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Integrating killer cell immunoglobulin-like receptor high-resolution genotyping for predicting transplant outcomes in allogeneic hematopoietic stem cell transplantation

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Keywords

killer cell immunoglobulin-like receptor (KIR); hematopoietic stem cell transplantation (HSCT); natural killer (NK) cell; alloreactivity; high-resolution

Key points:

- Functional KIR2DS4 interactions have detrimental effects on several transplant outcomes.
- KIR3DL1 and KIR2DS1 interactions are implicated in the risk of relapse in AML-transplanted recipients.

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Authorship Contributions

A.S., J.V. designed the study; H.B., S.F.L., S.M.L, V.M., O.K., G.N., J.H., T.G., D.S., Y.C., J.R.P. provided the clinical data; P.J.N. provided the KIR sequencing workflow, the KIR primers and the KIR gene and allele calling bioinformatic pipeline (PING); A.S., MP.H., Z.C.S., T.D.J.F., K.M.K. performed the experiments; B.M. performed the bioinformatic gene and allele calling; A.S. and S.B. performed the statistical analysis; A.S. and J.V. drafted the manuscript; all authors critically reviewed, edited the manuscript, and approved the final version.

Conflict of Interest Disclosures

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All other authors declare that they have no competing interests.

Data availability

Data is available on request from the corresponding author.

ABSTRACT

The success of hematopoietic stem cell transplantation (HSCT) partly relies on the beneficial graft-versus-leukemia effect, mediated by alloreactive NK cells through their killer cell immunoglobulin-like receptors (KIR). Conflicting results have been reported regarding the impact of the KIR immunogenetic system on HSCT outcomes with a scarcity of data interrogating the effect of KIR allelic polymorphism. With the aim to fill this gap, donor *KIR* genes derived from a national cohort of 1247 HLA-matched transplanted donor/recipient pairs were determined at a high-resolution and tested in Cox proportional hazards models. Donor/recipient (D/R) pairs bearing a KIR2DS4*00101 – HLA-C1/C2/A11 interaction showed a significant detrimental impact on progression-free survival (PFS), overall survival (OS), transplant-related mortality (TRM) and chronic graft-versus-host disease (cGvHD) in multivariable analysis. Strong KIR2DL2/L3 – HLA-C1 and especially KIR2DL3*00501 and *015 interactions showed a significant increase in the incidence of cGvHD compared to missing ligand D/R pairs. Highly inhibiting KIR3DL1 – HLA-B and HLA-A (Bw4) interactions were associated with a reduced relapse incidence as compared to weak and non-inhibiting interactions. Our study indicates that high-resolution *KIR* genotyping informs post-transplant outcomes with a seemingly higher protection of educated NK cells.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the standard of care for certain hematological malignancies and other immunological disorders. Despite major advancements, HSCT remains a high-risk treatment, and its success is significantly hampered by the occurrence of infections, immunological complications and disease relapse (1). Its therapeutic rationale partly relies on the beneficial graft-versus-leukemia (GvL) effect exerted by donor-derived alloreactive T and natural killer (NK) cells. NK cell immunosurveillance and killing capabilities are triggered by an HLA class I missing-self situation, mediated by their germline-encoded killer cell immunoglobulin-like receptors (KIR) (2). In a transplant setting, it has been hypothesized that a missing ligand situation could be mimicked by a donor and recipient KIR/HLA mismatch configuration, thus inducing alloreactivity against various target cells (3). Subsequent research to dissect this effect on modulating transplant outcomes, especially the relapse propensity, has yielded conflicting results (4).

Transplant-related features have been recognized as potential confounding parameters in this association, such as *in vitro* T-cell depletion (5), the type of the underlying diagnosis or the use of post-transplant cyclophosphamide. The genetic complexity of the *KIR* system encompassing gene, copy number and allelic variation combined with the lack of sequencing resolution depth and scalability have further challenged the interrogation of its effect. Recent studies have allocated beneficial or detrimental effects to selected *KIR* allele candidates such as *KIR3DL1* and *KIR2DL1* on HSCT outcomes (6-10) and a scarcity of studies has been investigating the entirety of *KIR* loci simultaneously (11, 12). In the largest genetic association study conducted to date with more than 5000 transplanted recipients included, authors were unable to replicate any of the proposed KIR models and were further unable to assess a link between *KIR* allelic polymorphism and the level of post-transplant NK cell alloreactivity (12).

Given the importance of NK cells in anti-tumor and antiviral immunity and the pressing need to improve post-transplant outcomes, we set out to revisit the predictive power of high-resolution *KIR* genotyping using a state-of-the-art *KIR* sequencing workflow in a large retrospective cohort of allogeneic HSCT transplanted recipients.

METHODS

Study cohort

For this multicenter retrospective study, 1247 patients who received a first allogeneic HSCT from an HLA-matched unrelated donor between January 2008 and April 2022 in one of the four transplant centers in Switzerland were selected. The study was approved by the local Ethics Committee for human studies of Geneva and the Geneva University Hospital (Commission Cantonale d'Ethique de la Recherche, CCER, CER 06-208 and 08-208) and was performed according to the Declaration of Helsinki principles. Informed consent was not necessary for this study.

High-resolution *KIR* genotyping

All donors were genotyped at high-resolution for the *KIR* loci. To this end, a DNA probe-based capture method was used as described (13). Further details are provided in the supplementary material.

Computational and statistical analysis

PING bioinformatic pipeline

Pushing Immunogenetics to the Next Generation (PING) pipeline was applied for sequence filtering, alignment, gene content and allelic genotype determination derived from the next-generation sequencing FASTQ files as developed by Norman *et al.* and Marin *et al.* (13, 14). High-resolution *KIR* genotype and copy number were determined for all *KIR* genes (*KIR2DS1, 2DS2, 2DS3, 2DS4, 2DS3/S5, 3DL1/3DS1, 2DL1, 2DL2/L3, 2DL4, 2DL5A/B, 3DL2, 3DL3*) and the two pseudogenes (*KIR2DP1, 3DP1*). The Immuno Polymorphism Database (IPD) IMGT/HLA database v3.25.0 was used as a source of reference sequences (15).

KIR alleles and haplotype assignment

Gene-level centromeric (cen) and telomeric (tel) haplotype assessment was performed manually based on the *KIR* gene presence and copy number variation following current haplotype

classification (16-18). *KIR* alleles ambiguities were manually curated. Haplotype, gene and allele frequencies were determined by direct, where the number observed was divided by 2*N* (alleles duplicated on a single haplotype were included as distinct loci, and gene absence was counted as a distinct allele). Further details on *KIR* haplotypes, alleles, allotypes assignment and KIR/HLA interactions are provided in the supplementary material.

Statistical endpoints and analysis

Statistical endpoints analyzed were overall survival (OS: time from transplantation until death), progression-free survival (PFS; survival without evidence of active malignancy after transplantation), relapse and progression, and transplant-related mortality (TRM; time from transplantation until death without evidence of relapse). OS, TRM and PFS were censored at the last reported follow-up date. Further outcomes were the incidence of acute graft-versus-host disease (aGvHD) and chronic graft-versus-host disease (cGvHD) (19).

A total of 16 variables were tested in univariable and multivariable analysis. Survival functions for OS and PFS were estimated according to the Kaplan-Meier method starting from the baseline date to the event or the last follow-up date available and compared between groups using a log-rank test. Cumulative incidence rates were estimated for events with competing risks (i.e., TRM, relapse/progression, aGvHD and cGvHD) and compared between groups using a log-rank test.

All models were then tested in a multivariable analysis using Cox proportional hazards regression to adjust for potential confounding covariates (see supplementary material).

All statistical tests were two-sided, with a threshold *p*-value of < 0.05 for statistical significance. All statistical analyses were computed using the statistical computing environment R, version 4.1.3 (R Core Team, Vienna).

RESULTS

Study cohort characteristics

The median age of recipients at the time of transplantation was 51.8 (IQR: 33.2 – 62). The primary diagnoses included hematologic malignancies (acute myeloid leukemia (AML): 39.9%,

myelodysplastic syndrome (MDS)/ myeloproliferative neoplasms (MPN): 20.4%, acute lymphoblastic leukemia (ALL): 13.5%), and non-malignant diseases (primary immunodeficiencies (PID): 4.57%). A majority (79.4%, *n* = 990) were 10/10 HLA matched transplants and 19.5% (*n* = 243) were 9/10 HLA-matched. Myeloablative conditioning regimen was applied in 50.3% (*n* = 627) of transplants and 84.4% (*n* = 1053) of recipients received a peripheral blood stem cell graft. A minority of patients (7.8%, *n* = 97) received an *in vitro* T-cell depleted graft. Further recipient and donor demographic and transplant-related characteristics are compiled in Table 1.

D/R pairs with a centromeric Bx portion show a higher overall survival probability

To analyze the predictive power of the *KIR* immunogenetic system on transplant outcomes, donor *KIR* genes derived from a cohort of 1247 HLA-matched transplanted donor/recipient (D/R) pairs were determined at a high-resolution. The detailed *KIR* allele characteristics are summarized in supplemental Tables S1 to S4. The allele frequencies were generally comparable, except for *KIR2DS4*: the frequencies of *KIR2DS4*00301* and *KIR2DS4*00601* were higher in our cohort (23.1% vs. 14.62% and 17.08% vs. 12.04%, respectively), while the frequency of *KIR2DS4*00101* was lower in our cohort (19.2% vs. 27.82%).

The *KIR* gene loci can be classified into group A and B haplotypes, and further divided into centromeric and telomeric fragments (see Methods section). Recipients receiving a graft from a donor with a Bx genotype had a significantly higher OS than D/R pairs with an AA genotype in univariable analysis (Fig. 1A) and in cause-specific multivariable regression model (hazard ratio [HR] 0.71, 95% confidence interval (CI) 0.59 – 0.87, $p < 0.001$). As the effect was more pronounced when analyzing the centromeric portion alone (Fig. 1B), this effect was likely driven by the presence of a centromeric portion of the B haplotypes. Presence of a centromeric B haplotype significantly increased the OS probability in univariable and cause-specific multivariable regression model (HR 0.76, 95% CI 0.63 – 0.92, $p = 0.004$) whereas no effect of the telomeric portion on OS was noted (HR 0.96, 95% CI 0.79 – 1.16, $p = 0.685$). The effect of the centromeric B portion on OS was indirectly confirmed by the significant impact of the copy number variation of *KIR2DL2* and *KIR2DS2* (HR 0.82, 95% CI 0.7 – 0.95, $p = 0.008$). In segregating the centromeric portion into cA01, cB01 and cB02 genotypes, cA01/cB02 bearing

D/R pairs showed a significantly increased OS probability than all other centromeric motifs by multivariable analysis (HR 0.72, 95% CI 0.56 – 0.92, p = 0.009), as previously observed (11).

As it is unknown whether the protective effect of cenB is due to the increased presence of activating KIRs or the differential presence of inhibitory KIRs (20, 21), we further investigated the effect of centromeric KIR interactions with their HLA ligands. We did not observe any impact on transplant outcomes in D/R pairs having a KIR2DS2*00101 – HLA-C*16/*01:02 and A*11:01 interaction (HR 1.15, 95% CI 0.9 – 1.46, p = 0.253). In line with the hypothesis that KIR2DL2 would be beneficial, we segregated the cohort according to whether D/R pairs have at least one inhibitory KIR2DL2 – HLA-C1 interaction compared to D/R pairs carrying only KIR2DL3 – HLA-C1 ligands or have a missing ligand status (C2/C2). D/R pairs bearing exclusively KIR2DL3 – HLA-C1 interactions had significantly lower overall survival probability in univariable (Fig. 1C) and cause-specific multivariable regression model (HR 1.38, 95% CI 1.13 – 1.69, p = 0.002) as compared to D/R pairs bearing at least one KIR2DL2 – HLA-C1 interaction.

KIR2DL1 and HLA-C2 interactions segregated according to whether they have a strong or weak signal transduction capacity had no effect on the OS probability (weak: HR 0.74, 95% CI 0.43 – 1.28, p = 0.284, strong: HR 1.06, 95% CI 0.87 – 1.3, p = 0.56).

KIR2DS4*001 represents the second most frequent allotype of KIR2DS4 and is the only one encoding a functional activating receptor (22). In our cohort, we observed KIR2DS4*00101 in combination with one or more of its ligands from HLA-C1/C2/A11 in 18.36 % (n = 229) of D/R pairs. This functional KIR2DS4 interaction had a significantly reduced OS both in univariable analysis (Fig. 1D) and cause-specific multivariable analysis (HR 1.26, 95% CI 1.0 – 1.59, p = 0.047). In addition, the interaction significantly negatively affected the following transplant outcomes: TRM (HR 1.65, 95% CI 1.2 – 2.27, p = 0.002) and PFS (HR 1.39, 95% CI 1.12 – 1.71, p = 0.002) when considered in cause-specific multivariable regression models. We did not observe an effect on the relapse/progression incidence.

Detailed results of the multivariable analysis for relapse incidence, OS, PFS, TRM, aGvHD and cGvHD are provided in the supplemental Table 5.

Inhibitory KIR2DL2 and KIR2DL3 – HLA-C1 interactions increase the likelihood of chronic GvHD

GvHD is thought to be modulated by NK cells through an indirect pathway targeting recipient's dendritic cells or alloreactive T cells (23). Analysis in a univariable and cause-specific multivariable regression model revealed that D/R pairs having a missing ligand configuration had a significantly reduced incidence of cGvHD as compared to recipients with inhibitory KIR2DL2 – HLA-C1 interactions (HR 0.69, 95% CI 0.5 – 0.94, p = 0.018).

Assuming that the inhibition threshold might play a role, we segregated *KIR2DL2/L3* alleles according to their binding affinity with HLA-C1 as previously described (24). Analysis in univariable (Fig. 2A) and cause-specific multivariable regression model revealed that – independently of the *KIR* gene type – the presence of two strongly or at least one strongly inhibitory allele significantly increased the risk of cGvHD as compared to D/Rs having only weakly binding *KIR2DL2/L3* alleles (one strong: HR 1.36, 95% CI 1.05 – 1.76, p = 0.02, two strong: HR 1.27, 95% CI 1.0 – 1.62, p = 0.049) and the presence of one strong interaction significantly increased the incidence of aGvHD (HR 1.38, 95% CI 1.01 – 1.87, p = 0.042). Stratified according to the *KIR* gene type, especially the presence of D/R pairs bearing at least one strongly inhibitory KIR2DL3 – HLA-C1 interaction encompassing *KIR2DL3*00501* and **015* alleles significantly increased the cumulative incidence of cGvHD in univariable (Fig. 2B) and cause specific multivariable analysis (HR 1.59, 95% CI 1.14 – 2.22, p = 0.006) as compared to D/R pairs without strong KIR2DL3 interactions.

Further, although not reaching statistical significance, D/R pairs with *KIR2DS1* alleles in a C2/C2 environment had a tendency towards a decreased cumulative incidence of cGvHD as compared to D/R pairs without a KIR2DS1 interaction (HR 0.63, 95% CI 0.37 – 1.05, p = 0.07).

KIR2DS4*00101 functional interactions were associated with a higher incidence of chronic GvHD in cause-specific multivariable analysis (HR 1.29, 95% CI 1.02 – 1.64, p = 0.035). It should be noted that there was no difference in the prediction of cGvHD based on the different HLA alleles (C2: HR 1.08, 95% CI 0.78 – 1.51, p = 0.632, C1: HR 1.18, 95% CI 0.69 – 2.01, p = 0.557, A11: HR 1.61, 95% CI 0.9 – 2.87, p = 0.11).

No significant correlation was observed with acute GvHD (Supplemental Table 5).

KIR3DL1 and KIR2DS1 interactions are predictive of relapse/progression incidence

Incremental research has been conducted into examination of KIR and HLA configurations on the relapse propensity in AML transplanted recipients, especially with regards to KIR3DL1 and KIR2DS1 (7, 25).

We started by interrogating the effect of KIR3DL1 stratifying the entire cohort based on the previous known classification (26) without considering D/R pairs who possess HLA-A allotypes containing the Bw4 motif. D/R pairs bearing weak (HR 1.7, 95% CI 1.3 – 2.21, $p < 0.001$) and non-inhibiting (HR 1.4, 95% CI 1.09 – 1.81, $p = 0.009$) KIR3DL1 – HLA-Bw4 interactions displayed a significantly increased incidence of relapse and progression compared to recipients bearing strong inhibiting KIR3DL1 – HLA-Bw4 interactions in cause-specific univariable (Fig. 3A) and multivariable analysis. We next thought to restrict this analysis only considering recipients transplanted in the context of an AML diagnosis (Table 1). In this subgroup, we confirmed that strong inhibiting interactions confer protection against the relapse/progression rate as compared to weak and non-inhibiting interactions in univariable (Fig. 3B) and cause-specific multivariable analysis (weak: HR 1.8, 95% CI 1.21 – 2.6, $p = 0.004$, non-inhibiting: HR 1.7, 95% CI 1.16 – 2.4, $p = 0.006$).

There is evidence that HLA-A allotypes with Bw4 motifs are potent educators for KIR3DL1^{+} NK cells, with exceptions being HLA-A*25:01 and HLA-A*23:01, which we considered being non-inhibiting interactors (27). In consideration of these results, we integrated D/R pairs with *HLA-A* alleles encoding Bw4 epitopes and confirmed that – in the entire cohort and in the AML subcohort – they were not significantly different than strong inhibiting KIR3DL1 – HLA-B Bw4 encoding D/R pairs (Cohort: HR 0.875 , 95% CI 0.559 – 1.37, $p = 0.557$, AML: HR 0.59, 95% CI 0.28 – 1.3, $p = 0.168$).

Within the entire cohort, there was no effect of KIR2DS1 – HLA-C2 interactions on the relapse/progression incidence (HR 0.99, 0.79 – 1.23, $p = 0.911$). However, within the AML subcohort, D/R pairs with a KIR2DS1 – HLA-C2 interaction had a significantly lower

relapse/progression incidence than D/R pairs lacking this interaction (HR 0.67, 95% CI 0.48 – 0.95, p = 0.024). We further refined D/R pairs segregating them according to whether they have a strong/weak or non-inhibiting KIR3DL1 – HLA-Bw4 interaction combined with the absence or presence of a KIR2DS1 – HLA-C2 interaction. D/R pairs with a strong inhibitory KIR3DL1 – HLA-Bw4 and a KIR2DS1 – HLA-C2 interaction confer the highest protection against relapse compared to D/R pairs with a weak KIR3DL1 – HLA-Bw4 interaction and the lack of KIR2DS1 – HLA-C2 interaction (weak/no 2DS1: HR 3.1, 95% CI 1.53 – 6.3, p = 0.002, none/no 2DS1: HR 2.5, 95% CI 1.25 – 5.2, p = 0.01). Interestingly, the group having weak and non-inhibiting KIR3DL1 – HLA-Bw4 interactions and the presence of a KIR2DS1 – HLA-C2 interaction did not have a significantly higher relapse/progression incidence (Weak/2DS1: HR 1.2, 95%CI 0.49 – 3.1, p = 0.659, None/2DS1: HR 2.0, 95% CI 0.91 – 4.4, p = 0.086) than the group with strong inhibiting KIR3DL1 – HLA-Bw4 interactions and the presence of a KIR2DS1 – HLA-C2.

Finally, it has to be noted that these KIR3DL1 and KIR2DS1 configurations did not aggravate the acute and chronic GvHD incidence in the entire cohort and in the AML subcohort, except for the KIR3DL1 – HLA-A interactions (Supplemental Tables S5, S6).

DISCUSSION

In this study, we aimed to challenge the immunogenetic hypothesis that specific allelic KIR/HLA configurations would enhance the NK cell alloreactivity propensity, thus modulating transplant outcomes. Integrating current paradigms and extending to further KIR/HLA configurations, we

could demonstrate that high-resolution *KIR* genotyping informs post-transplant NK cell alloreactivity mainly driven by *KIR2DS4, KIR2DL2/L3* and *KIR3DL1* alleles.

Donors with a centromeric B portion have been associated with better transplant outcomes, a finding we were only partially able to replicate in this study. This discrepancy between the previous cohort and this contemporary cohort might be due to differences in transplant-related factors such as the use of reduced-intensity conditioning regimens. This protocol could lead to a less inflammatory environment post-transplant, potentially resulting in decreased upregulation of stress ligands and delayed NK cell reconstitution. Another contributing factor could be a reduced incidence of cytomegalovirus (CMV) reactivation, which is known to induce KIR2DL2/L3/S2^{+} NK cells (28). This reduction may be attributed to the use of new CMV prophylaxis regimens, such as Letermovir, in the contemporary cohort.

We revealed a detrimental effect of KIR2DS4*00101 interactions on almost all transplant outcomes in terms of a lower OS rate, lower PFS and higher risk of cGvHD and TRM, while there was no effect on relapse/progression and aGvHD. These results partially overlap with recent studies demonstrating unfavorable transplant outcomes associated with *KIR2DS4*00101*, albeit none of these studies considered the HLA ligands for KIR2DS4 (29, 30). Poor outcomes were equally reported for patients carrying full-length *KIR2DS4* genes in non-transplant settings such as HIV-1 and SARS-CoV-2 (31, 32), substantiating its potential deleterious effect. We could hypothesize that NK cell activation by KIR2DS4 might lead to a sustained induced inflammatory setting with the secretion of proinflammatory cytokines leading to detrimental paracrine side effects. It remains, however, elusive as to why this detrimental effect is only present with KIR2DS4 and lacking with other activating KIRs. From a genetic point of view, *KIR2DS4* is the only activating KIR on the telomeric part from the A haplotype, which might hint towards either suppressing roles of co-receptors or a differential intrinsic mechanism.

We found that there is a strengthened protection for chronic GvHD development in KIR2DL2/L3^{+} D/R pairs with a missing ligand status and especially a higher risk conferred to recipients bearing strongly inhibiting KIR2DL3*00501 and *015 – HLA-C1 interactions. NK cell mediated alloreactivity has been shown to indirectly impact the development of chronic GvHD by targeting

recipient's dendritic and T cells (23). Thus, given the proinflammatory environment, NK cell tolerance could be broken, and missing ligand situations be favorable in selectively killing target cells. In addition, there is compelling evidence that HLA-C expression plays a key role in modulating the immune response. In stem cell transplantation settings, there is evidence that the highest expressed HLA-C allele (**14:02*) was associated with the most striking risk of acute GvHD (33), which might substantiate that an increased inhibitory threshold hinders NK cell targeted cytotoxicity against dendritic and T cells.

The observed lack of effect of KIR2DL1^{+} D/Rs pairs with a missing ligand status (C1/C1) might be allocated to a reduced reconstitution pattern, reducing the NK cell alloreactivity potential (34). Finally, another explanatory hypothesis that might account for this effect is based on a direct NK and T cell interaction. Indeed, a recent study showcased that the number of direct inhibitory KIR and HLA interactions between NK and T cells impacts the lifespan of T cells *in vitro*, with a higher number of inhibitory interactions leading to a prolonged longevity (35, 36). Thus, we might hypothesize that NK cells could potentiate the level of T cell alloreactivity, which might in turn render GvHD development.

With regard to the anti-leukemic NK cell alloreactivity, out of our analysis, we could speculate that activating and inhibitory KIRs do not contribute equally to leukemic control. We found that there is an increased protection against relapse in patients with highly inhibiting KIR3DL1 – HLA-Bw4 interactions, partially dissimilar to previous studies (7, 9, 37). Indeed, non-inhibiting KIR3DL1 – HLA-B (Bw4) interactions have been initially suggested to magnify anti-leukemic immunity through a reduced inhibition threshold and responsiveness acquired through a cytokine enriched environment (7). These findings could not be replicated in a following prospective study, where only recipients with weakly inhibiting interactions were subject to a decrease in the relapse incidence (37). Breaking of tolerance might not occur outside of specific settings, such as infections, and might thereby curtail the anti-leukemic effect.

The favorable association between strong inhibiting KIR3DL1 interactions and the relapse incidence in this study suggests that classical NK cell licensing might override missing ligand or non-inhibiting configuration states in anti-tumor immunity.

Recent data has demonstrated a higher frequency of HLA-A and HLA-B loss-of-function mutations but to a lesser extent HLA-C across multiple cancer types, which substantiates the implication of HLA-B specific KIR receptors in the sensing of transformed tumor cells (38). Further, the expression of HLA-A and B ligands on cells is approximately 5 times higher than HLA-C (39, 40). Following the educational tuning model of NK cells (41), we might hypothesize that strongly inhibiting KIR3DL1^{+} NK cells might show an enhanced sensitivity for discrete changes in the HLA-Bw4 ligand expression. A recent study has shown similar results with a beneficial effect conferred by the presence of highly expressed *KIR3DL1* alleles compared to non-expressed *KIR3DL1*004* and **019* alleles in recipients following haploidentical transplantation (8).

Furthermore, we found a significant additive effect of KIR2DS1 – HLA-C2 interactions on the relapse incidence in AML recipients with a lacking effect within the entire cohort, suggesting that the tumor microenvironment might render the expression of activating KIR ligands.

Despite the large size of our cohort, we need to acknowledge some limitations of the present study. The lack of biological knowledge may lead to potential misinterpretation in the associative analysis. Missing biological information includes the ligand identity for KIR2DS3 which might interfere with the analysis or the potential binding of activating KIRs such as KIR2DS4 to non-HLA ligands (42). Another major weakness is the fact that we ignore the HLA ligand distribution on target cells, a major component of NK cell licensing and responsiveness comprehension. Thus, our results do not preclude effects of KIR and HLA in subgroups of disease. The study design may have biased our results due to its retrospective nature and the heterogeneity of some transplant-related features hampering accurate comparability with other cohorts.

Notwithstanding these limitations, our results suggest that high-resolution *KIR* genotyping might be an additional immunogenetic stratification tool for use in clinical practice and might help clinicians in the donor allocation process. We don't believe that high resolution *KIR* genotyping should be performed for all volunteers enrolled in the registries similar to HLA typing, but one could envision a smarter and cost-efficient sequencing of selected *KIR* loci when several unrelated donors are available for a single patient.

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Table 1. Demographic and transplant related characteristics of the entire study cohort and the AML subcohort.

Parameter	**Cohort (*n* = 1247)**	**AML (*n* = 498)**
Recipient's age in yrs (median, IQR)	51.77 (33.2 – 62)	54.6 (41.5 – 63.2)
Recipient's gender (M:F)	836 : 411	277 : 221
Donor's age in yrs (median, IQR)	30.6 (25 – 39)	30.7 (24.2 – 39.1)
Donor's gender (M:F)	775 : 472	330 : 168
Year of transplantation, *n* (%)		
2008 – 2012	353 (28.3)	137 (27.5)
2013 – 2017	475 (38.1)	192 (38.5)
2018 – 2022	419 (33.6)	169 (33.9)
Transplant center, *n* (%)		
202	463 (37.1)	174 (34.9)
208	297 (23.8)	139 (27.9)
261	348 (27.9)	172 (34.5)
334	139 (11.1)	13 (2.6)
Underlying diagnosis, *n* (%)		
AML	498 (39.9)	498 (39.9)
ALL	168 (13.5)	-
CML	36 (2.9)	-
CLL	28 (2.3)	-
MDS	183 (14.7)	-
MPN	71 (5.7)	-
PCD	54 (4.3)	-
NHL	77 (6.2)	-
PID	57 (4.6)	-
BMF	35 (2.8)	-
Others	40 (3.2)	-
HLA-matching, *n* (%)		
10/10	990 (79.4)	400 (80.3)
9/10	243 (19.5)	95 (19.1)
Single mismatch at HLA-A	92 (7.5)	33 (6.6)
Single mismatch at HLA-B	33 (2.6)	8 (1.6)
Single mismatch at HLA-C	35 (2.8)	16 (3.2)

Single mismatch at HLA-DRB1	56 (4.5)	23 (4.6)
Single mismatch at HLA-DQB1	27 (2.2)	15 (3.0)
< 9/10	12 (1.0)	3 (0.6)
Missing information	2 (0.2)	-
Conditioning regimen, *n* (%)		
MAC	627 (50.3)	270 (54.2)
RIC	620 (49.7)	228 (45.8)
TBI		
No	887 (71.1)	379 (76.1)
Yes	358 (28.7)	118 (23.7)
Missing information	2 (0.2)	1 (0.2)
No *in vitro* T-cell depletion, *n* (%)	1150 (92.2)	454 (91.2)
Stem cell source, *n* (%)		
PBSC	1053 (84.4)	466 (93.6)
BM	186 (14.9)	31 (6.2)
CB	8 (0.6)	1 (0.2)
Disease state, *n* (%)		
Early	642 (51.5)	323 (64.9)
Intermediate	378 (30.3)	94 (18.9)
Late	227 (18.2)	81 (16.3)
Karnofsky Status, n (%)		
90 – 100%	970 (77.8)	405 (81.3)
≤ 80%	267 (21.4)	87 (17.5)
Missing information	10 (0.8)	6 (1.2)
EBMT risk score, *n* (%)		
0 – 1	62 (5.0)	14 (2.8)
2 – 3	539 (43.2)	291 (58.4)
4 – 5	523 (41.9)	166 (33.3)
6 – 7	123 (9.9)	27 (5.4)
CMV serostatus, *n* (%)		
D+ /R+	421 (33.8)	164 (32.9)
D- /R+	247 (19.8)	117 (23.5)
D+ /R-	129 (10.3)	57 (11.4)
D- /R-	436 (35)	160 (32.1)
Missing information	14 (1.1)	-

Gender matching (D/R), *n* (%)		
Male/Male	590 (47.3)	212 (42.6)
Female/Male	185 (14.8)	65 (13.1)
Male/Female	246 (19.7)	118 (23.7)
Female/Female	226 (18.1)	103 (20.7)
Progression-free survival, median (IQR)	1.8 (0.87 – 5.03)	1.79 (0.46 – 4.77)
Overall survival, median (IQR)	2.58 (0.88 – 5.21)	2.43 (0.87 – 5.03)

ALL; acute lymphoblastic leukemia, AML; acute myeloid leukemia, ATG; anti-thymocyte globulin, BM; bone marrow, BMF; bone marrow failure, CB; cord blood, CLL; chronic lymphocytic leukemia, CML; chronic myeloid leukemia, CMV; cytomegalovirus, D; donor, D/R; donor/recipient, IQR; interquartile range, MAC; myeloablative conditioning, MDS; myelodysplastic syndrome, MPN; myeloproliferative neoplasm, NHL; non-Hodgkin lymphoma, PBSC; peripheral blood stem cells, PCD; plasma cell disorder, PID; primary immunodeficiency, RIC; reduced-intensity conditioning, TBI; total body irradiation

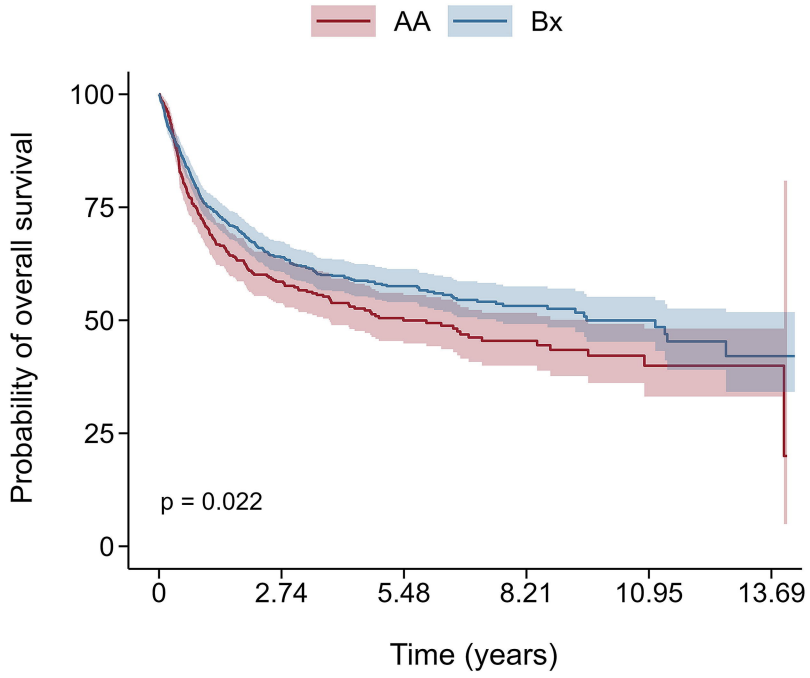
MAIN FIGURES LEGENDS

Figure 1. Impact of KIR genotypes, KIR2DL2/L3 and KIR2DS4 interactions on the overall survival probability. (A) Kaplan-Meyer estimates of the impact of AA (red) and Bx (blue) KIR genotypes on the overall survival probability. **(B)** Kaplan-Meyer estimates of the impact of centromeric AA (red) and Bx (blue) KIR genotypes on the overall survival probability. **(C)** Kaplan-Meyer estimates of the impact of the presence of ≥ 1 KIR2DL2 – HLA-C1 (red), only KIR2DL3 – HLA-C1 (blue) and missing ligand (lila) interactions on the overall survival probability. **(D)** Kaplan-Meyer estimates of the impact of the presence (blue) and absence (red) of KIR2DS4*00101 functional interactions on the overall survival probability. In the plots, shaded bands represent the 95% confident interval. Non-adjusted *p*-values indicate the significance of the log-rank test.

Figure 2. Impact of KIR2DL2/L3 interactions on the incidence of cGvHD. (A) Impact of two strong (green), one strong (blue), only weak (red) KIR2DL2/L3 – HLA-C1 interactions and missing ligand (lila) on the cumulative incidence of cGvHD. **(B)** Impact of strongly inhibitory KIR2DL3 – HLA-C1 interactions encompassing *KIR2DL3*00501* and **015* alleles (green), no strong KIR2DL3 – HLA-C1 interactions (blue) and missing ligand (lila) on the cumulative incidence of cGvHD. In the plots, shaded bands represent the 95% confident interval. Non-adjusted *p*-values indicate the significance of the log-rank test.

Figure 3. Impact of KIR3DL1 interactions on the risk of disease relapse. (A) Impact of strong inhibiting (red), weak inhibiting (blue) and non-inhibiting (lila) KIR3DL1 – HLA-B (Bw4) interactions on the cumulative incidence of relapse/progression within the entire cohort (*n* = 1247). **(B)** Impact of strong inhibiting (red), weak inhibiting (blue) and non-inhibiting (lila) KIR3DL1 – HLA-B (Bw4) interactions on the cumulative incidence of relapse/progression within the AML subcohort (*n* = 498). In the plots, shaded bands represent the 95% confident interval. Non-adjusted *p*-values indicate the significance of the log-rank test.

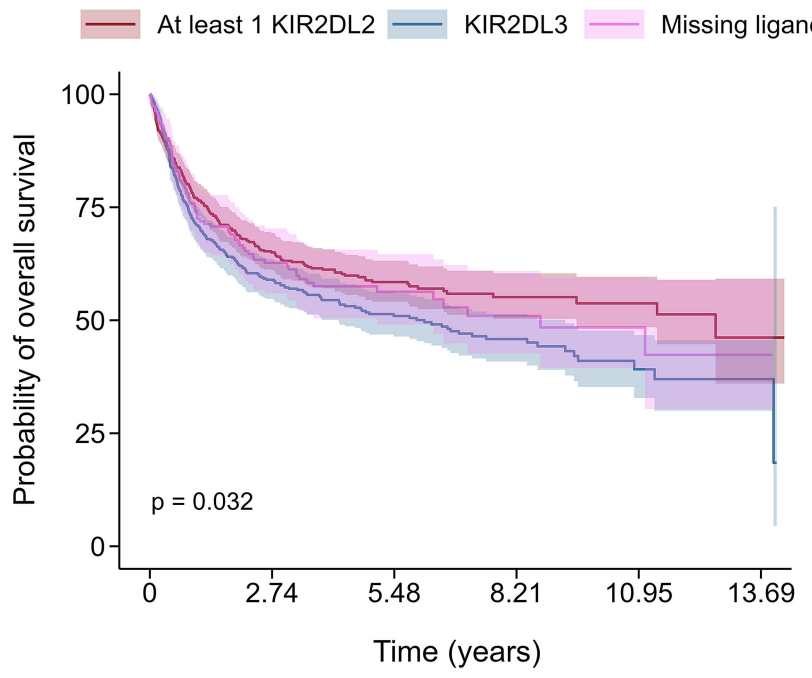
A



No. at risk:

406	188	96	49	18	2
841	426	195	94	34	4

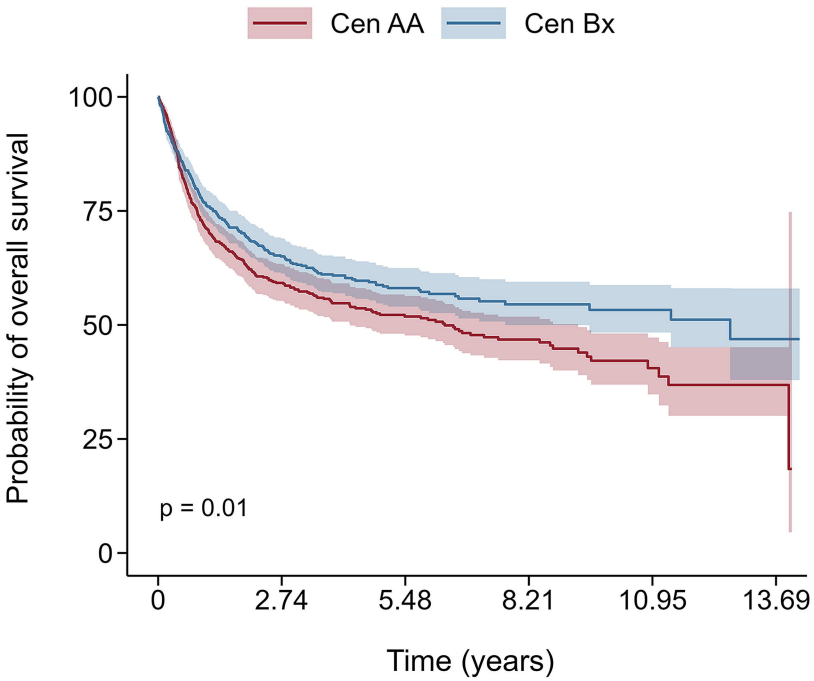
C



No. at risk:

549	277	128	60	23	3
512	243	120	61	21	2
186	94	43	22	8	1

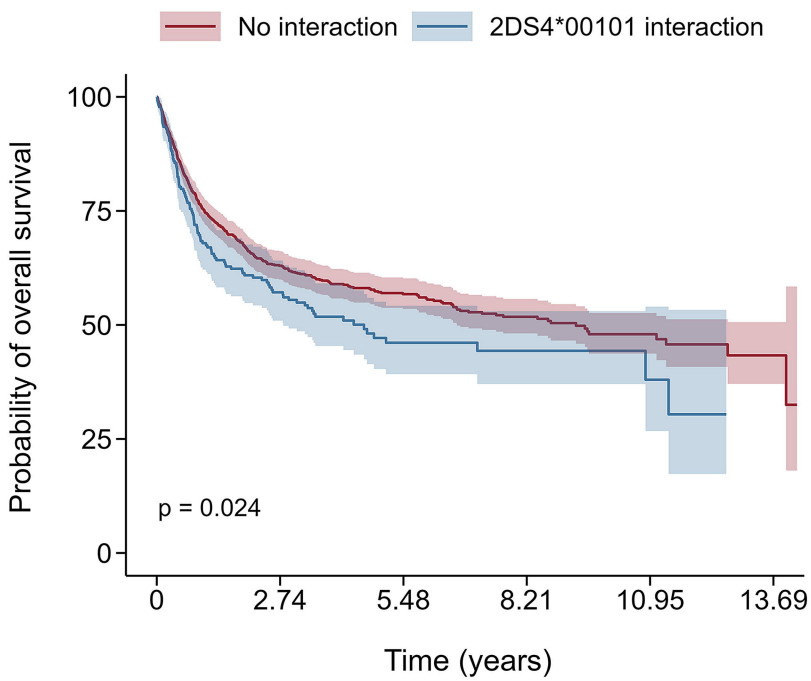
B



No. at risk:

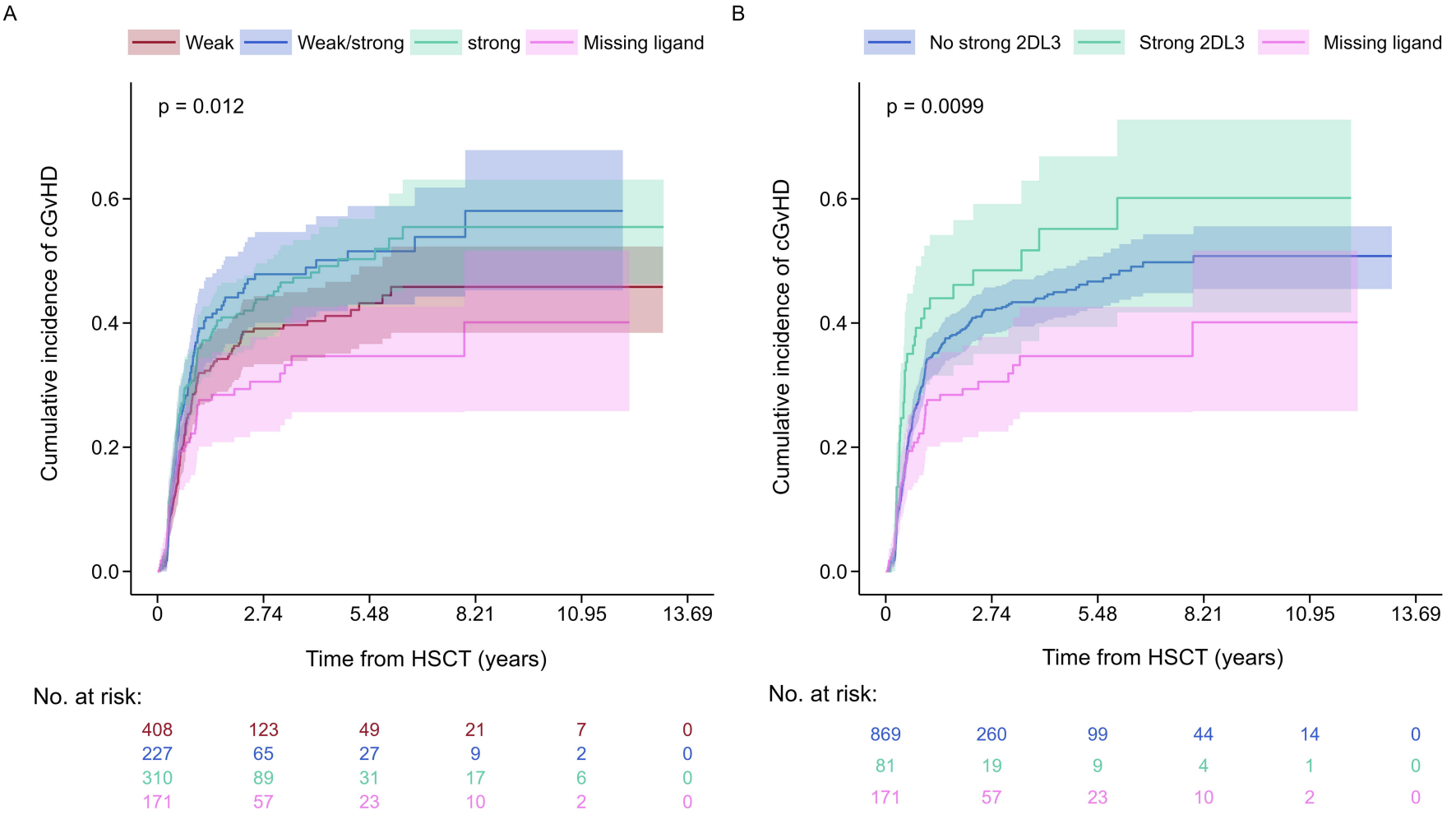
607	290	147	77	25	2
640	324	144	66	27	4

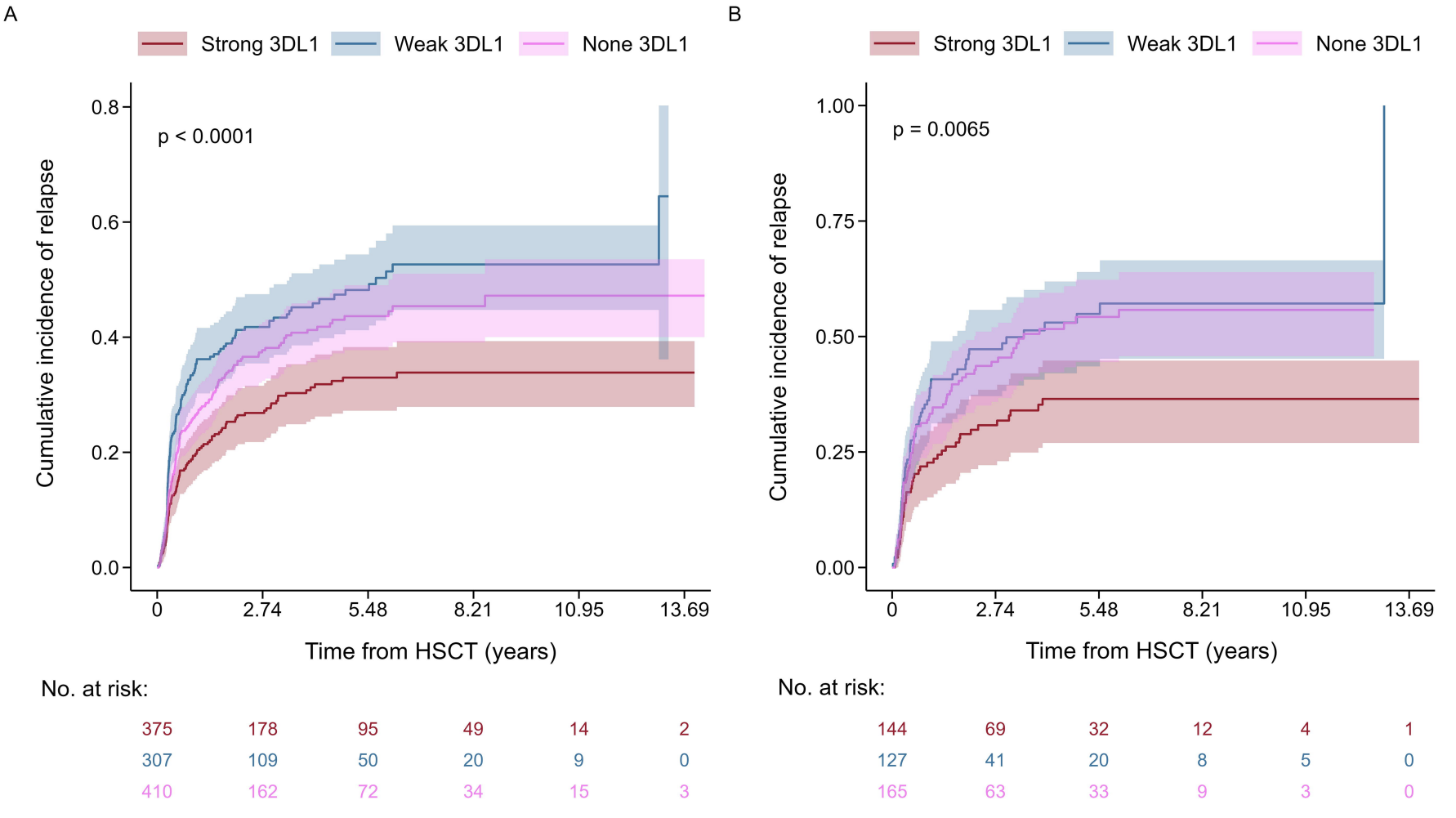
D



No. at risk:

1000	498	247	125	47	6
229	104	41	18	5	0


A
Weak
Weak/strong
strong
Missing ligand
p = 0.012
Cumulative incidence of cGvHD
0.0
0.2
0.4
0.6
0
2.74
5.48
8.21
10.95
13.69
Time from HSCT (years)
No. at risk:
408 123 49 21 7 0
227 65 27 9 2 0
310 89 31 17 6 0
171 57 23 10 2 0
B
No strong 2DL3
Strong 2DL3
Missing ligand
p = 0.0099
Cumulative incidence of cGvHD
0.0
0.2
0.4
0.6
0
2.74
5.48
8.21
10.95
13.69
Time from HSCT (years)
No. at risk:
869 260 99 44 14 0
81 19 9 4 1 0
171 57 23 10 2 0


A
Strong 3DL1
Weak 3DL1
None 3DL1
p < 0.0001
Cumulative incidence of relapse
0.0
0.2
0.4
0.6
0.8
0
2.74
5.48
8.21
10.95
13.69
Time from HSCT (years)
No. at risk:
375 178 95 49 14 2
307 109 50 20 9 0
410 162 72 34 15 3
B
Strong 3DL1
Weak 3DL1
None 3DL1
p = 0.0065
Cumulative incidence of relapse
0.00
0.25
0.50
0.75
1.00
0
2.74
5.48
8.21
10.95
13.69
Time from HSCT (years)
No. at risk:
144 69 32 12 4 1
127 41 20 8 5 0
165 63 33 9 3 0

Supplementary material

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Table of contents

Supplementary methods

1. Study cohort

The post-transplant immunosuppression protocol for GvHD prophylaxis consisted of either a calcineurin inhibitor with methotrexate or a calcineurin inhibitor with mycophenolate mofetil. Anti-thymocyte globulin (ATG) was used in 10/10 matched unrelated transplants and in 9/10 matched unrelated transplants until 2015. Post-transplant cyclophosphamide (PTCy) was used in 9/10 matched unrelated transplants from 2016 onward.

2. High-resolution *KIR* genotyping

Genomic DNA was directly extracted from whole blood samples and purified using the QIAGEN Blood and Tissue Kit according to the manufacturer's instructions. DNA purity and concentration were assessed by Qubit fluorometer. All samples were stored at 4°C until use. For library preparation, 500 ng of genomic DNA was first fragmented by digestive enzymes (New England Biolabs, Boston, MA, USA), followed by barcode ligation with unique adaptors (IDT, Coralville, Iowa, USA). After post-ligation cleanup, dual size selection was performed with AMPure magnetic beads (Beckman Coulter, Brea, California, USA) to acquire fragment sizes of 800 to 1200 bp length. In a second step, a pool of oligonucleotide probes specific for the KIR and HLA genomic regions was used for the targeted capture (1). Final enriched libraries were normalized to a concentration of 12 pmol/l. Paired-end sequencing was performed using a NovaSeq instrument with a sequencing length of 2 x 250 bp (Illumina, San Diego, CA, USA).

3. High-resolution *HLA* genotyping

High-resolution *HLA* genotyping was performed on all recipients and donors using reverse PCR-sequence-specific oligonucleotide microbead arrays, high-throughput sequencing (One Lambda, Canoga Park, CA, USA) or PCR-sequence-specific primers (Genovision, Milan Analytika AG, Switzerland).

4. *KIR* haplotype assignment

The centromeric portion is defined as *KIR* genes present in between the framework genes *KIR3DL3* and *KIR3DP1*, while the telomeric part encompasses genes from *KIR2DL4* to *KIR3DL2*. The presence of one or more of the following KIRs – *2DL2*, *2DL5*, *3DS1*, *2DS1*, *2DS2*, *2DS3* and *2DS5* – defines Bx haplotypes, whereas the presence of *2DL1*, *2DL3*, *3DL1* and *2DS4* marks A haplotypes.

5. KIR allotype assignment

KIR allotype refers to a distinct amino acid sequence and KIR alleles were grouped according to published known KIR allotypes: KIR3DL1 expression levels were classified into high, low or null as previously described (2, 3). KIR2DS4 alleles were classified into the full-length version or the truncated variant (4). KIR2DL1 alleles were segregated into strongly and weakly inhibiting based on the presence of an arginine or a cysteine group at position 245, respectively (5). Functionally stronger KIR2DL2 and KIR2DL3 alleles were defined as alleles having a glutamic acid at position 35, whereas weak alleles were defined by the presence of a glutamine at position 35 (6) (Supplemental Tables S3 and S4).

6. KIR and HLA interactions

HLA-A, -B and -C alleles were categorized according to their relevant epitopes following known classifications using the IPD database (7). KIR-HLA pairs were then summed up for each individual, with homozygous KIR or HLA alleles counted twice: HLA-C (C1 epitope) and HLA-B*46/B*73 with KIR2DL2 and KIR2DL3 (8), HLA-C (C2 epitope) with KIR2DL1 (8), HLA-C (C2 epitope) with KIR2DS1, HLA-C2 (**02:02*, **04:01, *05:01*), HLA-C1 (**01:02*, **14:02, *16:01*) and HLA-A*11 with *KIR2DS4*001* (8-10), HLA-C1 (**16*, **01:02*) and HLA-A**11:01* with KIR2DS2 (10-12), HLA-A (Bw4 epitope) and HLA-B (Bw4 epitope) with KIR3DL1 (13, 14). HLA-A25 and HLA-A23 molecules were not considered ligands for KIR3DL1 as they do not educate KIR3DL1$^+$ NK cells (15, 16). *KIR2DS5*003*, **004*, **005*, **006*, **007*, **008* with HLA-C2 (17). The interaction between KIR3DS1 and HLA-B was assumed based on its 97% sequence homology in the extracellular domain with the KIR3DL1 receptor, despite the lack of *in vitro* demonstration (18).

7. Statistical endpoints and analysis

The following covariates were tested by forward selection in univariable analysis and by backward selection to eliminate non-significant variables in multiple regression models: recipient and donor age, disease type, HLA matching, Karnofsky performance status, EBMT risk score, disease status, conditioning regimen, graft source, *in vitro* T-cell depletion, total body irradiation, comorbid conditions, donor/recipient cytomegalovirus matching, gender matching and transplant center.

Descriptive results are presented as medians and interquartile ranges (IQRs) for continuous variables and counts and percentages for categorical variables.

Table S1. *KIR* alleles characteristics of activating KIRs in the cohort (*n* = 1247).

KIR2DS1	*k*	%	% (Amorim *et al.*) *	*KIR2DS4*	*k*	%	% (Amorim *et al.*)
**null*	1951	78.23	77.63	**null*	551	22.1	22.65
**001*	1	0.04	-	**00101*	479	19.2	27.82
**00201*	462	18.52	17.42	**00104*	2	0.008	1.92
**00502*	21	0.84	0.02347	**00301*	576	23.1	14.62
**006*	31	1.24	0.28	**00401*	71	2.85	3.57
**008*	1	0.04	-	**00601*	426	17.08	12.04
**011*	2	0.08	-	**010*	359	14.4	14.34
**unresolved*	25	1.0	4.58	**016*	1	0.004	-
KIR2DS2	*k*	%	% (Amorim *et al.*)	**022*	2	0.008	-
**null*	1738	69.68	70.63	**unresolved*	27	1.08	2.89
**00101*	701	28.11	24.11	*KIR3DS1*	*k*	%	% (Amorim *et al.*)
**00102*	2	0.08	-	**null*	1950	77.97	-
**00104*	1	0.04	-	**01301*	525	21	18.87
**00106*	2	0.08	-	**014*	2	0.008	-
**00107*	1	0.04	-	**049N*	19	0.76	-
**002*	34	1.36	0.89	**1107*	1	0.004	-
**02001*	6	0.24	-	**unresolved*	4	0.16	-
**005*	1	0.04	-				
**007*	1	0.04	-				
**008*	1	0.04	0.0235				
**unresolved*	6	0.24	4.30				

*Comparative allele frequencies from the publication by Amorim *et al* .are shown (19).

Table S2. *KIR* alleles characteristics of inhibitory KIRs in the cohort (*n* = 1247).

KIR2DL1	*k*	%	% (Amorim *et al.*) *	*KIR2DL2/L3*	*k*	*%*	% (Amorim *et al.*)
**00101*	116	4.63	5.35	*3*00101*	957	38.7	38.36
**00201*	583	23.25	24.29	*3*00110*	14	0.56	1.10
**00302*	914	36.46	38.12	*3*00201*	591	23.6	25.21
**00303*	1	0.004	-	*3*003*	12	0.48	0.68
**00401*	281	11.21	11.48	*3*00501*	116	4.65	4.30
**00402*	12	0.48	0.26	*3*006*	1	0.04	0.07
**007*	34	1.36	1.17	*3*009*	3	0.12	0.07
**008*	4	0.16	0.40	*3*015*	2	0.08	0.12
**010*	1	0.04	-	*3*030*	1	0.04	0.0469
**01201*	1	0.04	-	**unresolved*	45	1.8	2.42
**01202*	2	0.08	-				
**014*	1	0.04	0.02347				
**020*	4	0.16	0.33				
**029*	1	0.04	-				
**3201*	8	0.32	0.40				
**03701*	9	0.36	-				
**040*	1	0.04	-				
**04301*	5	0.2	-				
**044*	4	0.16	-				
**049*	1	0.04	-				
**05401*	1	0.04	-				
**057*	5	0.2	-				
**063*	1	0.04	-				
**null*	425	16.95	16.76				
**unresolved*	92	3.67	1.03				
KIR2DL2/L3	*k*	%	% (Amorim *et al.*)				
*2*00101*	402	16.1	14.81				
*2*00301*	322	12.9	11.55				
*2*00303*	2	0.08	-				
*2*012*	3	0.12	0.05				
*2*unresolved*	23	0.92	-				

KIR3DL1	*k*	%	% (Amorim *et al.*) *
**00101*	385	15.4	16.38
**00103*	1	0.04	-
**00201*	286	11.43	9.46
**00401*	360	14.4	13.85
**00402*	30	1.2	1.57
**00501*	344	13.75	12.18
**00701*	64	2.56	2.68
**00801*	149	5.96	4.81
**00901*	18	0.72	0.99
**01501*	5	0.2	0.19
**01502*	149	5.96	6.24
**01702*	1	0.04	0.05
**019*	17	0.68	0.82
**02001*	59	2.36	-
**021*	1	0.04	0.05
**02901*	3	0.12	0.05
**03101*	2	0.08	-
**033*	3	0.12	0.05
**039*	1	0.04	0.16
**043*	2	0.08	0.02
**052*	1	0.04	0.05
**053*	14	0.56	-
**072*	1	0.04	0.02
**089*	1	0.04	-
**110*	1	0.04	-
** null*	43	1.72	-
** unresolved*	50	2	-

*Comparative allele frequencies from the publication by Amorim *et al* .are shown (19).

Table S3. Frequency of KIR2DS4, KIR2DL1, KIR2DL2/L3 and KIR3DL1 allotypes in the cohort according to their functional aspects (*n* = 1247).

KIR2DS4	*n*	%
Expressed/expressed	60	4.81
Expressed	361	28.9
Not expressed	808	64.8
Unresolved	18	1.44
KIR2DL1	*n*	%
Strong/strong or strong	854	68.48
Strong/weak	221	17.7
Weak/weak or weak	82	6.57
Unresolved	51	4.1
KIR2DL2/L3	*n*	%
E^{35} homozygous	157	12.6
E^{35} / Q^{35} heterozygous	545	43.7
Q^{35} homozygous	519	41.6
Unresolved	26	2.1
KIR3DL1	*n*	%
High/high or high	644	51.64
High/low	167	13.4
Low/low or low	215	17.24
Not expressed	130	10.4
Unresolved	30	2.41

E^{35}; glutamic acid at position 35, Q^{35}; glutamine at the position 35

Table S4. Stratification of *KIR2DS4*, *KIR2DL1*, *KIR2DL2/L3* and *KIR3DL1* alleles in the cohort according to their functional aspects (*n* = 1247).

KIR2DL1	Alleles	Reference
Strong	**00101, *00201, *00301, *00302, *00303, *00501, *008, *01201, *01202, *014, *010, *020, *040, *044, *049, *057, *063, *4301*	(5)
Weak	**00401, *00402, *00701,*007,*05401,*029*	
Not expressed	**03201*	
KIR2DL2/L3		
2DL2 E^{35}	**00101, *00301, *00303,*012*	(6)
2DL3 E^{35}	**00501, *015*	
2DL3 Q^{35}	**00101, *01101, *00201,*003,*00501,*006,*00701,*009,*015,*030*	
KIR3DL1		
High	**00101, *00103, *00201, *00801, *00901,*01501,*01502,*01702,*02001,*02901,*043,*052,*089*	(2, 3)
Low	**00501, *00701, *033, *03101, *053*	
Not expressed	**00401, *00402 *039, *019, *072*	
KIR2DS4		
Expressed	**00101, *00104*	(4)
Not expressed	**00301, *00401, *00601,*010, *016, *022*	

E^{35}; glutamic acid at position 35, Q^{35}; glutamine at the position 35

Table S5. Multivariable Cox regression analysis of the KIR variables tested on six different transplant outcomes (*n* = 1247).

		OS		**TRM**	
		Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities		*Adjusted for Karnofsky score, disease type, transplant center, source of transplant*	
Variable	*n*	HR (95%-CI)	*p*	HR (95%-CI)	*p*
KIR genotype					
AA	406	1		1	
Bx	841	0.71 (0.59 – 0.87)	**< 0.001**	0.73 (0.56 – 0.96)	**0.026**
Cen AA	607	1		1	
Cen Bx	640	0.76 (0.63 – 0.92)	**0.004**	0.76 (0.58 – 1.0)	0.05
Tel AA	742	1		1	
Tel Bx	505	0.96 (0.79 – 1.16)	0.69	0.93 (0.71 – 1.23)	0.63
Centromeric genotype					
AA	587	1		1	
cA01/cB01	212	0.82 (0.63 - 1.07)	0.16	0.79 (0.54 – 1.17)	0.24
cA01/cB02	276	0.72 (0.56 – 0.92)	**0.009**	0.82 (0.58 – 1.16)	0.26
cB01/x	61	0.8 (0.51 – 1.26)	0.34	0.47 (0.2 – 1.06)	0.07
cB02/cB02	40	0.78 (0.44 – 1.38)	0.39	1.1 (0.53 – 2.27)	0.8
B content score					
Neutral	860	1		1	
Better	267	1.01 (0.8 – 1.27)	0.93	1.12 (0.81 – 1.54)	0.49
Best	120	0.83 (0.59 – 1.17)	0.29	0.7 (0.44 – 1.24)	0.25
KIR2DS1 – HLA-C2					
Absence	947	1		1	
Presence	300	0.95 (0.76 – 1.18)	0.65	1.03 (0.76 – 1.41)	0.85
KIR2DS1 – HLA-C2					
Absence	947	1		1	
2DS1 – C1/x	233	0.93 (0.73 – 1.2)	0.59	0.98 (0.69 – 1.39)	0.9
2DS1 – C2/C2	67	1.00 (0.67 – 1.49)	1.0	1.21 (0.7 – 2.1)	0.49
KIR2DS2*00101 – HLA-C*16,C*01:02, A*11:01					

Absence	1035	1		1	
Presence	212	1.15 (0.9 – 1.46)	0.25	0.97 (0.68 – 1.39)	0.89
KIR2DS4*00101 – HLA-C1/C2/A11					
Absence	1000	1		1	
Presence	229	1.26 (1 – 1.59)	**0.047**	1.65 (1.2 – 2.27)	**0.002**
KIR3DS1					
Absence	779	1		1	
Presence	468	0.96 (0.79 – 1.17)	0.72	1.0 (0.76 – 1.31)	0.98
KIR3DS1 – Bw4 (HLA-B)					
Absence	946	1		1	
Presence	301	0.88 (0.71 – 1.1)	0.27	1.02 (0.75 – 1.4)	0.89
KIR2DL1 – HLA-C2					
Strong	671	1		1	
Weak	52	0.74 (0.43 – 1.28)	0.28	0.38 (0.14 – 1.03)	0.06
Missing ligand	429	1.06 (0.87 – 1.3)	0.56	0.83 (0.62 – 1.11)	0.2
KIR2DL2/L3 – HLA-C1					
≥ 1 KIR2DL2 – C1	549	1		1	
KIR2DL3 – C1	512	1.38 (1.13 – 1.69)	**0.002**	1.29 (0.96 – 1.73)	0.09
Missing ligand	186	1.0 (0.75 – 1.34)	1.0	1.2 (0.81 – 1.78)	0.36
KIR2DL2/L3 – HLA-C1					
Weak/weak	437	1		1	
Weak/strong	253	0.83 (0.65 – 1.07)	0.16	0.89 (0.62 – 1.29)	0.55
Strong/strong	342	0.72 (0.56 – 0.91)	**0.007**	0.68 (0.48 – 0.97)	**0.033**
Missing ligand	186	0.73 (0.55 – 0.99)	**0.041**	0.92 (0.62 – 0.97)	0.67
KIR2DL2/L3 – HLA-C1					
No strong 2DL3	949	1		1	
Strong 2DL3	88	0.95 (0.67 – 1.35)	0.77	0.97 (0.57 – 1.66)	0.92
Missing ligand	186	0.85 (0.65 – 1.12)	0.26	1.06 (0.73 – 1.52)	0.77
KIR3DL1 – Bw4 (HLA-B)					

Strong inhibiting	375	1		1	
Weak inhibiting	307	1.23 (0.95 – 1.59)	0.11	1.06 (0.75 – 1.51)	0.73
Non-inhibiting	410	1.05 (0.82 – 1.35)	0.69	0.83 (0.59 – 1.17)	0.3
KIR3DL1 – Bw4 (HLA-A and -B)					
Strong inhibiting	375	1		1	
Weak inhibiting	307	1.23 (0.96 – 1.59)	0.11	1.06 (0.74 – 1.51)	0.75
Non-inhibiting	434	1.06 (0.83 – 1.35)	0.65	0.83 (0.59 – 1.16)	0.27
HLA-A*24 and A*32	100	1.27 (0.88 – 1.83)	0.21	1.1 (0.67 – 1.8)	0.7
KIR3DL1 and KIR2DS1					
Strong + 2DS1	85	1		1	
Weak + 2DS1	75	1.0 (0.59 – 1.69)	1.0	1.74 (0.85 – 3.57)	0.13
None + 2DS1	112	0.86 (0.51 – 1.43)	0.56	1.08 (0.52 – 2.26)	0.84
Strong without 2DS1	290	0.92 (0.6 – 1.42)	0.72	1.35 (0.74 – 2.48)	0.33
Weak without 2DS1	232	1.22 (0.6 – 1.42)	0.37	1.21 (0.64 – 2.29)	0.56
None without 2DS1	298	1.04 (0.68 – 1.58)	0.87	1.05 (0.56 – 1.95)	0.88

		Relapse/progression		**PFS**	
		Adjusted for age of recipient, disease type, disease state, risk score, source of transplant, transplant center		*Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities, transplant center*	
Variable	*n*	HR (95%-CI)	*p*	HR (95%-CI)	*p*
KIR genotype					
AA	406	1		1	
Bx	841	1.04 (0.84 – 1.27)	0.73	0.82 (0.69 – 0.98)	**0.032**
Cen AA	607	1		1	
Cen Bx	640	0.98 (0.81 – 1.19)	0.83	0.88 (0.74 – 1.04)	0.13
Tel AA	742	1		1	
Tel Bx	505	1.02 (0.84 – 1.24)	0.83	0.96 (0.8 – 1.14)	0.63
Centromeric genotype					
AA	587	1		1	
cA01/cB01	212	1.23 (0.95 – 1.6)	0.12	1.01 (0.8 – 1.29)	0.87
cA01/cB02	276	0.88 (0.69 – 1.13)	0.32	0.83 (0.67 – 1.03)	0.09
cB01/x	61	0.95 (0.61 – 1.49)	0.83	0.72 (0.47 – 1.1)	0.13
cB02/cB02	40	1.0 (0.56 – 1.81)	0.99	1.02 (0.61 – 1.7)	0.95
B content score					
Neutral	860	1		1	
Better	267	0.99 (0.78 – 1.26)	0.95	1.09 (0.89 – 1.34)	0.41
Best	120	0.87 (0.62 – 1.23)	0.43	0.78 (0.57 – 1.06)	0.11
KIR2DS1 – HLA-C2					
Absence	947	1		1	
Presence	300	0.99 (0.79 – 1.23)	0.91	0.95 (0.78 – 1.16)	0.6
KIR2DS1 – HLA-C2					
Absence	947	1		1	
2DS1 – C1/x	233	0.97 (0.75 – 1.24)	0.78	0.94 (0.76 – 1.18)	0.62
2DS1 – C2/C2	67	1.06 (0.7 – 1.62)	0.77	0.9 6(0.67 – 1.39)	0.83

KIR2DS2*00101 – HLA-C*16,C*01:02,A*11					
Absence	1035	1		1	
Presence	212	1.17 (0.92 – 1.49)	0.19	1.15 (0.92 – 1.43)	0.22
KIR2DS4*00101 – C1/C2/A11					
Absence	1000	1		1	
Presence	229	1.25 (0.98 – 1.59)	0.07	1.39 (1.12 – 1.71)	**0.002**
KIR3DS1					
Absence	779	1		1	
Presence	468	1.04 (0.86 – 1.27)	0.67	0.99 (0.83 – 1.18)	0.88
KIR3DS1 – Bw4 (HLA-B)					
Absence	946	1		1	
Presence	301	1.0 (0.8 – 1.25)	0.97	0.97 (0.8 – 1.19)	0.8
KIR2DL1 – HLA-C2					
Strong	671	1		1	
Weak	52	0.78 (0.46 – 1.32)	0.36	0.67 (0.41 – 1.09)	0.11
Missing ligand	429	1.11 (0.91 – 1.37)	0.3	1.04 (0.86 – 1.25)	0.68
KIR2DL2/L3 – HLA-C1					
≥ 1 KIR2DL2 – C1	549	1		1	
KIR2DL3 – C1	512	1.08 (0.88 – 1.33)	0.46	1.19 (0.99 – 1.44)	0.06
Missing ligand	186	0.97 (0.72 – 1.3)	0.84	0.95 (0.73 – 1.23)	0.64
KIR2DL2/L3 – HLA-C1					
Weak/weak	437	1		1	
Weak/strong	253	0.97 (0.75 – 1.26)	0.81	0.91 (0.72 – 1.14)	0.41
Strong/strong	342	0.91 (0.71 – 1.16)	0.43	0.81 (0.65 – 1.0)	0.05
Missing ligand	186	0.89 (0.66 – 1.21)	0.46	0.79 (0.6 – 1.03)	0.08
KIR2DL2/L3 – HLA-C1					
No strong 2DL3	949	1		1	
Strong 2DL3	88	0.97 (0.67 – 1.42)	0.89	0.92 (0.66 – 1.27)	0.6
Missing ligand	186	0.93 (0.7 – 1.23)	0.61	0.86 (0.67 – 1.2)	0.23
KIR3DL1 – Bw4 (HLA-B)					

Strong inhibiting	375	1		1	
Weak inhibiting	307	1.7 (1.3 – 2.21)	**< 0.001**	1.44 (1.14 – 1.81)	**0.002**
Non-inhibiting	410	1.4 (1.09 – 1.81)	**0.009**	1.12 (0.89 – 1.4)	0.33
KIR3DL1 – Bw4 (HLA-A and -B)					
Strong inhibiting	375	1		1	
Weak inhibiting	307	1.69 (1.3 – 2.2)	**< 0.001**	1.44 (1.14 – 1.81)	**0.002**
Non-inhibiting	434	1.39 (1.08 – 1.78)	**0.011**	1.11 (0.89 – 1.38)	0.37
HLA-A*24 and A*32	100	0.88 (0.56 – 1.37)	0.56	1.08 (0.77 – 1.52)	0.65
KIR3DL1 and KIR2DS1					
Strong + 2DS1	85	1		1	
Weak + 2DS1	75	1.26 (0.73 – 2.19)	0.41	1.25 (0.77 – 2.03)	0.36
None + 2DS1	112	1.37 (0.83 – 2.27)	0.22	1.07 (0.67 – 1.7)	0.78
Strong without 2DS1	290	0.98 (0.62 – 1.55)	0.94	1.04 (0.7 – 1.56)	0.84
Weak without 2DS1	232	1.82 (1.18 – 2.82)	**0.007**	1.58 (1.06 – 2.35)	**0.025**
None without 2DS1	298	1.04 (0.9 – 2.15)	0.14	1.19 (0.8 – 1.76)	0.4

		Acute GvHD		**Chronic GvHD**		
		Adjusted for disease type, risk score, conditioning regimen, T-cell depletion, source of transplant, transplant center		*Adjusted for age of recipient, disease type, transplant center*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR genotype						
AA	348	1		377	1	
Bx	750	0.95 (0.74 – 1.21)	0.65	766	1.12 (0.91 – 1.39)	0.28
Cen AA	527	1		563	1	
Cen Bx	571	1.09 (0.87 – 1.37)	0.45	580	1.19 (0.98 – 1.44)	0.08
Tel AA	643	1		682	1	
Tel Bx	455	0.9 (0.72 – 1.14)	0.39	461	1.03 (0.83 – 1.24)	0.8
Centromeric genotype						
AA	508	1		545	1	
cA01/cB01	189	1.24 (0.91 – 1.69)	0.17	287	1.31 (1.0 – 1.7)	**0.047**
cA01/cB02	242	1.06 (0.79 – 1.43)	0.68	253	1.26 (0.99 – 1.6)	0.06
cB01/x	54	0.8 (0.43 – 1.48)	0.47	59	1.01 (0.64 – 1.6)	0.96
cB02/cB02	37	0.85 (0.43 – 1.68)	0.63	31	1.32 (0.79 – 2.21)	0.29
B content score						
Neutral	748	1		797	1	
Better	240	1.08 (0.82 – 1.43)	0.56	237	1.19 (0.95 – 1.5)	0.14
Best	110	0.78 (0.51 – 1.19)	0.25	109	0.95 (0.68 – 1.32)	0.74
KIR2DS1 – HLA-C2						
Absence	829	1		869	1	
Presence	269	1.01 (0.77 – 1.31)	0.96	274	1.07 (0.86 – 1.33)	0.55
KIR2DS1 – HLA-C2						
Absence	829	1		869	1	
2DS1 – C1/x	209	0.99 (0.74 – 1.33)	0.95	213	1.21 (0.96 – 1.52)	0.11
2DS1 – C2/C2	60	1.07 (0.65 – 1.75)	0.8	61	0.63 (0.37 – 1.05)	0.08

KIR2DS2*00101 – HLA-C*16,C*01:02, A*11						
Absence	912	1		946	1	
Presence	186	0.99 (0.74 – 1.34)	0.97	197	1.11 (0.87 – 1.42)	0.4
KIR2DS4*00101 – C1/C2/A11						
Absence	874	1		917	1	
Presence	208	1.23 (0.94 – 1.62)	0.13	208	1.29 (1.02 – 1.64)	**0.035**
KIR3DS1						
Absence	679	1		716	1	
Presence	419	0.9 (0.71 – 1.14)	0.38	427	1.06 (0.87 – 1.29)	0.54
KIR3DS1 – Bw4 (HLA-B)						
Absence	832	1		716	1	
Presence	266	0.96 (0.73 – 1.27)	0.79	427	1.03 (0.82 – 1.28)	0.84
KIR2DL1 – HLA-C2						
Strong	586	1		619	1	
Weak	48	0.68 (0.35 – 1.35)	0.27	49	1.13 (0.7 – 1.8)	0.62
Missing ligand	379	0.94 (0.73 – 1.2)	0.62	397	1.05 (0.85 – 1.29)	0.64
KIR2DL2/L3 – HLA-C1						
≥ 1 KIR2DL2 – C1	492	1		497	1	
KIR2DL3 – C1	447	0.92 (0.72 – 1.18)	0.54	475	0.84 (0.68 – 1.03)	0.09
Missing ligand	159	0.94 (0.67 – 1.34)	0.74	171	0.69 (0.5 – 0.94)	**0.018**
KIR2DL2/L3 – HLA-C1						
Weak/weak	380	1		408	1	
Weak/strong	227	1.38 (1.01 – 1.87)	**0.042**	227	1.36 (1.05 – 1.76)	**0.02**
Strong/strong	309	1.08 (0.81 – 1.46)	0.59	310	1.27 (1.0 – 1.62)	**0.049**
Missing ligand	159	1.06 (0.74 – 1.53)	0.75	171	0.87 (0.63 – 1.2)	0.4
KIR2DL2/L3 – HLA-C1						
No strong 2DL3	844	1		869	1	
Strong 2DL3	77	1.13 (0.73 – 1.74)	0.58	81	1.59 (1.14 – 2.22)	**0.006**
Missing ligand	159	0.95 (0.69 – 1.32)	0.78	171	0.77 (0.57 – 1.04)	0.09
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	330	1		342	1	
Weak inhibiting	276	1.06 (0.78 – 1.44)	0.72	279	1.11 (0.86 – 1.43)	0.44

Non-inhibiting	355	1.08 (0.81 – 1.44)	0.62	382	0.99 (0.77 – 1.26)	0.95
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	330	1		342	1	
Weak inhibiting	276	1.05 (0.77 – 1.43)	0.77	279	1.1 (0.86 – 1.42)	0.45
Non-inhibiting	375	1.05 (0.79 – 1.4)	0.73	405	0.99 (0.78 – 1.25)	0.91
HLA-A*24 and A*32	90	1.01 (0.66 – 1.56)	0.95	88	0.92 (0.63 – 1.37)	0.7
KIR3DL1 and KIR2DS1						
Strong + 2DS1	78	1		79	1	
Weak + 2DS1	67	0.91 (0.47 – 1.76)	0.77	68	1.52 (0.92 – 2.51)	0.1
None + 2DS1	98	1.17 (0.66 – 2.08)	0.59	102	1.07 (0.65 – 1.75)	0.8
Strong without 2DS1	252	0.97 (0.59 – 1.58)	0.89	263	1.1 (0.72 – 1.68)	0.67
Weak without 2DS1	209	1.06 (0.65 – 1.75)	0.81	211	1.08 (0.7 – 1.68)	0.73
None without 2DS1	257	1.0 (0.61 – 1.64)	0.99	280	1.07 (0.7 – 1.64)	0.77

Cen; centromeric, CI; confidence interval, GvHD; graft-versus-host disease, HR; hazard ratio, OS; overall survival, PFS; progression-free survival, Tel; telomeric, TRM; transplant-related mortality

Table S6. Multivariable cox regression analysis of KIR3DL1 and KIR2DS1 interactions tested on six different transplant outcomes in the AML subcohort (*n* = 498).

		Acute GvHD		**Chronic GvHD**		
		Adjusted for disease type, risk score, conditioning regimen, T-cell depletion, source of transplant, transplant center		*Adjusted for age of recipient, disease type, transplant center*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.17 (0.78 – 1.76)	0.87	127	1.17 (0.78 – 1.76)	0.45
Non-inhibiting	165	1.31 (0.9 – 1.91)	0.86	165	1.31 (0.9 – 1.91)	0.16
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	0.96 (0.6 – 1.62)	0.88	127	1.1 (0.86 – 1.42)	0.51
Non-inhibiting	172	0.95 (0.58 – 1.56)	0.84	172	0.99 (0.78 – 1.25)	0.23
HLA-A*24 and A*32	42	2.18 (1.19 – 3.99)	**0.01**	42	0.92 (0.63 – 1.37)	0.98
KIR3DL1 and KIR2DS1						
Strong + 2DS1	38	1		38	1	
Weak + 2DS1	32	0.65 (0.25 – 1.7)	0.38	32	1.33 (0.62 – 2.85)	0.46
None + 2DS1	47	0.7 (0.29 – 1.66)	0.41	47	1.63 (0.82 – 3.24)	0.16
Strong without 2DS1	106	0.58 (0.27 – 1.23)	0.15	106	0.97 (0.51 – 1.84)	0.93
Weak without 2DS1	95	0.66 (0.32 – 1.38)	0.27	95	1.07 (0.56 – 2.06)	0.84
None without 2DS1	118	0.64 (0.31 – 1.31)	0.22	118	1.14 (0.61 – 2.14)	0.67

		Relapse/progression		**PFS**		
		Adjusted for age of recipient, disease type, disease state, risk score, source of transplant, transplant center		*Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities, transplant center*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.8 (1.21 – 2.6)	**0.004**	127	1.37 (0.96 – 2.0)	0.08
Non-inhibiting	165	1.7 (1.16 – 2.4)	**0.006**	165	1.28 (0.92 – 1.8)	0.15
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.77 (1.2 – 2.6)	**0.004**	127	1.37 (0.96 – 2.0)	0.08
Non-inhibiting	172	1.63 (1.13 – 2.4)	**0.009**	172	1.24 (0.89 – 1.7)	0.2
HLA-A*24 and A*32	42	0.59 (0.28 – 1.3)	0.168	42	0.73 (0.4 – 1.3)	0.31
KIR3DL1 and KIR2DS1						
Strong + 2DS1	38	1		38	1	
Weak + 2DS1	32	1.2 (0.49 – 3.1)	0.659	32	0.9 (0.42 – 1.9)	0.78
None + 2DS1	47	2.0 (0.91 – 4.4)	0.086	47	1.16 (0.59 – 2.3)	0.66
Strong without 2DS1	106	1.6 (0.75 – 3.3)	0.23	106	1.14 (0.63 – 2.1)	0.66
Weak without 2DS1	95	3.1 (1.53 – 6.3)	**0.002**	95	1.84 (1.01 – 3.4)	**0.046**
None without 2DS1	118	2.5 (1.25 – 5.2)	**0.01**	118	1.54 (0.86 – 2.8)	0.15

		OS		**TRM**		
		Adjusted for Karnofsky score, disease state, disease type, presence of comorbidities		*Adjusted for Karnofsky score, disease type, transplant center, source of transplant*		
Variable	*n*	HR (95%-CI)	*p*	*n*	HR (95%-CI)	*p*
KIR3DL1 – Bw4 (HLA-B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.39 (0.95 – 2.0)	0.09	127	0.8 (0.43 – 1.5)	0.49
Non-inhibiting	165	1.23 (0.85 – 1.8)	0.26	165	0.72 (0.4 – 1.28)	0.26
KIR3DL1 – Bw4 (HLA-A and -B)						
Strong inhibiting	144	1		144	1	
Weak inhibiting	127	1.4 (0.95 – 2.0)	0.09	127	0.8 (0.43 – 1.48)	0.47
Non-inhibiting	172	1.21 (0.84 – 1.7)	0.3	172	0.68 (0.38 – 1.2)	0.18
HLA-A*24 and A*32	42	0.82 (0.43 – 1.6)	0.57	42	0.69 (0.26 – 1.81)	0.45
KIR3DL1 and KIR2DS1						
Strong + 2DS1	38	1		38	1	
Weak + 2DS1	32	0.71 (0.32 – 1.6)	0.4	32	1.43 (0.46 – 4.47)	0.54
None + 2DS1	47	0.8 (0.39 – 1.6)	0.54	47	0.94 (0.31 – 2.82)	0.91
Strong without 2DS1	106	0.79 (0.42 – 1.5)	0.46	106	1.37 (0.55 – 2.43)	0.5
Weak without 2DS1	95	1.39 (0.75 – 2.6)	0.29	95	0.88 (0.32 – 2.47)	0.8
None without 2DS1	118	1.16 (0.63 – 2.1)	0.64	118	0.9 (0.34 – 2.37)	0.83

CI; confidence interval, GvHD; graft-versus-host disease, HR; hazard ratio, OS; overall survival, PFS; progression-free survival, TRM; transplant-related mortality

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