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

Will P. Bultitude1,2 • Jennifer Schellekens1,2 • Richard M. Szydlo1,3 • Chloe Anthias1,4 • Sarah A. Cooley5 • Jeffrey S. Miller6 • Daniel J. Weisdorf6 • Bronwen E. Shaw7 • Chrissy H. Roberts8 • Christian A. Garcia-Sepulveda9 • Julia Lee10 • Rachel M. Pearce10 • Marie C. Wilson10 • Michael N. Potter4 • Jenny L. Byrne11 • Nigel H. Russell11 • Stephen MacKinnon12 • Adrian J. Bloor13 • Amit Patel14 • I. Grant McQuaker15 • Ram Malladi16 • Eleni Tholouli17 • Kim Orchard18 • Victoria T. Potter19 • J. Alejandro Madrigal1,2 • Neema P. Mayor1,2 • Steven G. E. Marsh1,2

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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.
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 utilised 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, 5 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 vs. 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 vs. 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 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 and Cellular Therapy. 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 the 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). Cen and 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 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 \( p \leq 0.05 \) inclusion criteria. Statistical significance was denoted at \( p \leq 0.05 \), whilst statistical trend was signified by \( p \leq 0.1 \). 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, Fig. 1). 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.

For the whole cohort, the probabilities of survival and relapse at 5 years post-transplant were 38.6% and 34.5% respectively, whilst the probability of NRM at 1 year post-transplant was 23.0%. All such univariate analyses were performed using methods of Kaplan–Meier and cumulative incidence as described in the Materials and Methods. 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 5 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 1 year post-transplant (KIR AA: 9%, 95%
Categorical variables were compared by the Chi-squared test (or the Fisher’s Exact test when \( n \leq 5 \) for any subgroup). Continuous variables were compared by the Mann–Whitney test. Statistically significant \( p \) values are denoted in italics.

CMV cytomegalovirus, BM bone marrow, PBSC peripheral blood stem cells.

Confidence interval [CI] = 2.9–26.1 vs. KIR Bx: 29%, CI = 20.6–40.6; \( p = 0.019 \); Fig. 2a, 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 5 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 with 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 1 year post-transplant (Cen-AA: 9%, CI = 4.0–21.7 vs. Cen-Bx: 34%, CI = 24.4–48.4; \( p = 0.001 \), Fig. 2b). This observation correlated with significantly improved 5 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 with 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 1 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; \( p = 0.005 \), Fig. 3a). 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; \( p = 0.01 \), Fig. 3b) 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 5 years post-transplant.

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

To further investigate how donor-encoded Cen 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 were missing for three further transplants. Accordingly, a competing risk analysis assessing the risk of death by infection
at 1 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 were 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

### Table 2: Univariate analyses of recipient and donor factors on OS, relapse, DFS and NRM.

| Variable                      | Valid cases (n) | 5 year OS % P value | 5 year relapse % P value | 5 year DFS % P value | 1 year NRM % 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 0.79                | 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              | 31.9                 | 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                  |

Statistically significant results (≤0.05) are italicised.

OS overall survival, NRM non-relapse mortality, CMV cytomegalovirus, BM bone marrow, PBSC peripheral blood stem cells.

aNRM/DFS/Relapse data missing for one transplant.

bEstimated incidence of OS, relapse and DFS at latest clinical follow-up (4 years) reported.
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

| Variable                        | 5 year OS HR (95% CI) | 1 year NRM HR (95% CI) | 1 year death by infection HR (95% CI) |
|---------------------------------|-----------------------|------------------------|---------------------------------------|
| 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                  | –                      | 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                                  | –                                      |
| 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 italicised.

OS: overall survival, NRM: non-relapse mortality.

aNRM data missing for one transplant.

bCause-of-death data missing for three transplants.

Fig. 3 Effect of donor Cen-B is dose-dependent. a Univariate probability of NRM at 1 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. As described in the footer of Table 2, the total number of transplants included in this NRM analysis is one less than listed in Table 2 as a result of one transplant missing relapse data.
owing to expression of at least one inhibitory receptor for a host-encoded HLA class I molecule, recognise 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]. Furthermore, 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 collecting complete clinical follow-up data, including those relating to co-morbidities and the types of viral infections that occurred post-transplant. 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 allorreactivity 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 Cen 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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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Mohty M. Acute myeloid leukaemia. In: Apperley JF, Carreras E, Gluckman E, Masszti T, editors. The EBMT handbook on haematopoietic stem cell transplantation, 6th edn. Genoa: Forum Service Editore; 2012. p. 316–29.
2. Crivello P, Zito L, Sizzano F, Zino E, Maiers M, Mulder A, et al. The impact of amino acid variability on alloreactivity defines a functional distance predictive of permissive HLA-DPB1 mismatches in hematopoietic stemcell transplantation. Biol Blood Marrow Transpl. 2015;21:233–41. https://doi.org/10.1016/j.bbmt.2014.10.017.
3. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007;110:4576–83. https://doi.org/10.1182/blood-2007-06-097386.
4. Shaw BE, Mayor NP, Szydlo RM, Bultitude WP, Anthias C, Kirkland K, et al. Recipient/donor HLA and CMV matching in recipients of T-cell-depleted unrelated donor haematopoietic cell transplants. Bone Marrow Transpl. 2017;52:717–25. https://doi.org/10.1038/bmt.2016.352.
Donor Cen-B increases NRM in matched adult MAC AML patients

5. Fleischhauer K, Shaw BE, Gooley T, Malkki M, Bardy P, Bignon JD, et al. Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study. Lancet Oncol. 2012;13:366–74. https://doi.org/10.1016/S1470-2045(12)70004-9.

6. D’Souza A, Fretham C. Current Uses and Outcomes of Haplo- poietic Cell Transplantation (HCT): CIBMTR Summary Slides, 2019. Available at https://www.cibmtr.org. Accessed 10 Mar 2020.

7. Shaw BE, Logan BR, Spellman SR, Marsh SGE, Robinson J, Pidala J, et al. Development of an unrelated donor selection score predictive of survival after het: donor age matters most. Biol Blood Marrow Transplant. 2018;24:1049–56. https://doi.org/10.1016/j.bbmt.2018.02.006.

8. Kollman C, Spellman SR, Zhang MJ, Hassabrook A, Anasetti C, Antin JH, et al. The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy. Blood. 2016;127:260–7. https://doi.org/10.1182/blood-2015-08-663823.

9. Vierra-Green C, Roe D, Jayaraman J, Trowsdale J, Traherne J, et al. Comparison between antithymocyte globulin and alemtuzumab for the conditioning regimen for patients with multiple myeloma. Br J Haematol. 2005;129:631–8. https://doi.org/10.1111/j.1365-2141.2005.05513.x.

10. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. Blood. 2004;104:52–5. https://doi.org/10.1182/blood-2003-01-0091.

11. Ruggeri L, Capanni M, Casucci M, Volpi I, Tosti A, Perruccio K, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999;94:333–9.

12. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295:2097–2100. https://doi.org/10.1126/science.1068440.

13. Giebel S, Locatelli F, Lamparelli T, Velardi A, Davies S, Frumento G, et al. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. Blood. 2003;102:814–9. https://doi.org/10.1182/blood-2003-01-0091.

14. Mancusi A, Ruggeri L, Urbani E, Pierini A, Massei MS, Carotti A, et al. Haploidentical hematopoietic transplantation from KIR ligand-mismatched donors with activating KIRs reduces non-relapse mortality. Blood. 2012;125:3173–82. https://doi.org/10.1182/blood-2014-09-599933.

15. Kroger N, Shaw B, Iacobelli S, Zabelina T, Peggs K, Shimoni A, et al. Comparison between antithymocyte globulin and alemtuzumab and the possible impact of KIR-ligand mismatch after dse-reduced conditioning and unrelated stem cell transplantation in patients with multiple myeloma. Br J Haematol. 2005;129:631–43. https://doi.org/10.1111/j.1365-2141.2005.05513.x.

16. Schaffer M, Malmberg KJ, Ringden O, Ljunggren HG, Remberger M. Increased infection-related mortality in KIR-ligand-mismatched unrelated allogeneic hematopoietic stem-cell transplantation. Transplantation. 2004;78:1081–5.

17. Kroger N, Binder T, Zabelina T, Wolschke C, Schieder H, Renges H, et al. Low number of donor activating killer immunoglobulin-like receptors (KIR) genes but not KIR-ligand mismatch prevents relapse and improves disease-free survival in leukemia patients after in vivo T-cell depleted unrelated stem cell transplantation. Transplantation. 2006;82:1024–30. https://doi.org/10.1097/01.tp.0000235589.24513.43.

18. Sobecks RM, Wang T, Askar M, Gallagher MM, Haagenson M, Spellman S, et al. Impact of KIR and HLA genotypes on outcomes after reduced-intensity conditioning hematopoietic cell transplantation. Biol Blood Marrow Transpl. 2015;21:1589–96. https://doi.org/10.1016/j.bbmt.2015.05.002.

19. Kanga U, Mourya M, Seth T, George J, Sood P, Sharma R, et al. Role of killer immunoglobulin-like receptor-ligand interactions in human leukocyte antigen-matched sibling hematopoietic stem cell transplantation. Transpl Proc. 2012;44:919–21. https://doi.org/10.1016/j.transproceed.2012.03.036.

20. Venstrom JM, Pittari G, Gooley TA, Chewing JH, Spellman S, Haagenson M, et al. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. N Engl J Med. 2012;367:805–16. https://doi.org/10.1056/NEJMoa1200503.

21. Park S, Kim K, Jang JH, Kim SJ, Kim WS, Kang ES, et al. KIR alloreactivity based on the receptor-ligand model is associated with improved clinical outcomes of allogeneic hematopoietic stem cell transplantation: result of single center prospective study. Hum Immunol. 2015;76:636–43. https://doi.org/10.1016/j.jhimmun.2015.09.009.

22. Leung W, Iyerag R, Turner V, Lang P, Bader P, Conn P, et al. Determinants of antileukemia effects of allogeneic NK cells. J Immunol. 2004;172:644–50.

23. Neuchel C, Furst D, Niederwieser D, Bunjes D, Tsmadou C, Wulf G, et al. Impact of donor activating KIR genes on HSCT outcome in C1-Ligand negative myeloid disease patients transplanted with unrelated donors—a retrospective study. PLoS ONE. 2017;12:e0169512. https://doi.org/10.1371/journal.pone.0169512.

24. Cooley S, Trachtenberg E, Bergemann TL, Saetuek T, Klein J, Le CT, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. Blood. 2009;113:726–32. https://doi.org/10.1182/blood-2008-07-171926.

25. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Marsh SGE, et al. Donor killer cell Ig-like receptor B haplotypes, recipient HLA-C1, and HLA-C mismatch enhance the clinical benefit of unrelated transplantation for acute myelogenous leukemia. J Immunol. 2014;192:4592–4600. https://doi.org/10.4049/jimmunol.1302517.

26. Mayor NP, Hayhurst JD, Turner TR, Sydullo RM, Shaw BE, Bulitwde WP, et al. Recipients receiving better HLA-matched hematopoietic cell transplantation grafts, uncovered by a novel HLA typing method, have superior survival: a retrospective study. Biol Blood Marrow Transpl. 2015;21:443–50. https://doi.org/10.1016/j.bbmt.2018.12.768.

27. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.

28. Vilches C, Castano J, Gomez-Lozano N, Estefania E. Facilitation of KIR genotyping by a PCR-SSP method that amplifies short DNA fragments. Tissue Antigen. 2007;70:415–22. https://doi.org/10.1111/j.1399-0039.2007.00923.x.

29. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53:457–81. https://doi.org/10.1080/01621459.1958.10501452.

30. Cox DR. Regression models and life-tables. J R Stat Soc Ser B (Methodol). 1972;34:187–220.

31. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat. 1988;16:1141–54.

32. Fine JP, Gray RJ. A proportional hazards model for the sub-distribution of a competing risk. J Am Stat Assoc. 1999;94:496–509. https://doi.org/10.1080/01621459.1999.10474144.

33. Cooley S, McCullar V, Wangen R, Bergemann TL, Spellman S, Weisdorf DJ, et al. KIR reconstitution is altered by T cells in the graft and correlates with clinical outcomes after unrelated donor transplantation. Blood. 2005;106:4370–6. https://doi.org/10.1182/blood-2005-04-1644.

34. Smith LE, Olszewski MA, Georgoudaki AM, Wagner AK, Haggllof T, Karlsson MC, et al. Sensitivity of dendritic cells to...
NK-mediated lysis depends on the inflammatory environment and is modulated by CD54/CD226-driven interactions. J Leukoc Biol. 2016;100:781–9. https://doi.org/10.1189/jlb.3A0615-271RR.

35. Zaia JA, Sun JY, Gallez-Hawkins GM, Thao L, Oki A, Lacey SF, et al. The effect of single and combined activating killer immunoglobulin-like receptor genotypes on cytomegalovirus infection and immunity after hematopoietic cell transplantation. Biol Blood Marrow Transpl. 2009;15:315–25. https://doi.org/10.1016/j.bbmt.2008.11.030.

36. Tomasec P, Braud VM, Rickards C, Powell MB, McSharry BP, Gadola S, et al. Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. Science. 2000;287:1031.

37. Welte SA, Sinzger C, Lutz SZ, Singh-Jasuja H, Sampaio KL, Eknigk U, et al. Selective intracellular retention of virally induced NKG2D ligands by the human cytomegalovirus UL16 glycoprotein. Eur J Immunol. 2003;33:194–203. https://doi.org/10.1002/eji.200390022.

38. Halenius A, Hauka S, Dolken L, Stindt J, Reinhard H, Wick C, et al. Human cytomegalovirus disrupts the major histocompatibility complex I peptide-loading complex and inhibits tapasin gene transcription. J Virol. 2011;85:3473–85. https://doi.org/10.1128/jvi.01923-10.

39. Ljunggren HG, Karre K. In search of the ‘missing self’: MHC molecules and NK cell recognition. Immunol Today. 1990;11:237–44.

40. Frazier WR, Steiner N, Hou L, Dakshanamurthy S, Hurley CK. Allelic variation in KIR2DL3 generates a KIR2DL2-like receptor with increased binding to its HLA-C ligand. J Immunol. 2013;190:6198–208. https://doi.org/10.4049/jimmunol.1300464.

41. Bari R, Thapa R, Bao J, Li Y, Zheng J, Leung W. KIR2DL2/2DL3-E(35) alleles are functionally stronger than -Q(35) alleles. Sci Rep. 2016;6:23689. https://doi.org/10.1038/srep23689.

42. Fischer JC, Ottinger H, Ferencik S, Sribar M, Punzel M, Beelen DW, et al. Relevance of C1 and C2 epitopes for hemopoietic stem cell transplantation: role for sequential acquisition of HLA-C-specific inhibitory killer Ig-like receptor. J Immunol. 2007;178:3918–23.

43. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, et al. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science. 2004;305:872–4. https://doi.org/10.1126/science.1097670.

44. Vidal-Castineira JR, Lopez-Vazquez A, Diaz-Pena R, Alonso-Arias R, Martinez-Borra J, Perez R, et al. Effect of killer immunoglobulin-like receptors in the response to combined treatment in patients with chronic hepatitis C virus infection. J Virol. 2010;84:475–81. https://doi.org/10.1128/jvi.01285-09.

45. Fauriat C, Ivarsson MA, Ljunggren HG, Malmberg KJ, Michaelsson J. Education of human natural killer cells by activating killer cell immunoglobulin-like receptors. Blood. 2010;115:1166–74. https://doi.org/10.1182/blood-2009-09-245746.

46. Bultitude WP, Gyrer AW, Robinson J, Anthias C, Potter MN, Russell NH, et al. The effect of donor KIR2DL1 allelic diversity on the outcomes of HSCT is influenced by conditioning regimen. HLA. 2019;94:122–3.