Shedding light on the complicated world of alloreactive NK cells

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In this issue of *Haematologica*, Schäfer and colleagues report their attempts to evaluate the impact of killer cell immunoglobulin-like receptor (KIR) high-resolution genotyping on outcomes in a large cohort of patients who underwent HLA-matched hematopoietic cell transplantation (HCT).[1] Indeed, they hypothesize that specific donor/recipient KIR/HLA configurations would modify the post-transplant natural killer (NK)-cell activation status, affecting transplant outcomes.

To better understand the relevance of their findings it is useful to underline a key principle of donor NK-cell functionality in the HCT context: NK cells need to be "educated" by the interaction with self-HLA molecules to become fully functional and "educated" NK cells react against allogeneic targets when they lack self-HLA expression. When donor alloreactive NK cells (not inhibited by HLA epitopes) arise from infused donor hematopoietic stem cells after HCT, they can exert a potent antileukemic activity by killing allogeneic leukemic cells. Such an event occurs in the presence of specific donor/recipient HLA combinations and was demonstrated to have great clinical impact in T-cell-depleted HLA-haploidentical HCT in the absence of post-transplant immune suppression.[2] In fact, a lower rate of relapse occurred in patients affected by acute myeloid leukemia who received an HLA-haploidentical T-cell-depleted HCT from a NK-cell alloreactive donor. The value of the finding was so great that the possibility of exerting NK-cell donor alloreactivity became a major criterion for donor selection in the T-cell-depleted HLA-haploidentical transplant setting.[3] The effect of alloreactive NK cells was maintained even when donor T-cell adoptive immunotherapy was added to the transplant platform as long as no post-transplant immune suppressive drugs were used.[4] Furthermore, the role of activatory KIR has also been studied in the T-cell-depleted HLA-haploidentical transplant setting and a possible impact on viral infection rate and transplant-related mortality when specific activating KIR (e.g., KIR2DS1)/HLA interactions occurred has been reported.[5]

The discovery of NK-cell alloreactivity and the new insights into KIR/HLA interactions paved the way for the development of novel NK-cell-based immunotherapies to treat acute leukemia and other cancers.[6] Many studies have been of interest and, more recently, engineered chimeric antigen receptor NK cells are under investigation with promising results.[7] Unfortunately, to date, none of these treatments has reached approval for routine clinical use because of some key limitations such as NK-cell number, expansion protocols, *in vivo* persistence, and killing capacity.

Many attempts have been made to understand whether donor NK-cell alloreactivity could be useful in transplant settings other than T-cell-depleted HLA-haploidentical transplants, such as HLA-matched and unmanipulated transplants. Specific inhibitory or activatory KIR/HLA interactions have been shown to have some impact on major transplant outcomes such as leukemia relapse and survival,[8,9] but conflicting results have been reported and no clear and strong evidence that supports routine use of donor KIR genotyping is available to date. In fact, the lack of T-cell depletion and the use of post-transplant immune suppressive agents (e.g., post-transplant cyclophosphamide), contributed to eliminate alloreactive NK cells *in vivo* and limited their clinical value.[10]

Therefore, because of many transplant-related confounding factors (e.g., donor/recipient HLA-matching, type of conditioning regimen, use of post-transplant immune suppression), pre-transplant KIR genotyping is not generally adopted as a valuable tool to predict transplant outcomes and guide clinical choices. Indeed, no clear link between KIR allelic polymorphism and the level of post-transplant NK-cell alloreactivity was found in a large study with more than 5,000 transplant recipients.[11]

Another major limitation is the genetic complexity of the KIR system. The number of KIR genes and allelic variations that might occur combined with the lack of sequencing resolution depth are unresolved issues that further challenge the possible routine use of KIR genotyping information. While

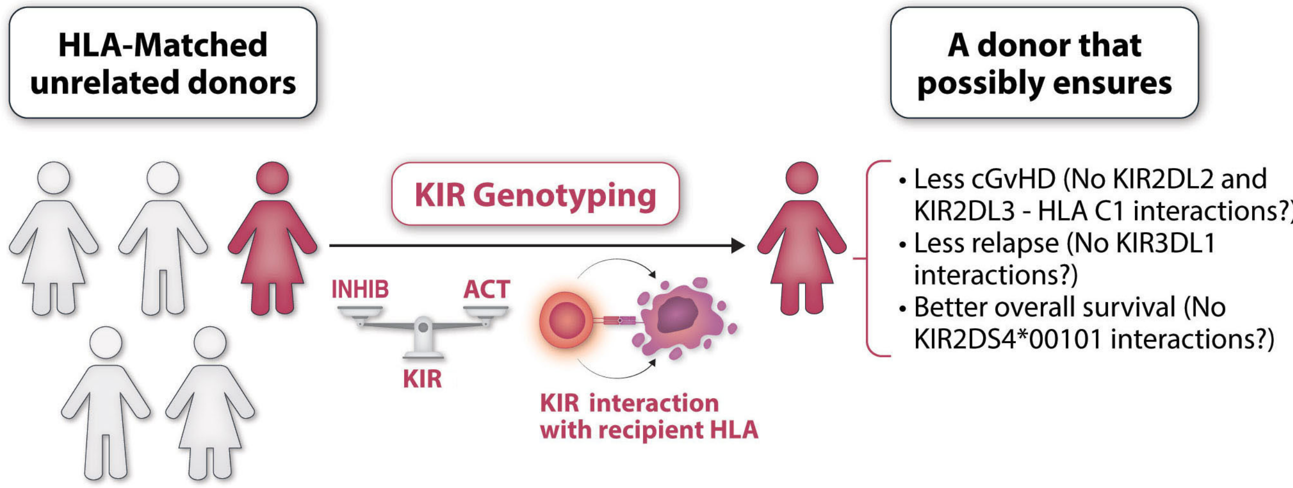

Figure 1. A schematic representation of suggested unrelated donor selection through KIR genotyping. KIR genotyping of HLA-matched unrelated donors informs about inhibitory and activatory KIR that can interact with recipient HLA molecules. Specific KIR/HLA interactions might help to assess the probability of some transplant outcomes such as chronic graft-*versus*-host disease and overall survival. This information could help to improve unrelated donor selection. HLA: human leukocyte antigen; KIR: killer cell immunoglobulin-like receptors; INHIB: inhibitory; ACT: activatory; cGvHD: chronic graft-*versus*-host disease.

some beneficial or detrimental effects on HCT outcomes have been associated with selected KIR allele candidates, such as KIR3DL1 and KIR2DL1, very few studies investigated the entirety of the KIR system in transplantation. The advent of easily usable high-resolution genotyping tools might help to overcome such issues.

Applying this modern technology, the study by Schäfer and colleagues challenged the hypothesis that specific allelic KIR/HLA configurations would modulate transplant outcomes by predisposing (or not) the patient to donor NK-cell alloreactivity in a large retrospective multicenter study of HLA-matched unrelated transplants. The authors demonstrated that post-transplant NK-cell alloreactivity was mainly driven by KIR2DS4, KIR2DL2/L3 and KIR3DL1 alleles. They found some relevant effect of specific KIR interactions such as the detrimental effect of KIR2DS4*00101 interactions on survival and transplant-related mortality and powerful protection against chronic graft-*versus*-host disease (GvHD) in KIR2DL2/L3-positive donor/recipient pairs with a missing ligand status. These findings further support the evidence that NK-cell-mediated alloreactivity protects from the development of chronic GvHD by targeting recipient's dendritic cells and T cells.[2,12] The authors also clarified that activatory and inhibitory KIR do not contribute equally to leukemic control in this setting with findings that largely differ from what was previously observed in T-cell-depleted HLA-haploidentical transplants and that remain contradictory and controversial, therefore warranting further investigation.

In conclusion, thanks to the deep analysis and the large dataset, the study by Schäfer and colleagues elucidates important clinical aspects of donor/recipient KIR/HLA interactions in unrelated HLA-matched transplants (Figure 1). Although the study does not provide enough evidence to support the use of whole donor KIR genotyping before transplant, it does foresee that a smart sequencing of selected KIR loci would be of great help in unrelated donor selection, especially when several options are available.

Disclosures

No conflicts of interest to disclose.

Contributions

All authors contributed equally.

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