05$. The Fine and Gray statistical test implemented in R version 3.4.1 (cmprsk package, version 2.2-7) was used to examine associations between haplotypes, competing risks and the cumulative incidence of death.[23] Multivariate analysis for relapse free survival was performed using Cox-proportional hazard model implemented in R version 3.4.1 (survival package, coxph function, version 2.41-3).

Results

Transplant Characteristics

Patients were consecutively recruited to the study and considered eligible if they were undergoing HSCT for hematological malignancies or severe aplastic anaemia and over 18 years of age. Patients who were undergoing T-depleted HSCT or were Hepatitis B, C or HIV positive by serology or DNA testing were excluded.

Transplant characteristics of those with complete clinical data (n=145) are summarised in Table 1. Ninety-five men and 50 women received hematopoietic stem cell transplants donated by an HLA-matched sibling. Eighty-five patients received myeloablative conditioning and 60 reduced intensity conditioning (RIC). The majority of patients received peripheral blood as the source of the stem cells and all were T-cell replete with the median time of follow-up being 2 years (9 days - 4.1 years). Of the 145 patients, 69 (47%) had been diagnosed with AML, the second largest group were those diagnosed with Non-Hodgkin Lymphoma (13%).

Seventy-eight percent of recipients (n=114) received GvHD prophylaxis for consisting of cyclosporine A/tacrolimus and short course methotrexate (MTX). CMV management involved either a prophylactic strategy using gancyclovir or oral valacyclovir or a pre-emptive strategy, involving weekly monitoring of blood for CMV DNA and treatment with gancyclovir or foscarnet where appropriate.

Multivariate analysis did not reveal any significant associations with relapse free survival when disease diagnosis, age at transplant, CMV reactivation or GvHD were considered (Supplementary Figure 1). There was a trend towards more males and the type of conditioning (RIC) received and relapse, however this did not reach statistical significance.

Donor KIR Haplotype alone was not associated with improved survival

To determine if there was a relationship between KIR haplotype and survival after sibling allogeneic HSCT, the KIR gene content of both donors and recipients was assessed and used to infer the associated KIR haplotypes in a cohort of 145 transplant recipients. Without disease stratification, there were no significant differences in overall survival associated with the presence of distinct KIR haplotypes in the donor (Figure 1A). Similarly, when limited to patients receiving transplants for AML, there was also no significant difference in relapse rates based on donor KIR haplotype either when all patients with AML were assessed or when the analyses were limited to those who received myeloablative conditioning (Figure 1A).

The presence of donor Cen-B genes was associated with improved overall survival.

While there was no significant association between the B haplotype and overall survival in this cohort, previous studies had suggested that the centromeric cluster of the B haplotypes was more strongly associated with survival in a number of contexts. Furthermore, in our patient cohort, the

presence in the donor of KIR2DS2, the only activating KIR within the centromeric end of B haplotypes, was also associated with significantly improved overall survival in both the AML cohort (P = 0.03) and those AML patients that received myeloablative conditioning (P=0.01) (Figure 1B).

Consequently, the cohort was stratified based on the presence of donor-derived centromeric or telomeric genes. Again, when the entire cohort was assessed, there were no significant differences in outcomes associated with presence of donor derived B genes, irrespective of whether they were encoded within the centromeric or telomeric region of the locus. However, for patients with AML, the presence of centromeric genes of B haplotypes (Cen-B) was associated with improved overall survival (P=0.01) (Table 2 and Figure 2A, centre). When limited to patients who received myeloablative conditioning, this association was also evident (P=0.01) (Table 2 and Figure 2A, right).

The presence of Cen-B genes was not associated with reduced rates of relapse.

To better understand the mechanisms responsible for the improved overall survival in patients receiving transplants from donors with Cen-B genes, the proportion of patients who relapsed was also assessed. As with overall survival, there was no significant association between the presence of Cen-B genes and relapse when the entire cohort was assessed. Furthermore, there was no significant reduction in the number of AML patients who experienced relapse associated with presence of Tel-B or Cen-B genes, or when the analyses were focused on patients who had received only myeloablative

conditioning (Table 2 and supplementary figure 2). Similarly, comparison of the frequency of individual KIR genes in patients who relapsed compared to those who did not showed no marked differences with the possible exception of KIR2DS3 which was found in higher frequency in patients who did not relapse (data not shown). Cumulative incidence of death was considered with relapse as a competing risk. Significant associations were found when the donor had a Cen-B haplotype with improved overall survival in patients diagnosed with AML (P = 0.03) and those with AML and receiving myeloablative conditioning (P = 0.05) (Figure 3). Thus, the data suggests that the improved overall survival associated with Cen-B genes may be linked to reduced levels of relapse.

No improvement in the incidence of CMV reactivation with the presence of KIR Cen-B genes

In the absence of an association between the presence of Cen-B genes and disease relapse, we next considered whether the presence of these genes was associated with better control of cytomegalovirus replication, a common complication in HSCT where both primary infection and reactivation are associated with adverse outcomes.[24] Of the 145 recipients for which full clinical data was available, 97 were CMV seropositive prior to transplant. No seronegative patients had evidence of CMV viremia after transplantation. In patients that were seropositive for CMV at diagnosis, there was no significant decrease in the timing or frequency of CMV reactivation that could be linked to the presence of Cen-B haplotypes. In contrast, CMV reactivation in patients that received a transplant from donors that were Tel-B/x was markedly less

than those receiving a transplant from a donor that possessed the A haplotype (P=0.02) (Table 2, supplementary figure 3).

All recipients either received a suppressive prophylactic treatment or pre-emptive treatment to control CMV. When stratified according to CMV treatment and donor KIR haplotype, only those receiving pre-emptive prophylaxis exhibited any significant differences, with those having an AA donor being associated with increased CMV reactivation relative to those receiving Tel-B (P=0.03) (Table 2, supplementary figure 3).

CMV reactivation was considered as a competing risk by cumulative incidence of death, without stratification for IgG positivity or prophylaxis. Cen-B was associated with improved survival in patients with AML (*P* = 0.012) and AML with myeloablative conditioning (*P* = 0.018) however Tel-B was associated with poor survival when the entire cohort was considered (*P* = 0.004) (Supplementary figure 4).

Donor KIR Cen-B was protective against aGvHD in patients with AML.

A similar approach was taken to determine if KIR haplotype had an effect on the occurrence of GvHD. When the effect of KIR haplotype on GvHD was considered for the entire cohort, for those treated for AML or according to pre-transplant conditioning there were no significant differences.

Acute GvHD (aGvHD) was defined as grades II-IV aGvHD up to day 50 post-transplant. When the effect of donor KIR haplotype on aGvHD was

considered for the entire cohort, no significant differences were noted (Table 2). However, when this was stratified to include only those receiving treatment for AML, a significant difference was seen (P = 0.04), with an increased proportion of recipients receiving grafts from Cen-A donors having aGvHD (Table 2 and supplementary figure 5A).

Cumulative incidence of death was considered with GvHD as a competing risk, without stratification of grade and considered up to 100 days post-transplant. GvHD was also a significant competing risk factor to overall survival when the donor had a Tel-B haplotype for the entire cohort (P = 0.01) and AML (P = 0.02) (Supplementary figure 6).

Discussion

In this study, donor and recipient sibling HSCT pairs were prospectively recruited from four major transplant hospitals in Australia, to investigate the role of KIR in transplant outcome. Prior studies examining the role of NK cell mediated alloreactivity and the outcome of HSCT have yielded conflicting data.[9,17,25-28] Improvement in HSCT outcome by alloreactive donor NK cells was first reported in the haploidentical setting where decreased relapse rates and a protective effect against GvHD were observed.[3] Since then, many groups have sought to correlate KIR haplotype with the outcome of HSCT. As with other studies [11,29-31], when our entire cohort of MHC-matched cases was examined no significant associations of either donor or recipient KIR haplotype were evident with overall survival, relapse free survival or GvHD.

However, when just those receiving treatment for AML were examined, statistically significant associations were found.

Significant associations were found with clinically relevant aGvHD and overall survival when the donor had both the AA haplotype and Cen-A KIR, and therefore limited numbers of activating KIR. All transplants were T cell replete and this may be a confounding factor, as when T cells are included in the graft this can affect the reconstitution of NK cells.[32,33] A proposed mechanism for the beneficial effect of NK cell alloreactivity in the setting of HSCT is the ability to lyse recipient antigen presenting cells (APCs) that might otherwise prime donor derived T cells to induce GvHD.[3] The presence of only one activating KIR or indeed stronger inhibitory receptors that are associated with A haplotypes [34] may limit their capacity to lyse recipient APCs resulting in inferior survival due to increased aGvHD. Consistent with this, studies in the haploidentical setting have found that the presence of the activating KIR2DS1 on NK cells is associated with an enhanced capacity to kill mature allogeneic myelomonocytic dendritic cells.[35] These same studies also found increased lysis of T cell blasts by NK cells expressing KIR2DS1 compared with those that lacked KIR2DS1. Thus, an additional mechanism by which donors with B haplotypes might limit GvHD, is through direct NK recognition of activated recipient reactive T cells.

The impact of KIR haplotype on survival in HSCT prompted an analysis of the effect of KIR haplotype on CMV reactivation. Patients who received stem cells from AA donors had significantly more CMV reactivation than those from Bx

donors, but this effect was limited to those that received myeloablative conditioning and pre-emptive treatment. Similar results are reported in the myeloablative setting in both T-depleted or non-T-depleted studies and sibling or unrelated transplant cohorts.[14,15] In contrast, another unrelated and T depleted study has reported no effect of KIR haplotype on CMV reactivation.[27] This advantage was limited to Tel-B and to a subset of those with CMV reactivation, in contrast to overall survival and aGvHD where this was a Cen-B effect. Together with the mixed results in the cumulative incidence of death analyses, this may suggest that KIR haplotype in relation to CMV reactivation is not an important driver of survival in this cohort and potentially that the immune mechanisms for CMV control are different to those for GvHD.

Despite studies to the contrary we were unable to find any direct association between KIR haplotype and reduced relapse rates notwithstanding also examining missing ligands and the number of B haplotype genes as described in other studies.[11,17,18,26] However, we did demonstrate that significantly improved overall survival with donor Cen-B when relapse was considered as a competing risk. These mixed results may be due to heterogeneity of this cohort and small sample size. While samples were collected prospectively from all eligible patients over a period of four years, it fell short of the planned 250 sibling pairs, impacting on the ability to obtain adequate power and this may have affected the statistical analysis. Differences among conditioning protocols and post-transplant treatment may also contribute to the discrepancies seen between NK alloreactivity and survival across cohorts. While similar trends for were evident for patients undergoing RIC, small

numbers in this study restricted meaningful analysis of outcomes. Of interest, relapse rates for the entire cohort was 20% and in the AML group was 23% whereas generally the relapse rates for AML patients exceed 40%.[36] Multivariate analysis did not reveal any independent factors for improved relapse-free survival. Although limited sample size makes multivariate analysis difficult to interpret, these figures would suggest that this is lower than expected perhaps reflecting the prospective nature and best practice of this cohort at the time of the study.

While the initial studies examining HSCT outcome in KIR ligand mismatched recipients were encouraging, the interpretation of this data has become increasingly complex. The previous understanding of the range of HLA-C ligands for KIR2DL2/3 was somewhat limited, excluding group 2 HLA-C alleles such as HLA-Cw6 that have been shown to function as ligands for KIR2DL2.[37] The promiscuous nature of KIR2DL2 has been demonstrated through binding to a number of HLA-C alleles while allelic diversity has also been shown to impact their specificity.[34,38,39] While the ligands for activating KIR remain poorly characterised in general, KIR2DS1 has been demonstrated to mediate alloreactivity while KIRDS2 also appears to recognise discrete subsets of HLA class I allotypes.[35,40-43] KIR3DS1 has been shown to reduce mortality and despite structural similarity to 3DL1 appears to recognise the non-classical molecule HLA-F.[40,44,45] Secondly, it is now evident that MHC class I expression impacts on the function of NK cells via licensing.[34,46-48] Consequently, the altered environment present within the allogeneic recipient may itself impact the composition of the NK cell compartment, being

shaped by an education process distinct from that of T cells but nevertheless important in the acquisition of effector function by mature NK cells.[49]

While differences in HSCT protocols may continue to confound results, the data presented in this limited, small study add to reports that B haplotype genes are associated with improved outcomes following HSCT.[10,11,31,50] Furthermore, centromeric and telomeric clusters were associated with improved overall survival and decreased incidence of CMV reactivation respectively in patients diagnosed with AML. A greater understanding of the contribution of these distinct arms of the KIR haplotype awaits larger scale studies with high-resolution genetics.

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Figure Legends

Figure 1. Donor KIR 2DS2/2DL2 was associated with improved survival in patients with AML.
Kaplan Meier analysis with log-rank statistic. Donor KIR genes with assigned to AA or Bx (BB and BA) haplotypes (panel A) or the presence or absence of KIR2DS2/2DL2 (panel B) and the analysis was determined for entire cohort (left), for AML patients only (centre) and those receiving myeloablative conditioning (right). AA haplotype is shown in red and Bx (BB and BA) is shown in black. The presence of KIR2DS2/2DL2 is shown in blue and the absence in purple.

Figure 2. Overall survival was significantly improved in AML patients receiving a HSCT from a sibling with KIR Cen-B haplotype.
Kaplan Meier analysis of overall survival with log rank statistic. Donor KIR genes were assigned to centromeric (A) or telomeric (B) haplotypes and the analysis was determined for the entire cohort (left), for AML patients only (centre) and those receiving myeloablative conditioning (right). AA haplotype is shown in red and Bx (BB and BA) in black.

Figure 3. Significant improvement in overall survival with donor Cen-B with relapse as a competing risk in AML patients.
Fine and Grey statistical method with relapse as a competing risk to overall survival. Donor KIR genes were assigned to centromeric (A) or telomeric (B) haplotypes and the analysis was determined for the entire cohort (left), for AML patients only (centre) and those receiving myeloablative conditioning (right). AA haplotype is shown in solid line and Bx (BB and BA) in dashed line. The number at risk in each group is shown with 0 = alive, 1 = death and 2 = relapse.

Supplementary Figure Legends

Supplementary Figure 1. Multivariate analysis of relapse free survival.
Multivariate analysis of relapse free survival using Cox proportional hazard modelling in R. The following variables were considered: disease status, type of conditioning, CMV reactivation, GvHD (any grade), recipient sex and age at transplant (>50 years or below).

Supplementary Figure 2. Donor KIR haplotype did not impact on the inncidence of relapse in recipients undergoing HSCT.
Kaplan Meier analysis of day to relapse with log rank statistic. Donor KIR genes were assigned to centromeric (A) or telomeric (B) haplotypes and the analysis was determined for the entire cohort (left), for AML patients only

(centre) and those receiving myeloablative conditioning (right). AA haplotype is shown in red and Bx (BB and BA) in black.

Supplementary Figure 3. The presence of donor KIR Tel-B genes in conjunction with pre-emptive CMV treatment was significantly associated with decreased CMV reactivation.
Kaplan Meier analysis with log rank statistic. CMV reactivation was monitored until day 100 post transplant. Donor KIR genes were assigned to centromeric (A) or telomeric (B) haplotypes and the analysis was determined for patients that were IgG positive for CMV at diagnosis(left), IgG positive and receiving pre-emptive treatment (centre) and IgG positive and on the prophylaxis treatment arm (right). AA haplotype is shown in red and Bx (BB and BA) in black.

Supplementary Figure 4. Cumulative incidence of death with CMV as a competing risk.
Fine and Grey statistical method with CMV as a competing risk to overall survival. Donor KIR genes were assigned to centromeric (A) or telomeric (B) haplotypes and the analysis was determined for the entire cohort (left), for AML patients only (centre) and those receiving myeloablative conditioning (right). AA haplotype is shown in solid line and Bx (BB and BA) in dashed line. The number at risk in each group is shown with 0 = alive, 1 = death and 2 = CMV.

Supplementary Figure 5. Severe aGVHD was significantly improved when the donor had KIR Cen-B haplotype in patients with AML.
Kaplan Meier analysis with Gehan-Breslow-Wilcoxon test. Severe aGvHD was defined as grades II-IV within the first 50 days after HSCT. Donor KIR genes were assigned to centromeric (A) or telomeric (B) haplotypes and the analysis was determined for the entire cohort (left), for AML patients only (centre) and those receiving myeloablative conditioning (right). AA haplotype is shown in red and Bx (BB and BA) in black.

Supplementary Figure 6. Cumulative incidence of death with GvHD as a competing risk.
Fine and Grey statistical method with GvHD (any grade to day 100) as a competing risk to overall survival. Donor KIR genes were assigned to centromeric (A) or telomeric (B) haplotypes and the analysis was determined for the entire cohort (left), for AML patients only (centre) and those receiving myeloablative conditioning (right). AA haplotype is shown in solid line and Bx (BB and BA) in dashed line. The number at risk in each group is shown with 0 = alive, 1 = death and 2 = GvHD.

	Conditioning	
	Myeloablative (n=86)	**Reduced Intensity (n=59)**
Mean age at transplant (years)	39.4 (19-58)	51 (19-64)
Recipient sex	55 M, 31 F	39 M, 20 F
Disease		
Acute lymphoblastic leukemia	11	0
Acute myeloid leukemia	44	25
Chronic lymphoid leukemia	0	3
Chronic myeloid leukemia	7	1
Hodgkin lymphoma	1	5
Myelodysplastic syndrome	2	1
Multiple myeloma	1	12
Non-Hodgkin lymphoma	11	8
Aplastic anemia	5	0
Other	4	4
Conditioning regimen		
Busulphan plus cyclophosphamide	22	0
Cyclophosphamide plus TBI	54	0
Etoposide plus TBI	8	0
Fludarabine plus cyclophosphamide	0	27
Fludarabine plus melphalan	0	24
ATG pus cyclophosphamide	1	0
Melphalan	0	5
Low dose TBI	0	4
Immunosuppresion		
Cyclosporin/tacrolimus plus Methotrexate	69	45
Cyclosporin plus mycophenolate mofetil	0	6
Cyclosporin	14	4
Other	2	5
CMV Prophylaxis		
Pre-emptive	61	32
Suppressive	21	28
Unknown	3	0

Table 1 Transplant characteristics
Myeloablative total body irradiation (TBI) consisted of doses of 12 to 13.2 Gy while low dose was 2 Gy.

A

		Overall survival					
		Centromere			Telomere		
		% survival proportion			% survival proportion		
	n=	AA	Bx	P =	AA	Bx	P =
Entire cohort	145	45.6	56.3	**0.53**	51.2	51.3	**0.12**
AML only	69	46.3	53.6	**0.01**	56.5	43.4	**0.81**
AML, myeloablative	44	52.2	47.7	**0.01**	54.5	45.4	**0.96**
AML, RIC	25	40.04	62.5	**0.48**	47.6	60	**0.70**

B

		Relapse free survival					
		Centromere			Telomere		
		% survival proportion			% survival proportion		
	n=	AA	Bx	P =	AA	Bx	P =
Entire cohort	145	33.2	32.2	**0.54**	33.8	27	**0.98**
AML only	69	31.6	24.0	**0.39**	26.5	30.1	**0.50**
AML, myeloablative	44	23.1	21.9	**0.63**	23.0	24.3	**0.70**
AML, RIC	25	48.1	27.4	**0.35**	32.8	40	**0.49**

C

		aGVHD (grades II-IV)					
		Centromere			Telomere		
		% survival proportion (day 50)			% survival proportion (day 50)		
	n=	AA	Bx	P =	AA	Bx	P =
Entire cohort	145	36.3	23.8	**0.09**	31.9	27.9	**0.75**
AML only	69	43.1	19.6	**0.04**	35.4	23.3	**0.33**
AML, myeloablative	44	42.3	17.6	**0.13**	35.8	22.3	**0.59**
AML, RIC	25	50	20	**0.11**	35.3	22.2	**0.40**

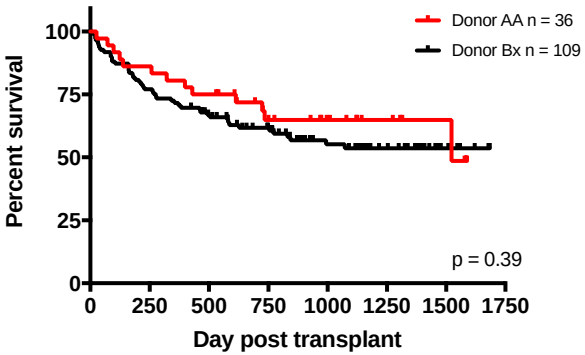
D

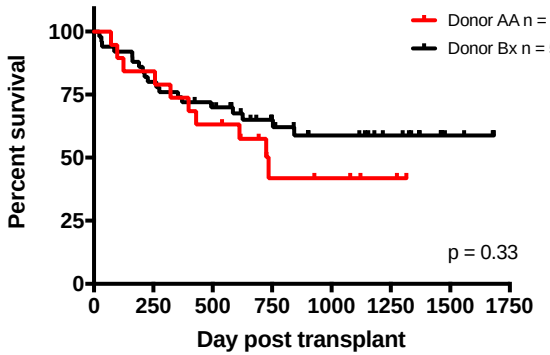
		CMV Reactivation					
		Centromere			Telomere		
		% survival proportion (day 100)			% survival proportion (day 100)		
	n=	AA	Bx	P =	AA	Bx	P =
IgG positive	97	51.1	42.2	**0.28**	58.3	36.7	**0.02**
IgG pos, Pre-emptive arm	53	55.1	55	**0.79**	73	44.4	**0.03**
IgG pos, prophylaxis arm	44	42.8	30	**0.28**	40.9	27.2	**0.26**

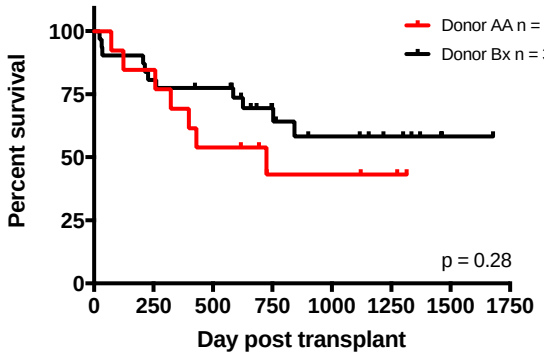
Table 2. Summary of Outcomes

Summary of Kaplan Meier analyses with log rank statistic for A - overall survival, B – relapse free survival, C – severe graft versus host disease and D - CMV reactivation according to donor KIR centromere or telomere haplotype. P <0.05 was considered significant and underlined.

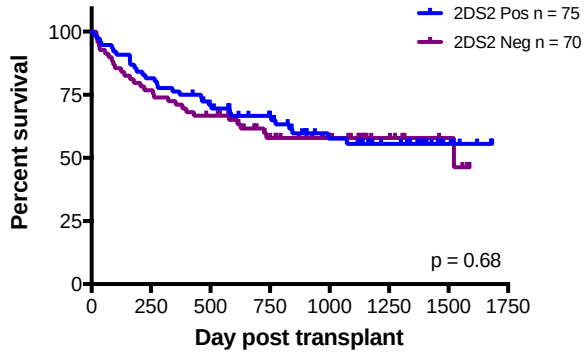
A

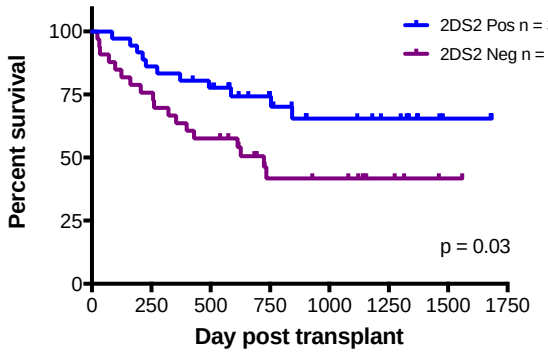


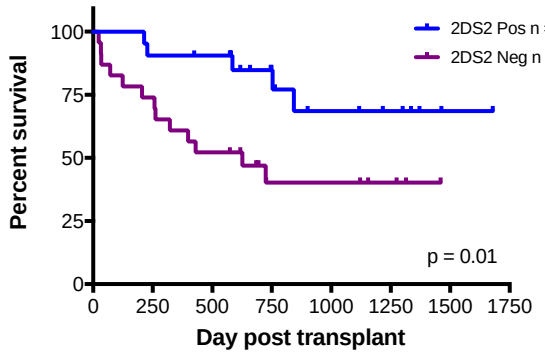




B







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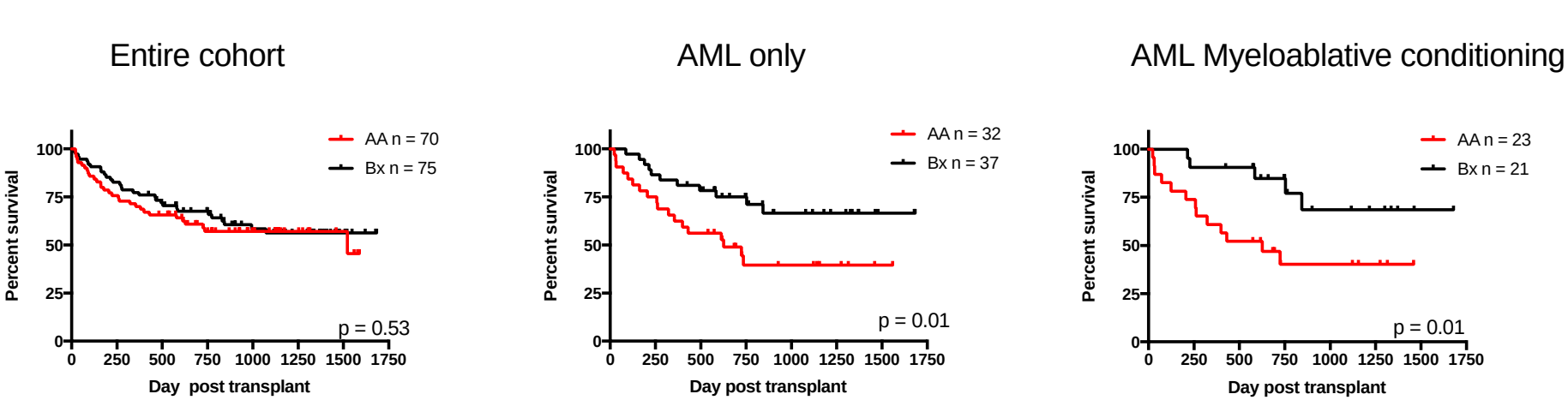
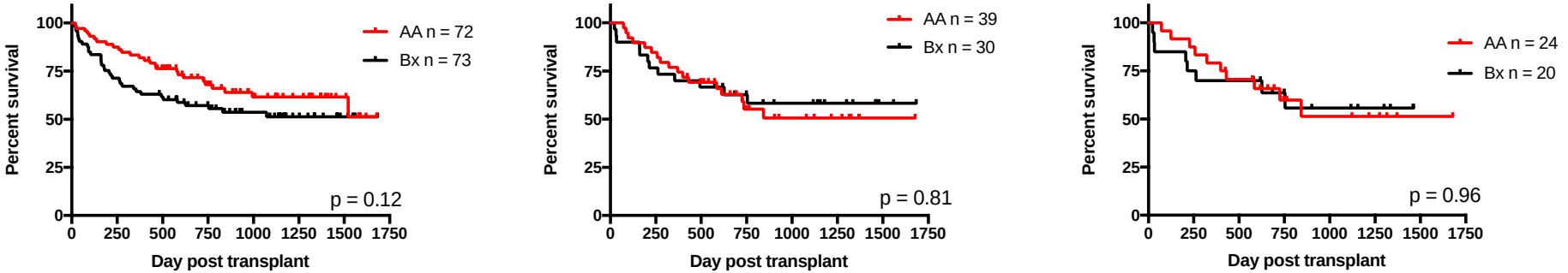
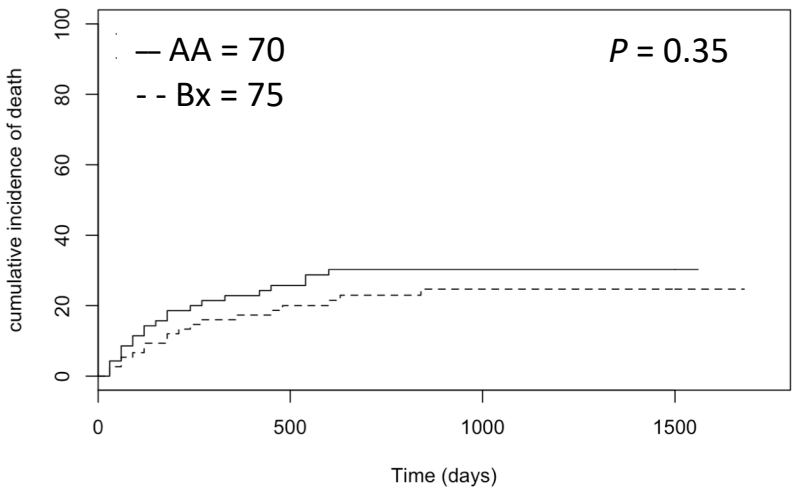


B



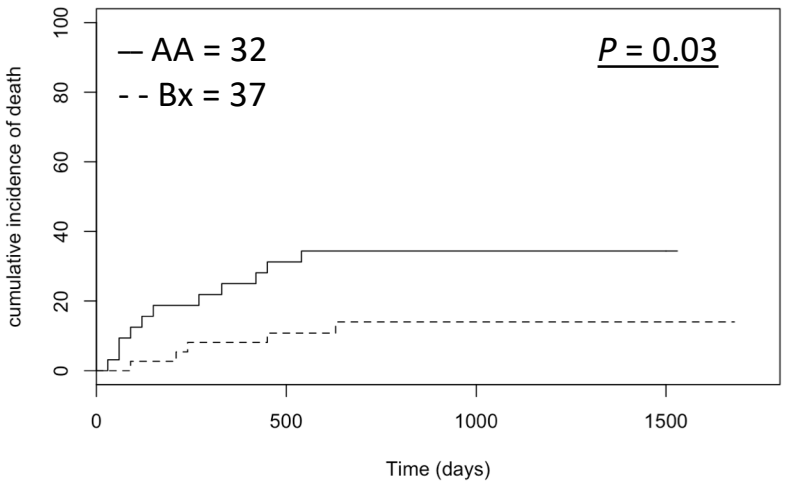
A

Entire cohort



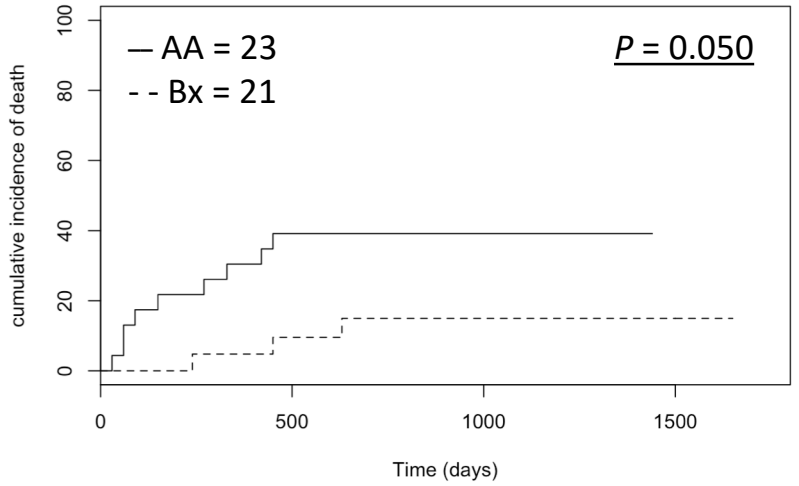
Number at risk 0 = 76, 1 = 39, 2 = 30

AML only



Number at risk 0 = 37, 1 = 16, 2 = 16

AML myeloablative conditioning



Number at risk 0 = 24, 1 = 12, 2 = 8

B