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Selection of Allogeneic Hematopoietic Cell Transplant Donors to Optimize Natural Killer Cell Alloreactivity

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Abstract

Natural killer (NK) cells are potent mediators of the graft *versus* leukemia (GVL) phenomenon critical to the success of allogeneic hematopoietic cell transplantation (allo HCT). Central to calibrating NK effector function via their interaction with class I human leukocyte antigens (HLA) are the numerous inhibitory killer Ig-like receptors (KIR). The KIR receptors are encoded by a family of polymorphic genes, whose expression is largely stochastic and uninfluenced by *HLA* genotype. These features provide the opportunity to select hematopoietic cell donors with favorable *KIR* genotypes that confer enhanced protection from relapse via NK-mediated GVL. Over the last two decades, a large body of work has emerged examining the use of *KIR* genotyping to stratify potential donors based on anticipated NK alloreactivity. Overall, these results support *KIR*-based donor selection for patients undergoing allo HCT for a diagnosis of acute myelogenous leukemia (AML). Despite this, the underlying factors that control NK cell responsiveness are not completely understood, and opportunities remain to refine donor selection using NK cell receptor genotyping. In this review, we will summarize the relevant findings with respect to *KIR* genotyping as a selection parameter for allogeneic hematopoietic cell donors and address practical considerations with respect to *KIR*-based selection of donors for patients with myeloid neoplasia.

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1.0 Introduction

Natural killer cells are lymphocytes defined by their innate capacity to recognize and exert cytotoxicity against malignant or virally infected cells without prior sensitization.[1–3] This attribute distinguishes NK cells from T and B cells, which rely on receptor gene rearrangement for antigen specificity and expansion of antigen-experienced clones to effect adaptive immunity. Recently, the discovery of “memory” populations of NK cells that form after pathogenic stimuli, as well as the recognition that NK cells play a role in recruiting and maintaining other lymphocytes at the immune reaction site, have blurred the line between the innate and adaptive immune systems.[4–7] It is clear that NK cells are a surprisingly diverse family of specialized cell types, responsible for protection from viral infection, maintenance of the placental immune barrier, and recognition of cells that have undergone malignant transformation.[8–10] This latter property has spurred significant interest in NK cells as an agent of cancer immunotherapy, either via adoptive transfer or in the context of allogeneic hematopoietic cell transplantation.

Central to NK cell immunity is the detection of cellular stress in target cells, whereby loss of HLA class I combined with increased expression of NK activating receptor ligands on the target cell tilts the NK cells towards cytotoxicity.[11–13] Discovery of the Ly49 receptor family in mice and the killer Ig-like receptor (KIR) family in humans led to the first understanding of how interaction between inhibitory receptors on the NK cell and HLA class I molecules on adjacent cells maintains NK tolerance to normal cells, but unleashes cytotoxicity abnormal cells with altered HLA expression.[14, 15] Many viral species induce HLA loss in infected cells as a mechanism for T-cell immune evasion, thus sensitizing the target cell to NK mediated immunity.[16] Cancer cells as well may downregulate or lose HLA class I expression [17–19], but readily upregulate HLA class I expression in a minimally inflamed setting [20, 21], causing NK cells in this setting to rely on increased activating signals to trigger cytotoxicity. Sensing immune pressure, malignant cells may then down-regulate NK activating receptor ligands.[22, 23] These observations provide compelling evidence for the narrow margin for cytotoxic response created by simultaneous inhibitory and activating interactions between the target cell and the NK cell. In this review, we will explore how natural variations in inhibitory and activating signaling can be used to manipulate the threshold for NK activation in the setting of HLA-expressing target cells.

Effectively the first cellular therapy to control cancer, allogeneic hematopoietic cell transplantation (allo HCT) exerts a graft-versus-leukemia (GVL) effect mediated by alloreactive donor lymphocytes. A growing body of clinical evidence supports a significant contribution of NK cells to the GVL phenomenon in patients with acute myeloid leukemia (AML)[24], and increasingly, NK cells have demonstrated potential as a platform for adoptive cellular therapies[25–28]. Adoptively transferred allogeneic NK cells thus far have demonstrated limited efficacy, however, in part due to poor *in vivo* persistence.[29] Providing a continuous renewal of allogeneic NK cells, allo HCT remains the most effective means by which to provide durable NK alloreactivity and disease control. Moreover, it is increasingly clear that promoting NK activation does not induce graft *versus* host disease (GVHD) in allo HCT recipients, offering the possibility of harnessing a GVL effect without inciting GVHD. The use of immunogenetic biomarkers, such as those encoding KIR and

their HLA ligands, to predict NK alloreactivity is a cost-effective and increasingly available means in the selection of donors to improve tumor control and transplant outcomes.

2.0 KIR: a diverse receptor family responsible for calibrating NK cell responsiveness

KIR receptors are a diverse group of cell surface receptors represented by both inhibitory and activating isoforms. Collectively, KIR are central to the NK cell maturation process and are important in maintaining and calibrating NK cell effector function in the peripheral tissues.[30–32] Inhibitory and activating KIR receptors are encoded by 14 different genes clustered with pseudogenes in the leukocyte receptor complex on chromosome 19q13.4.[33] While a diversity of gene content can populate the *KIR* gene cluster, two broad haplotype groups exist: the canonical “A” haplotype with fixed *KIR* gene content enriched for genes encoding inhibitory KIR, and the variable “B” haplotype group, comprised of haplotypes diverse in number of *KIR* genes and rich in genes encoding activating KIR.[33, 34] Allelic polymorphism, particularly among the inhibitory *KIR* genes, lends an additional layer of diversity.[35] Considerable differences in *KIR* genotype therefore exist between individuals, both at the level of gene content and allelic identity. Importantly, because the genetic regions encoding KIR and their HLA ligands are located on distinct chromosomes and segregate according to classic Mendelian patterns of inheritance, individuals do not always inherit the genes encoding HLA ligands for their given KIR; and, likewise, others may exhibit HLA for which they lack the genes encoding cognate KIR. In the context of allo HCT donors, therefore, *KIR* genotype cannot be imputed from *HLA* genotype, and donors with similar *HLA* genotypes will frequently have very different *KIR* genotypes.

Interaction between inhibitory KIR and their cognate HLA ligands not only prevents auto-aggression of NK cells toward autologous cells, it is also critical for the “education” of NK cells for effector function, whereby cells expressing KIR for self-HLA ligands are more responsive to NK stimulation.[32] Expression of KIR is largely stochastic, leading to NK populations within the NK repertoire with widely differing capacities for response.[36, 37] The inhibitory KIR include KIR3DL1, which interacts with the Bw4 epitope on HLA-B and some HLA-A allotypes; KIR2DL2 and KIR2DL3, which recognize the HLA-C group 1 allotypes (C1) characterized by Ser77/Asn80; and KIR2DL1, which recognizes the HLA-C group 2 allotypes (C2) characterized by Asn77/Lys80.

Educated NK cells, which express inhibitory KIR for autologous HLA class I, are far more reactive to target cells in both *in vitro* and *in vivo* models compared to uneducated NK cells, which express KIR for which the individual lacks the HLA ligand.[38–40] Even among educated NK cells, those with a high degree of inhibitory signal from self-HLA are even more capable of effector function against transformed or infected cells, but are also more easily inhibited upon engagement of cognate HLA ligand on the same target cell.[41, 42] It is important to note that under highly inflammatory conditions, uneducated NK cells can overcome this deficit to become reactive against target cells, contributing to the immune response.[43] While NK education is central to establishing an NK cell’s threshold for

response, it is still “tunable” by alterations in the HLA environment, one aspect of a complex and still poorly understood process of establishing the response capacity of an NK cell.[44]

3.0 Clinical significance of HLA and KIR interaction in *HLA*-mismatched allo HCT

The clinical significance of donor *KIR* genotype diversity first became apparent in the context of HLA-haploidentical transplantation using CD34⁺-selected allograft products (Table 1).[45, 46] In this setting, it was reported that absence of donor *KIR* ligand in the recipient was associated with decreased relapse of myeloid neoplasia following allo HCT, fulfilling the “missing self” mechanism of NK activation. Isolated donor NK cells expressing cognate *KIR* for the missing donor *KIR* ligand in the patient demonstrated reactivity to recipient leukemia cells, supporting NK tumor toxicity due to missing-self and revealing the previously unrecognized contribution of NK cells to the graft *versus* leukemia (GVL) phenomenon.

The recent introduction of post-transplant administration of cyclophosphamide (PT-Cy) as a GVHD prophylaxis strategy has obviated the use of isolated hematopoietic progenitor cells in HLA-mismatched adult donor allo HCT. The underlying hypothesis of PT-Cy is to activate and then selectively eliminate alloreactive T-cells by withholding immune suppression for three days after the allograft infusion prior to administration of PT-Cy on days 3 and 4 post-transplant. Recently published data in PT-Cy recipients suggest that this strategy also eliminates alloreactive NK cells, potentially dampening the overall contribution of the NK repertoire to GVL.[47] It is important to note, however, that in this study a robust recovery of *KIR*⁺ NK cells was still protective from relapse. Together, these findings suggest that PT-Cy based GVHD prophylaxis may be improved with strategies that either promote NK immune reconstitution or supplement the NK repertoire with donor NK cells infused after PT-Cy. At the current time, the data are insufficient to support *KIR*-based selection of haploidentical related donors when using a PT-Cy based approach, outside of a clinical trial.

Umbilical cord blood (UCB)-derived progenitor cells are another graft source to facilitate HLA-mismatched allo HCT. The role of *KIR* ligand mismatching in the graft *versus* host (GvH) direction has been tested in several relatively large, retrospective studies, yielding inconsistent results.[48–51] For example, a retrospective study of 218 patients with acute leukemia undergoing single-unit UCB performed by the European Society for Blood and Marrow Transplantation (EBMT)/Eurocord-NetCord consortium demonstrated protection from relapse in recipients that underwent HCT using a *KIR* ligand mismatched donor. Three other studies of similar size, however, did not identify a benefit from *KIR*-ligand mismatched UCB donors. Indeed, data from the single center study performed at the University of Minnesota suggested an association between *KIR* ligand mismatching in the GvH direction and increased acute GVHD in recipients of reduced intensity conditioned allo HCT from double UCB recipients.[51] Despite the association with increased GVHD, *KIR* ligand mismatching did not provide protection from relapse in this study. Finally, the impact of *KIR* allelic polymorphism on UCB allo HCT outcomes remains largely unexplored.

Overall, significant heterogeneity of clinical and transplant-specific parameters in UCB allograft recipients limits broad conclusions from these collective results.

4.0 *KIR*-based selection of *HLA*-matched adult unrelated donors

Facilitated by the growth of volunteer donor registries, unrelated donor (URD) transplantation has increased dramatically over the past four decades.[46] Between 2013 – 2017, as reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) registry, 12,683 URD transplants were performed in the United States for the diagnosis of AML or MDS.[52] Allele matching for the *HLA-A*, *HLA-B*, *HLA-C*, and *HLA-DRB1* loci is now the standard of care to define an “8/8 *HLA*-matched” URD.[53] The likelihood of having an available 8/8 *HLA*-matched URD is highly dependent on the overall diversity of *HLA* haplotypes within the patient population, resulting in wide disparities of donor availability for any given patient.[54] Among 528 patients with AML who underwent a formal URD search at our center, 77 (14.6%) had only one evaluated URD whereas 389 (73.7%) patients had three or more URDs evaluated for allo HCT. These numbers illustrate the underlying reality of URD availability: many patients have no matched donors available, but among those that do have at least one URD available, most will have several donors from whom to choose.

URD transplants matched for *HLA* genotype are, by definition, also *KIR*-ligand matched, precluding donor NK alloreactivity due to missing-self activation. Most individuals lack at least one *HLA* ligand for their cognate *KIR* (e.g. individuals homozygous for *HLA-C1* lack the *HLA-C2* ligand for *KIR2DL1*). In the context of allo HCT, this so-called “missing ligand” circumstance leaves some donor NK cells uneducated but also uninhibited upon contact with malignant cells.[43] The realization that uneducated NK cells can induce a potent GVL effect led to the hypothesis that recipient missing-ligand may be beneficial in the context of allo HCT. Several large scale observational studies have demonstrated that recipients missing one of the *KIR* ligands among *HLA-C1*, *-C2*, or *-Bw4* had lower incidence of relapse and improved relapse-free survival after *HLA*-matched allo HCT (Table 1).[55–57] Again, the observed benefit was confined to recipients with a diagnosis of AML, supporting the unique role of NK cells in the GVL effect against this disease.

4.1 Activating *KIR*-based donor selection: *KIR* haplotypes and *KIR2DS1/HLA-C1*

HLA genotype in individuals is fixed and therefore cannot be manipulated to induce missing self or missing ligand donor NK activation in HCT. In contrast, however, donor *KIR* genotypes can vary greatly, leading investigators to examine whether donor *KIR* genotypes might increase allogeneic NK response to leukemia in HCT. Among the two *KIR* haplotype groups, the *KIR*-haplotype A contains only one activating *KIR* gene, *KIR2DS4*, which is frequently represented by an allele encoding a truncated non-signaling mutant.[58] In contrast, the *KIR* haplotype-B group possesses a more diverse membership, with haplotypes containing up to six activating *KIR* genes, including *KIR3DS1*, *KIR2DS1*, *KIR2DS2*, *KIR2DS3*, *KIR2DS4*, and *KIR2DS5*. Although the ligands for many of the activating *KIR* remain unknown, a reasonable hypothesis posits that donors with B haplotypes may result in a global NK repertoire with a lower threshold for activation, increasing the likelihood of a

successful GVL response, and preventing leukemia relapse following allo HCT. Several older retrospective studies using CIBMTR registry data support this hypothesis, with some evidence that donors with B haplotypes with the centromeric-residing *KIR2DS2* (*cenB*) are associated with relapse protection, particularly donors homozygous for *cenB* (*cenBB*). [59–62] An evaluation of patients treated in the modern era (2010–2016), could still demonstrate an effect of *KIR* haplotype-B on AML relapse, but only in patients receiving non-myeloablative conditioning regimens. [59] A prospective study evaluating donor selection based on *KIR* haplotypes has been completed for unrelated donor transplantation (NCT01288222); while initial results suggest that *KIR* haplotype-based donor selection is feasible, efficacy results from this study are pending. [63] Finally, retrospective studies in UCB allo HCT did not identify an impact of UCB donor haplotype B content on unit dominance or transplant outcomes following double-unit UCB transplantation. [64, 65]

KIR2DS1, found in the telomeric portion of B-haplotypes, encodes the only activating *KIR* known to contribute to NK education. [38, 66] *KIR2DS1*⁺ NK cells are progressively hyporesponsive in the presence of increasing amounts of ligand HLA-C2 in the environment. [67] Consequently, *KIR2DS1*⁺ NK cells from HLA-C2 homozygous individuals are tolerized and hyporesponsive to an HLA-C2⁺ target. [68] Venstrom and colleagues found in 1,272 patients undergoing transplant for AML that the presence of *KIR2DS1* in allo HCT donors was protective from relapse, but only in the context of *HLA-C1/x* donors and not in the context of *HLA-C2* homozygous donors. [69] Interestingly, donor *HLA-C2* homozygosity also reverses the beneficial effects of donor *KIR* B-haplotype independent of *KIR2DS1*, suggesting that an environment rich in HLA-C2 ligands is generally suppressive toward NK cells with activating *KIR*. [62, 69, 70]

Donor activating *KIR* may have impacts on transplant outcomes separate from AML relapse. Donors possessing *KIR3DS1* were associated with protection from acute GVHD, but did not affect relapse in a population of 1,087 patients with AML undergoing *HLA* well-matched URD allo HCT. [71] An activating isoform of *KIR3DL1*, *KIR3DS1* binds to HLA-F open conformers in lieu of HLA-Bw4, leading to an uneducated status and insensitivity toward HLA-Bw4 for *KIR3DS1*⁺ NK cells. [72]

4.2 *KIR3DL1* allele-based donor selection

KIR3DL1 and *HLA-B* alleles encoding the Bw4 epitope are the most polymorphic of the *KIR/KIR* ligand pairs, and different pairings of each exhibit significant differences in NK education and strength of inhibition. [73–76] *KIR3DL1* allotypes may be organized into biologically relevant subtype groups based on surface expression: *KIR3DL1*-h subtypes (such as those encoded by *KIR3DL1**001, *002 and *015) are highly expressed on the NK cell surface; *KIR3DL1*-l subtypes (including those encoded by *KIR3DL1**005 and *007) are weakly expressed on the NK cell surface; and the *KIR3DL1*-null allotype (encoded by *KIR3DL1**004) are retained intracellularly (Figure 2). [73–77] *KIR3DS1* is considered a *KIR3DL1* activating variant that does not have measurable binding to HLA-Bw4. As a general principle, *KIR3DL1*-h subtypes encode receptors that bind with higher affinity to HLA-Bw4⁺ subtypes with Ile⁸⁰ (Bw4–80I) compared to HLA-Bw4⁺ subtypes with Thr⁸⁰ (80T). Consequently, NK cells bearing *KIR3DL1*-h receptors are more sensitive to

inhibition by target cells expressing Bw4–80I ligands. In contrast, NK cells bearing KIR3DL1-I subtypes demonstrate higher inhibition with Bw4–80T allotypes.[42] Finally, neither the KIR3DL1-n allotype nor KIR3DS1 is inhibited by HLA-Bw4 on the target cell. This range of NK education and inhibitability among the KIR3DL1 subtypes for the different HLA-Bw4 subtypes presents an opportunity for selecting donors whose KIR3DL1 receptors will signal the least inhibition upon encountering patient HLA-Bw4.

Two large-scale retrospective studies have examined the role of KIR3DL1/HLA-Bw4 subtype interactions in allo HCT (Table 1). The first was an analysis of 1,328 AML patients undergoing HCT from 9/10 and 10/10 *HLA*-matched unrelated donors conducted by our group and facilitated by the National Marrow Donor Program and CIBMTR.[41] In this population, donors with weakly interacting or low inhibition KIR3DL1-Bw4 subtype combinations were protective from relapse and beneficial for overall survival, even after adjustment for other KIR interactions. A more recent analysis conducted by Schetelig and colleagues in the German Cooperative Transplant Study Group and the Deutsches Register für Stammzelltransplantationen failed to demonstrate a low inhibition KIR3DL1/HLA-Bw4 benefit, or any other KIR-related benefit, in 2,222 patients transplanted for AML.[78] It is important to note that in the latter study, lower-intensity conditioning and the use of anti-thymocyte globulin were more common than in studies evaluating patients treated primarily in the United States. It is important to note that the above analyses only examined the contribution of HLA-B allotypes with the Bw4 KIR epitope, but several HLA-A allotypes also exhibit the Bw4 epitope. A recent study by van der Ploeg and colleagues demonstrated that the Bw4⁺ HLA-A allotype HLA-A*24 is capable of strong inhibition of KIR3DL1⁺ NK cells.[79] Furthermore, in 1,729 recipients of an HLA well-matched URD allo HCT, the combination of *HLA-A*24* and donor *KIR3DL1* was associated with increased incidence of AML relapse. These findings suggest that including the contribution of Bw4⁺ HLA-A allotypes may refine the discriminatory capability of this model in future analyses. At the current time, it is unclear whether *KIR3DL1/HLA-Bw4* may be used as a predictive biomarker for donor selection. Results of a prospective study evaluating donor selection based on a tiered framework prioritizing KIR3DL1/HLA-Bw4 interactions (NCT02450708) will add some clarity to this issue.

5.0 The NKG2 family of receptors

Apart from KIR, members of the NKG2 cell surface receptors may also contribute to NK education. The inhibitory NKG2A and the activating NKG2C receptors form heterodimers with CD94, and the heterodimeric pairs bind HLA-E on target cells to convey their respective signals. NKG2A functions similarly to inhibitory KIR in that interaction with its HLA ligand contributes to NK cell education and inhibition.[30, 80] The availability of a blocking antibody to NKG2A and its prevention of interaction with HLA-E and the ensuing inhibitory signaling, presents an alternative pathway by which to recruit NKG2A⁺ NK cells for cytotoxicity.[81] Polymorphism in *HLA-E* or in its peptide, the leader sequence of HLA class I, may also influence clinical outcomes via NK cells expressing NKG2A.[82, 83] In a retrospective study involving 1,840 recipients of 10/10 *HLA*-matched unrelated donor allografts, Tsamadou and colleagues demonstrated that donor homozygosity for the more highly expressed *HLA-E*01:03* allele is associated with decreased disease-free survival and

increased treatment related mortality.[84] These results are conflicted by a smaller study conducted by Hosseini and colleagues, where *HLA-E*01:03* homozygosity was associated with decreased relapse and improved disease-free survival.[85] Overall, further studies are needed to understand how *HLA-E*, and potentially *NKG2A*, genotypes can be used to guide donor selection for allo HCT before this approach can be used in clinical practice.

6.0 Practical considerations when using *KIR* to select unrelated donors

A common and practical concern for the transplant physician is whether a “good *KIR*” donor is available only at the expense of other parameters that may also influence transplant outcome, such as donor age, parity, or ABO blood type. A second consideration is whether *KIR* allele or gene typing will delay donor workup, thereby exposing the patient to increased risk of relapse. To address these questions, we prospectively type all *HLA*-matched/single-antigen mismatched URDs evaluated for patients undergoing allo HCT workup with a diagnosis of MDS or AML for *KIR* genes and *KIR3DL1* subtype. Since 2013, our center has performed *KIR* genotyping typing for 2,079 potential donors for 527 patients. We found that for *HLA-Bw4*⁺ recipients, the probability of having a donor with low-inhibition *KIR3DL1*/*HLA-Bw4* interaction was 43% if only one donor was available for typing but increased significantly to 79.6% if three donors were typed. Among *HLA-C1*⁺ recipients, the probability of having a *KIR2DS1*⁺ or a *cenBB* donor was 45% and 11%, respectively, if only one donor was typed, and increased to 69% and 22%, respectively, if three donors were typed. Importantly, there was no difference in the time from formal search initiation to date of transplant in patients with advantageous donors compared to those without advantageous donors. The median time at our center from search to transplant was 86.5 days (32 – 1408 days) in patients with low-inhibition *KIR3DL1*/*HLA-Bw4* donors compared to 88 days (21 – 975 days) in patients with high or neutral inhibition donors. Furthermore, there was no difference in donor age or CMV seropositivity between these two groups. These results suggest that *KIR* genotyping can be integrated into the URD search without increasing time to transplant or altering other donor parameters that may be important to the treating clinician.

6.1 Where should we place *KIR* when selecting an unrelated donor?

Selection of unrelated donors is complicated by a wide diversity of factors that may guide the clinician’s choice. Recently, a CIBMTR study examined what donor-specific factors are most likely to influence outcomes in allo HCT.[86] In this analysis, which did not include donor *KIR* genotype, only donor age was predictive of survival outcomes. Other factors, such as donor parity, ABO, or CMV status could influence quality of life due to their impacts on transplant-specific outcomes, but none of them altered the key outcome of overall survival. The mixed data supporting *KIR*-based URD selection for patients with AML suggest that previously unrecognized factors may alter *KIR*-mediated NK effects. Indeed, some of these factors are only now being identified: NK alloreactivity due to missing self in haploidentical HCT is severely blunted in the age of post-transplant cyclophosphamide [47]; *HLA-C2* homozygosity erases the effect of activating *KIR* on HCT outcomes [62, 69]; and idiosyncrasies of conditioning regimens may favor or diminish the impact of alloreactive NK cells.[59] As we continue to seek to identify additional factors

that compromise NK alloreactivity, the available evidence indicates that an alloreactive NK benefit is not only possible, but that taking steps to capture this effect does not compromise outcomes.

Presently, there are no standardized therapeutic interventions for reducing the risk of relapse following HCT, a major cause of transplant failure that accounts for nearly 50% of late mortality.[87] Given the potential benefit of durable disease control and relapse prevention, *KIR*-based donor selection is an appropriate, cost-effective intervention to implement while we await results of prospective studies. Moreover, unlike other maintenance strategies, it does not require patient compliance and does not impose adverse effects.

7.0 Role of NK immunogenetics in tailoring adoptive cellular therapies

NK cells are a natural platform for development of immune effector cellular therapies (IECTs). Due to their lack of rearranged, HLA-specific cell surface receptor expression *HLA*-mismatched cells may be infused without the consequence of severe GVHD. A promising example of this approach was demonstrated by Liu and colleagues, who transfected UCB-derived NK cells with genes encoding a CD19 specific chimeric antigen receptor and IL-15, as well as an inducible caspase-9 suicide gene.[88] In an early phase clinical trial, these investigators reported an overall response rate of 73% in 11 patients with CD19⁺ malignancy treated with this cell product.[89] Donors were selected to maximize the potential of missing-self given the respective KIR ligand profile of the recipient, but the small number of patients treated precludes any conclusions as to whether this selection was beneficial. Similarly, Ciurea and colleagues did not find a benefit to *KIR* haplotype-based selection of donors used to generate a membrane-bound IL-21 *ex vivo* expanded haploidentical NK cell product used in conjunction with allo HCT from the same donor, when PT-Cy-based GVHD prophylaxis was used.[90] Lee and colleagues found a non-statistically significant benefit from both *KIR* haplotype B content and KIR-ligand mismatched donors with infusion of third-party haploidentical related donor NK cells prior to HLA-matched allo HCT.[91] Bachanova and colleagues did not find an association between KIR-ligand mismatch and response in patients with refractory AML treated with haploidentical NK cells with or without T-regulatory cell depleting IL-2/diphtheria toxin conjugate.[92] Romee and colleagues found that a cytokine induced memory-like NK cell, generated via brief activation step with IL-12, IL-15, and IL-18, were reactive against AML cell line targets *in vitro* regardless of expression of KIR.[28] These results collectively suggest that pro-inflammatory manipulation to engineered IECT products may overcome any inhibitory KIR-HLA signaling, obviating the need to select NK-IECT donors based on this parameter. More data from clinical trials are required to address this question.

8.0 Concluding remarks

The past three decades have witnessed a rapid increase in our understanding of NK cell biology, and in particular, the role of *HLA* class I and *KIR* in calibrating NK cell effector function. Following biologically based hypotheses, there are now multiple retrospective clinical studies that support the beneficial impact of *KIR* and *HLA*-based URD selection in the context of allo HCT. The most proximal goal of future work will to be to define how NK

immunogenetics can be used in prospective donor selection, with the knowledge that other considerations will compete for priority in the choice of a final donor. Looking forward, the use of PT-Cy-based GVHD prophylaxis will likely increase in both *HLA*-matched and -mismatched URD, as will the use of haploidentical related donor allo HCT. A major challenge exists in developing methods to promote the survival and expansion of alloreactive donor NK cells in recipients receiving PT-Cy. Finally, NK cell-based IECTs will emerge to be delivered as independent therapies as well as in combination with allo HCT, and the interaction of KIR-*HLA* in promoting the activity of these cell products will need to be explored. Decades of advancements in donor-recipient *HLA* matching have led to a dramatic reduction in T-cell mediated transplant-related mortality. Whether we can now use our understanding of KIR receptors, a complex layer of human immunogenetics beyond *HLA*, to open a door to innate immunity and the promise of long-lasting tumor control is an open and exciting question.

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Figure 1:

Inhibitory and activating killer Ig-like receptors found to influence allo HCT outcomes and their cognate HLA ligands. KIR3DL1 receptors interact with HLA-B and HLA-A allotypes exhibiting the Bw4 epitope. KIR2DL1 interacts with HLA-C2 allotypes. KIR2DL2 and KIR2DL3 interact with HLA-C1 allotypes. KIR2DS1 interacts with HLA-C2 allotypes and KIR3DS1 interacts with HLA-F open conformers.[72]

DESCRIPTION	GRAPHICAL ILLUSTRATION	CLINICAL RESULTS
<p>HIGH INHIBITION PAIRS</p> <p>KIR3DL1 – HLA-Bw4 combinations that result in educated but highly inhibitable NK cells. NK alloreactivity is dampened.</p>		<p>Increased relapse</p> <p>Decreased overall survival</p>
<p>LOW INHIBITION PAIRS</p> <p>KIR3DL1 – HLA-Bw4 combinations that result in educated NK cells but are poorly inhibitable. NK alloreactivity is increased.</p>		<p>Decreased relapse</p> <p>Improved overall survival</p>
<p>NON-INTERACTING PAIRS</p> <p>KIR3DL1 allele groups that result in uneducated NK cells. NK cells may become alloreactive in highly inflammatory conditions.</p>		<p>Intermediate effects.</p>
<p>NULL Expressing KIR3DL1</p> <p>KIR3DL1 null allele groups are not expressed at the cell surface. NK cells are capable of education and are uninhibitable. NK alloreactivity is increased <i>in vitro</i>.</p>		<p>Unclear. This is a rare clinical occurrence and robust data are lacking.</p>

Figure 2:

Donor KIR3DL1 and recipient HLA-Bw4 subtype combinations, their effects on NK function, and their associations with outcomes following allo HCT for AML.

Table 1:

NK Cell Alloreactivity Models	Hypothesis	Example	References
Inhibitory KIR			
Missing Self	Donor has a KIR ligand that is missing in the recipient AND donor exhibits the cognate KIR. Loss of inhibitory signal to donor KIR ⁺ NK cells in the recipient activates them for cytotoxicity.	HLA-C1/x, KIR2DL3 ⁺ donor used for HLA-C2 homozygous recipient (missing HLA-C1 ligand for KIR2DL3).	[45, 46, 93]
Missing Ligand	Donor and recipient lack an inhibitory KIR ligand, rendering donor NK cells expressing the cognate KIR to be uneducated. Uneducated NK cells can participate in GVL reaction if sufficiently activated.	Recipient is HLA-C1 homozygous, HLA-C2 homozygous, or HLA-Bw6 homozygous. Donor exhibits inhibitory KIR but lacks HLA ligand.	[55–57]
Education/inhibition	When an inhibitory KIR ligand is present, variation in the cognate inhibitory KIR allows for selection of donors, with KIR that exhibit weak inhibitory interaction. NK cells remain educated but are minimally inhibited and can offer maximum participation in GVL.	Recipient is HLA-Bw4–80I. Donor is selected for KIR3DL1-L, KIR allotypes with weak interaction for HLA-Bw4–80I.	[41, 42, 78]
Activating KIR			
Haplotype based selection	KIR B-haplotypes have more activating receptors, potentially leading to lower threshold for activating cells in the NK repertoire.	Donors are selected for KIR B-haplotypes, particularly those donors with activating receptors in the centromeric portion of the haplotype.	[59–63, 94]
HLA-C1/KIR2DS1 based selection	KIR2DS1 educates NK cells via interaction with HLA-C2. Overstimulation with HLA-C2 “disarms” NK cells, rendering them hyporesponsive.	For <i>HLA-C1</i> /x recipients, donors exhibiting <i>HLA-C1</i> and <i>KIR2DS1</i> are selected.	[68–70, 78]