

## \* CHAPTER 3

# Immunogenetics of allogeneic HSCT

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## \* 3.2 KIR: Beneficial effects of natural killer cell alloreactivity in haploidentical HSCT

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## 1. Introduction

Recent studies have shown natural killer (NK) cells influence the outcome of haploidentical haematopoietic transplantation in a remarkable way, with a favourable outcome where NK cells are alloreactive in the donor-recipient direction (1-4).

NK cell activation is regulated by a balance between inhibitory and activating receptors, called killer-cell Ig-like receptors (KIRs). In humans, currently 16 inhibitory KIR genes and pseudo-genes are known, which codify for inhibitory and activating KIRs. Inhibitory KIRs recognise amino acids in the COOH-terminal portion of the MHC class I  $\alpha 1$  helix (reviewed in refs. 3, 5, 6). They possess two (KIR2D) or three (KIR3D) extra-cellular C2-type Ig-like domains and a long cytoplasmic tail (L) containing immunoreceptor tyrosine-based inhibition motifs (ITIM) which recruit and activate SHP-1 and SHP-2 phosphatases for inhibitory signal transduction. KIR2DL1 recognises HLA-C alleles characterised by a Lys80 residue (HLA-Cw4 and related, "Group 2" alleles). KIR2DL2 and KIR2DL3 (which are allele variants) recognise HLA-C with an Asn80 residue (HLA-Cw3 and related, "Group 1" alleles). KIR3DL1 is the receptor for HLA-B alleles sharing the Bw4 supertypic specificity (Table 1).

Another type of human NK cell inhibitory receptor involved in HLA recognition is CD94-NKG2A. It binds to the non-conventional class I molecule HLA-E. Several HLA class I alleles provide signal sequence peptides that bind HLA-E and allow its expression at the cell surface. Consequently, it is expressed in every individual. Inhibitory KIRs, CD94/NKG2 and HLA-class I genes determine individual NK cell repertoires during development. As they are located on different chromosomes, receptors and ligands segregate independently in human pedigrees. The HLA class I genotype selects a self-tolerant repertoire by dictating which KIR and/or NKG2A

**Table 1: HLA-class I allele specificity of the main KIRs expressed by human NK cells**

KIR gene	Encoded protein	HLA specificity
KIR2DL1	P58.1 receptor	HLA-C group 2 (e.g., -Cw2, -Cw4, -Cw5, -Cw6) (Lys80)
KIR2DL2/3	P58.2 receptor	HLA-C group 1 (e.g., -Cw1, -Cw3, -Cw7, -Cw8) (Asn80)
KIR3DL1	P70/NKB1 receptor	Bw4 alleles (e.g., HLA-B27)

receptor combinations are to be used as inhibitory receptors for self HLA class I (7). Consequently, every functional NK cell in the mature repertoire expresses at least one inhibitory receptor for self HLA: co-expression of two or more receptors is less frequent.

## 2. Inhibitory KIRs and alloreactivity

Since inhibitory KIRs recognise specific groups of HLA class I molecules, i.e., HLA-C group 1, HLA-C group 2, HLA-Bw4 alleles, NK cells with the potential to exert alloreaactions use KIRs as inhibitory receptors for self (1-6). NK cells which express, as their only inhibitory receptor for self, a KIR for the HLA class I group which is absent on allogeneic targets, sense the missing expression of the self class I KIR ligand and mediate alloreaactions ("missing self" recognition). Importantly, most individuals possess a full complement of inhibitory KIR genes and can exert NK cell alloreaactions (3, 4, 6). In particular, 100% of individuals possess the KIR2DL2 and/or KIR2DL3 receptors for HLA-C group 1 alleles. If they have HLA-C group 1 allele(s) in their HLA type, these individuals possess HLA-C1-specific NK cells which are alloreactive against cells from individuals who do not express HLA-C group 1 alleles. Ninety-seven percent of individuals possess the KIR2DL1 receptor for HLA-C group 2. If they possess HLA-C group 2 allele(s) in their HLA type, these individuals have HLA-C2-specific NK cells which mediate alloreaactions against cells from individuals who do not express HLA-C group 2 alleles. Finally, ~90% of individuals possess the KIR3DL1 receptor for HLA-Bw4 alleles. When they have HLA-Bw4 allele(s) in their HLA type, these individuals may have HLA-Bw4-specific NK cells that are alloreactive against Bw4-negative cells. These KIR ligand mismatches often occur in haploidentical donor recipient transplant pairs.

## 3. Clinical effects of NK cell alloreactivity

When exerted in the donor-versus-recipient direction, NK cell alloreactivity emerged as a crucial factor in improving outcomes of haploidentical transplantation (1-4). It reduced the risk of leukaemia relapse, did not cause graft versus host disease (GvHD) and markedly improved event-free survival (EFS) in a series of haploidentical transplants (57 acute myeloid leukaemia (AML) patients, 20 of whom were transplanted from NK alloreactive donors) (2). In an updated analysis (4), 112 high-risk AML patients received haploidentical transplants from NK alloreactive (n=51) or non-NK alloreactive donors (n=61). Transplantation from NK-alloreactive donors was associated with:

- a significantly lower relapse rate in patients transplanted in any CR (3 vs. 47%) ( $p<0.003$ );

- better EFS whether patients transplanted in relapse (34 vs. 6%,  $p=0.04$ ) or in remission (67 vs. 18%,  $p=0.02$ );
- overall reduced risk of relapse or death (relative risk vs. non-NK-alloreactive donor: 0.48 [95%CI: 0.29-0.78],  $p<0.001$ ).

The 67% probability of surviving event-free for acute myeloid leukaemia patients transplanted in any remission from NK alloreactive donors is in the range of best survival rates after transplantation from unrelated donors and cord blood units. The 34% EFS of patients transplanted in chemo-resistant relapse from NK alloreactive donors is also satisfactory. Transplantation from non-NK alloreactive haploidentical donors appears justified only for AML patients in remission and even in these patients it is associated with only an 18% EFS. Lack of an NK alloreactive donors is a contra-indication to transplant for patients in chemo-resistant relapse as very few survive.

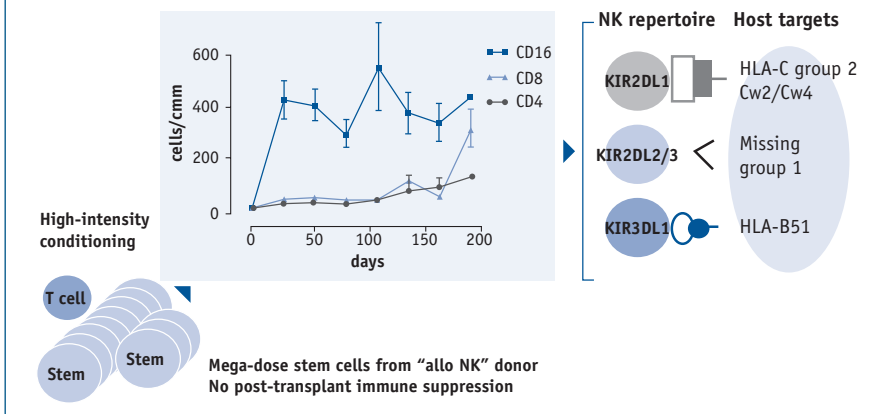
Several observations suggest alloreactive NK cells are responsible for favourable transplantation outcomes. Transfer of human alloreactive NK cells to NOD-SCID mice eradicated previously transplanted human AML cells (2). KIR ligand mismatches correlated with the ability of donor NK cell clones to kill cryopreserved haematopoietic recipient cells, including leukaemia cells (1, 2, 4). Most importantly, as depicted in [Figure 1](#), upon transplantation from NK alloreactive donors the engrafted stem cells give rise to an NK cell repertoire which includes donor-vs-recipient alloreactive NK clones which kill cryopreserved haematopoietic recipient cells, including leukaemia cells (1). Donor versus recipient alloreactive NK clones are detectable in vivo in recipients for up to 1 year after transplant (4).

#### 4. Selection of an NK alloreactive donor

In the clinical studies above, NK alloreactive donors were found for ~50% of patients, which approaches the maximum because, in fact, 1/3 of the population expresses class I alleles belonging to all three class I groups recognised by KIRs and block NK cells from every donor.

How is an NK alloreactive donor selected? Recipients who express alleles belonging to 1 or 2 of the 3 class I allele groups recognised by KIRs may find NK alloreactive donors. Donors who are HLA-C group mismatched with their recipients possess high-frequency NK clones which are alloreactive against recipients' target cells (1-4). Thus, high-resolution HLA-C typing is a good predictor of NK cell alloreactivity. Since 3% of individuals do not possess the KIR2DL1 gene, the combination of a KIR2DL1-negative donor and a recipient without HLA-C group 2 alleles could result in a 1.5% incidence of false positivity. KIR2DL1 gene typing of the donor may be necessary to assess the NK alloreactive potential of this combination.

Figure 1



*Haematopoietic stem cell transplantation from a KIR ligand-mismatched donor who possesses donor versus recipient alloreactive NK clones in his/her repertoire gives rise to transient post-transplant generation of a repertoire which is identical to the donor's, including the alloreactive clones. In fact, alloreactive clones can be isolated from the circulation of successfully engrafted recipients and can kill cryopreserved recipient targets, including leukaemic cells (1, 4)*

In HLA-Bw4 mismatches, even when the KIR3DL1 gene is present in the donor (~90% of individuals), NK repertoire studies show alloreactive NK clones are non-detectable ~1/3 of individuals (4). In some allelic variants in the HLA-Bw4 inhibitory NK receptor gene KIR3DL1 may not allow full receptor expression at the cell membrane and affect NK cell inhibition by HLA-Bw4 ligand (8); others apparently express alloreactive NK clones in very low frequencies. Thus, for HLA-Bw4 mismatches, functional assessment of the donor NK repertoire appears necessary.

As approximately half of unrelated donor transplants are mismatched for one or more HLA class I alleles, donor vs. recipient NK cell alloreactivity may also occur in this setting. However, some retrospective studies show no advantage in transplantation from KIR ligand-mismatched donors (9, 10). Unrelated donor transplant protocols are heterogeneous in conditioning regimens, patient populations and underlying diseases. They use T-cell-deplete bone marrow harvests (or, less frequently, peripheral blood progenitors) which contain ~4 log more T-cells and up to 1 log fewer stem cells than haploidentical grafts. Relatively few transplanted stem cells, combined with the high T-cell graft content and post-transplant immune suppression have been associated with poor reconstitution of potentially alloreactive, KIR-bearing NK cells (11). Nonetheless, other studies have observed an increased GvL effect (12–15).

A marked survival advantage was reported in patients who received anti-thymocyte globulins pre-transplant (which provided *in vivo* T-cell depletion) and a graft containing 2-3-fold more nucleated cells than usual in unrelated-donor transplants (12). Prospective studies are needed to determine whether strategies (high doses of stem cells, T-cell depletion, no post-transplant immune suppression), which in haploidentical transplantation harness donor-vs-recipient NK cell alloreactivity, can be implemented to improve outcome in unrelated donor transplants.

### 5. The “missing ligand” model

Since the original report that NK cell alloreactivity in haploidentical transplantation rests upon KIR ligand mismatching and donor NK cell recognition of “missing self” on recipient targets (2), the “missing ligand” model has been proposed as a powerful algorithm for predicting favourable transplant outcomes not only in haploidentical transplants (16) but also in matched sibling (17) and in unrelated donor transplants (18). Under the perturbed conditions that exist after a haematopoietic stem-cell transplant, it hypothesises that NK alloreactions occur when KIR ligand-matched donors possess an “extra” KIR for which neither donor nor recipient have an HLA ligand. These donors may carry KIR-bearing NK cells in an anergic/regulated state which, upon transfer into the recipient, are hypothesised to become activated and exert a GvL effect.

However, even though self-tolerant NK cells which do not express inhibitory receptors for self-MHC have been described (19) no studies have as yet determined whether tolerant NK cells acquire/resume cytotoxic effector function after transplant. When an adult series of AML patients who were transplanted from haploidentical donors was analysed according to the “missing ligand” algorithm, the “missing ligand” transplant recipients disappointingly had a worse prognosis than patients transplanted from NK-alloreactive (KIR ligand mismatched) donors (4). While differences in diseases, age of patients and transplantation protocols, such as ATG vs. no ATG in the conditioning or peripheral blood CD34<sup>+</sup> cells vs. bone marrow as a source of haematopoietic cells, may account for these conflicting results, the analysis shows that donor NK cell recognition of “missing self” on recipient targets is essential for triggering powerful NK cell alloreactions that impact beneficially on transplantation outcomes.

### 6. Effects of donor activating KIR genetics on donor vs. recipient NK cell alloreactivity

Activating KIRs, which regulate NK and T-cell functions, are molecular homologues of the inhibitory KIRs with shorter cytoplasmic tails (S) (reviewed in refs. 3, 5, 6) and a charged residue in their transmembrane domain that allows association with

ITAM containing signalling polypeptides. Knowledge of their ligand specificity is limited. Studies have reported a weak interaction between KIR2DS1 and Lys80 HLA-C molecules, despite its homology to KIR2DL1, and an even weaker interaction between KIR2DS2 and Asn80 HLA-C molecules, despite its homology to KIR2DL2 and KIR2DL3. Unlike inhibitory KIRs, activating KIRs exhibit extensive variation in gene number and content, which leads to heterogeneity within the general population and diverse ethnic groups (reviewed in ref. 6). Indeed, activating KIRs may not even be present in approximately 25% of Caucasians who are homozygous for the so-called group A KIR gene haplotypes which contain inhibitory KIR genes and the KIR2DS4 activating KIR gene (encoding for a non-functional protein in 2/3 of individuals). On the other hand, 75% of Caucasians are either heterozygous or homozygous for B haplotypes which carry not only inhibitory KIR genes but also various combinations of activating KIR genes (KIR2DS1-2-3-5 and KIR3DS1).

## 7. Activating KIRs and results of haploidentical HSCT

We, therefore, evaluated the role of donor activating KIR genetics in haploidentical haematopoietic transplantation (Mancusi et al., manuscript submitted for publication). In a series of 84 haploidentical transplants for AML, the impact of donor KIR genetics (group A vs. group B KIR gene haplotypes) was assessed separately in NK alloreactive and non-NK alloreactive transplants. Forty-seven recipients were transplanted from NK alloreactive donors (12 with group A KIR gene haplotypes vs. 35 with B haplotypes) and 37 recipients from non-NK alloreactive donors (8 with group A KIR gene haplotypes vs. 29 with B haplotypes). KIR gene haplotypes had no impact in non-NK alloreactive transplants. In transplants from NK alloreactive donors, presence of group B haplotype KIR genes in the donors was associated with reduced incidence of TRM (largely infection-related) (B vs. A haplotypes: 20 vs. 67% TRM,  $p < 0.005$ ). In multivariate analyses against disease status at transplant, age, patient and donor sex, conditioning regimens, and the number of CD34+ and CD3+ cells in the graft, it was the only significant variable predicting protection from TRM (RR: 0.24; 95%CI: 0.14-0.42;  $p < 0.01$ ) and resulted in a trend towards better EFS (60 vs. 33%,  $p < 0.1$ ). When the number of activating KIR genes in the donor was taken into account, donors carrying  $\geq 3$  activating KIR genes provided significant protection from TRM and significantly better EFS compared with A haplotype donors (TRM: 12 vs. 67%,  $p < 0.003$ ) (EFS: 71 vs. 33%,  $p = 0.02$ ). In multivariate analysis, transplantation from alloreactive donors carrying  $\geq 3$  group B haplotype activating KIR genes was the only variable predicting protection from TRM (RR: 0.40; 95%CI: 0.27-0.58;  $p < 0.02$ ) and significantly improved EFS (RR: 0.56; 95%CI: 0.32-0.98;  $p < 0.05$ ). Thus, while NK-alloreactive donors protect against leukaemia relapse,

those who also carry activating KIRs protect against infectious mortality and help improve survival. The chance of finding an NK alloreactive donor is ~ 50% of haploidentical transplants and the odds of finding an NK alloreactive donor carrying activating KIRs are ~ 30% of haploidentical transplants.

### 8. Activating KIRs and protection against infection

Protection against infection may be mediated directly by NK cells or indirectly through other mechanisms. Activating KIRs could enhance NK cell cytokine secretion and cytotoxicity against pathogen infected cells in the context of missing self. A notable example of direct recognition of a pathogen by activating NK receptor is provided by murine CMV protein m157 and the murine Ly49H NK receptor (20). In humans, progression to AIDS is slower in patients who have both KIR3DS1 and the HLA-Bw4 allotype, the putative ligand of KIR3DS1 (21). More NK cells expressing NKG2C are present in CMV-exposed individuals, suggesting this activating NK cell receptor plays a role in the immune response to this infection (22). Therefore associations between activating NK receptors and enhanced immunity against infections have been documented. Activating KIRs could also help control infections indirectly through the interaction between NK cells and dendritic cells (DCs) (reviewed in ref. 23). NK cells regulate DC homeostasis and maturation. Mature DCs can, in turn, activate NK cells. In vivo NK/DC interactions in lymphoid organs or non-lymphoid tissues can lead to Th1 polarisation. NK cells in lymph nodes provide the early IFN- $\gamma$  production, which is essential for Th1 polarisation. Consequently, the interaction between NK cells and DCs influences the quality and the strength of adaptive immune response. The clinical data suggest either or both these mechanisms could operate in haploidentical transplants from NK-alloreactive donors who possess activating KIRs. Whatever the mechanisms, these studies have improved criteria for donor selection.

### 9. Conclusions

Transplantation from a full HLA haplotype mismatched family member is nowadays a viable option for patients with acute leukaemia at high risk of relapse who urgently need a transplant and who do not have a matched donor (24). The mismatched transplant relies for its success on the combined action of:

- high-intensity conditioning regimens to ensure the lowest possible residual leukaemia burden and optimal immunosuppression;
- high doses of haematopoietic cells to ensure engraftment across the HLA barrier;
- extensive T-cell depletion of the graft to prevent GvHD (with no post-transplant immunosuppression);

- donor versus recipient NK cell alloreactivity to enhance anti-leukaemia effects;
- NK-alloreactive donors who also carry activating KIRs to protect against infectious mortality.

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## Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

### 1. Which molecules are recognised by inhibitory KIRs?

- a) HLA class II molecules .....
- b) HLA-E .....
- c) HLA class I molecules .....
- d) All of the above .....

### 2. Which of the following statements is correct in pre-clinical models and in the clinical practice of haploidentical haematopoietic transplantation?

- a) Donor vs. recipient alloreactive NK cells cause GvHD .....

- b) Donor vs. recipient alloreactive NK cells increase the incidence of leukaemia relapse.....
- c) Donor vs. recipient alloreactive NK cells mediate a GvL effect .....
- d) Donor vs. recipient alloreactive NK cells cause rejection.....

**3. From the data in ref. 4 of this Chapter, the event-free survival of acute myeloid leukaemia patients transplanted in any remission from haploidentical NK alloreactive donors is:**

- a) 30-40%.....
- b) 40-50%.....
- c) 50-60%.....
- d) 60%.....

**4. From the data in ref. 4 of this Chapter, the event-free survival for acute myeloid leukaemia patients transplanted in chemo-resistant relapse from NK alloreactive donors is:**

- a) 10%.....
- b) 10-20%.....
- c) 20-30%.....
- d) 30%.....

**5. From the study by Mancusi et al. reported in this Chapter, transplantation from NK alloreactive donors who also possess activating KIR genes is associated with:**

- a) Decreased incidence of leukaemia relapse.....
- b) Increased incidence of GvHD.....
- c) Decreased incidence of infectious mortality.....
- d) Increased incidence of infectious mortality.....

## NOTES