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NK Cell - KIR-TREGs: How to Manipulate a Graft for Optimal GVL

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Abstract

Two of the major complications that limit the efficacy of allogeneic hematopoietic cell transplantation (allo-HCT) are disease relapse and graft vs. host disease (GVHD). Due to their rapid recovery early after allo-HCT and ability to kill malignant targets without prior exposure, natural killer (NK) cells have been considered one of the main effector cells that mediate early graft vs. leukemia (GVL) reactions. Conversely, regulatory T cells (Treg) have proven to be critical in facilitating self-tolerance. Both murine and human studies demonstrate a significant role for Tregs in the modulation of GVHD after allo-HCT. Here I will review the mechanisms of how these two cell types carry out these functions, focusing on the post-allo-HCT period. Surprisingly, relatively few studies have addressed how Tregs and NK cells interact with one another and whether these interactions are antagonistic. While pre-clinical studies suggest active cross-talk between NK cells and Tregs, early clinical studies have not shown a detrimental impact of Treg therapy on relapse. Despite this, interruption of tolerogenic signals may enhance the efficacy of NK effector functions. Methods to transiently impair Treg functions and augment NK cell allo-reactivity will be discussed.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HCT) is uniquely curative for a variety of hematological malignancies that are at high-risk for relapse or refractory to conventional chemotherapy. Allo-HCT leads to leukemia eradication through both the pre-transplantation preparative regimen and post-transplant graft vs. leukemia (GVL) responses. The exact contribution of each to leukemia eradication is unknown, but numerous studies support the concept that GVL reactions account for a significant proportion of the anti-leukemia activity of allo-HCT. The cells that mediate GVL in humans have not been clearly defined, but the rapid recovery of natural killer (NK) cells after transplantation has led some investigators to focus on their role in GVL reactions. While donor NK cells mediate anti-host reactions that translate into GVL, regulatory T cells (Tregs) are also present in both the recipient and the allo-HCT graft and function to induce tolerance. While Tregs are quite beneficial in protecting from graft vs. host disease (GVHD), they also have the potential to suppress allo-reactive effector responses, such as those associated with GVL. Given that relapse and GVHD are two of the major obstacles that impede successful outcomes after allo-HCT, understanding and manipulating these two cell populations may have a significant

impact on transplant outcomes. While detailed mechanistic biological paradigms for both NK cells and Tregs have been established in murine models, the purpose of this review is to discuss and highlight the interactions between human NK cells and Tregs in the setting of allo-HCT. Additionally, I will summarize recent advances in the clinical use of these cell populations and focus on how they interact with one another to highlight potential opportunities to prevent either disease recurrence or GVHD after allo-HCT.

Natural Killer Cells: Development and Function

NK cells are phenotypically defined as CD3-CD56+ cells and account for ~5-10% of the peripheral blood lymphocyte population. NK cells develop from common lymphoid progenitors which emerge from the bone marrow and seed the secondary lymphoid tissues (lymph nodes). There, they proceed through a series of developmental intermediates under the influence of instructive cytokines (i.e., IL-15) ^{1,2}. Mature NK cells are then released into the peripheral circulation where they can rapidly produce inflammatory cytokines (i.e., IFN- γ and TNF- α) or mediate cytotoxicity in response to virally infected or malignant cells. In support of this, rare individuals who lack or have dysfunctional NK cells suffer from repeated herpes or papillomavirus infections ³ and some are at risk for hematopoietic malignancies ⁴. These two situations (infection or oncogenesis) both result in the reduction in surface major histocompatibility (MHC) class I molecules and/or the increase in other surface molecules that are associated with cell distress (see below). Accordingly, NK cells display a variety of both inhibitory and activating receptors that recognize MHC class I and/or stress-associated molecules. These receptors are diverse and have been outlined in previous reviews ⁵⁻⁷ and a full listing their names and function is beyond the scope of this review, but some are listed in table 1.

NK cells use their receptors to assess the status of a potential target (health or distress). In a simplified view of this, healthy cells display sufficient MHC class I and have no expression of distress molecules. In contrast, upon viral infection or malignant transformation, cells down-modulate MHC class I and/or acquire distress receptors, thereby becoming NK cell targets. Thus, the *lack* of MHC class I and/or the *presence* of distress receptors, triggers NK cell activation resulting in cytokine production (IFN- γ) and/or cytotoxicity (via granzyme/perforin or FasL/TRAIL). Such a system allows for self-tolerance while still assuring that deranged cells are recognized and killed. To add further complexity, NK receptors are stochastically expressed by individual NK cells. This seemingly random expression of NK cell receptors results in clones of NK cells that display enormous receptor combinations, accounting for NK cell diversity, which in turn endows these clones with the capacity to broadly recognize virally infected or malignant cells without the need for germ-line recombination of antigen receptors (that occurs with either B or T cells). Considering that no one receptor dominates over another, the triggering of an individual NK cell depends on the summation of the activating and inhibitory signals a cell receives.

As described above, NK cells undergo differentiation in the lymphoid tissues and are released into the peripheral circulation. Until recently, it has been assumed that once developed, NK cells are poised to kill malignant targets. However, pioneering work by Kim and Yokoyama has clearly demonstrated that recognition of self-MHC (through MHC class I

recognizing NK cell receptors) is required for the acquisition of NK cell functionality⁸. This process has been referred to as NK cell “licensing.” While complex, most NK cells have a receptor that recognizes self-MHC. Thus a significant proportion of blood NK cells are “licensed” or cytotoxic. NK cell clones that *lack* receptors that recognize self-MHC class I are also present within individuals and are “unlicensed.” These hypofunctional NK cells can become activated upon exposure to cytokines (IL-12, IL-15 and/or IL-18), which are present during periods of pancytopenia and infections. Once activated, these cells would not be restrained by recipient MHC (since it is lacking). In fact, in a small study examining autologous transplant recipients by Leung and coworkers patients who had NK cells that expressed KIR which recognized MHC which was not present in the patient had less disease recurrence, perhaps supporting an autologous NK mediated GVL⁹. Studies are ongoing to decipher the differences between licensed and unlicensed NK cells, but such concepts are particularly relevant in the allo-HCT setting where bacterial and viral infections are common. Thus, there may be a dynamic interplay between recipient MHC class I, donor NK cells and infectious complications that may modulate early, NK cell mediated graft vs. leukemia reactions after allo-HCT. However, mechanisms that control these reactions have not been entirely elucidated and present opportunities to enhance GVL.

NK cells and Allo-HCT

A variety of clinical studies have linked NK cells to successful outcomes following allo-HCT. For instance, HCT grafts with higher numbers of NK cells are associated with a less acute and chronic GVHD^{10,11}. These findings are consistent with murine studies showing that donor NK cells eradicate recipient antigen presenting cells (APCs)¹² or allo-reactive donor T cells¹³ thereby preventing GVHD. Other research has linked donor NK characteristics with post allo-HCT infectious diseases. For instance, the number of KIR genes in an individual varies. Transplantation with donors that have large numbers of KIR genes was associated with fewer bacterial infections in the post-transplant than those with a small number of KIR genes¹⁴. Individuals with large numbers of KIR genes typically have more activating genes, possibly implicating these receptors in the control of infections. Another emerging area involving NK cells and post-transplant infections is the interaction between NK cells and CMV reactivation. A number of groups have now demonstrated that CMV reactivation leads to the emergence of population of terminally differentiated NK cells that express NKG2C and acquire CD57 expression. These “memory” NK cells expand in response to CMV reactivation and have potent cytotoxicity and IFN- γ production in response to both AML cell lines and primary leukemic blasts^{15,16}. On a related note, Elmaagacli and coworkers have shown that early CMV reactivation after allo-HCT is associated with reduced relapse rates in AML patients¹⁷, however, other investigators have not reproduced these findings^{18,19}. Thus, the influence of CMV reaction on NK cells and GVL reactions is not yet understood.

Since NK cells make up the majority of the lymphocytes recovering early after transplantation, investigators have examined the impact of the absolute lymphocyte count (ALC) or the absolute NK cell count on transplant outcomes. Rapid lymphocyte or NK recovery (at D28 after allo-HCT) has been linked to reductions in relapse rates or improved disease free survival (DFS)^{20,21}. Whether this rapid recovery represents expansion of the

mature NK cells in the graft (homeostatic expansion) or newly generated, stem cell-derived NK cells is not known and is difficult to determine. However, based on this data, methods to enhance NK cell recovery, such as the use IL-15 may warranted to prevent relapse and are currently being tested (Haploidentical Donor Natural Killer Cell Infusion With IL-15 in Acute Myelogenous Leukemia (AML), NCT01385423).

Prior high profile studies demonstrate that incompatibilities between donor KIR and recipient MHC class I (i.e., KIRL mismatch) are associated with reductions in AML relapse following haploidentical transplantation¹², implying that interruption of inhibitory KIR signals will lead to less restrained, more active NK cells. However, recent work, also implicates the presence of activating KIR in the donor and protection from acute myeloid leukemia (AML) relapse. In two large registry reports by Cooley²² and Venstrom²³, the presence of activating KIR in the donor was associated with a significantly less AML relapse and improved DFS. While there were subtle differences between the two studies, both suggest that activating KIR recognize a heretofore uncharacterized ligand on AML cells. This work raises the possibility that allo-HCT donors might be selected for enhanced GVL. The feasibility of this concept is currently being testing in a clinical trial where allo-HCT donors are prospectively being assessed for KIR gene content to determine whether, in addition to HLA typing, KIR gene content can be used in donor selection ("Selecting a Favorable KIR Donor in Unrelated HCT for AML," NCT01288222). Further evidence for a direct GVL effect of NK cells is provided by Miller and coworkers who demonstrated in the non-transplant setting that lymphodepleting chemotherapy followed by the infusion of a NK cells from a haploidentical family member lead to transient *in vivo* NK expansion and remissions in ~30% of chemotherapy refractory AML patients²⁴.

Adoptive Therapy with NK cells

One significant limitation is that the numbers of NK cells/kg recipient weight obtained by leukaphoresis relatively small ($\sim 2 \times 10^7$ /kg). No study has linked human GVHD with NK cell infusions, thus increasing the NK cell dose is one obvious approach to improve the anti-leukemia activity. In this regard, a number of promising methods are currently being tested including expanding NK cells with artificial antigen presenting cells (aAPCs) transduced with cytokines and costimulatory receptors. In particular, Imai and coworkers have pioneered the use of K562 cells transduced with IL-15, the IL-15 receptor α chain and a potent costimulatory molecule, 4-1BBL. Therefore, these cells mimic the interactions that NK cells receive from activated APCs and induced significant NK cell expansion and functionality²⁵. Similar to this approach, work by the Lee group has used the same concept of aAPCs with the 4-1BBL costimulatory molecule, but instead of IL-15, they transduced aAPCs with IL-21/IL-21R. Prior to these studies, the role of IL-21 on NK cell function was controversial, but surprisingly, these aAPCs have shown profound NK cell activation and proliferation ($>40,000$ fold over 3 weeks)²⁶. These IL-21 activated NK cells are also being tested in humans ("IL-21-Expanded NK Cells for Induction of Acute Myeloid Leukemia (AML)," NCT01787474). Still other approaches to expand NK cells *ex vivo* include the co-culture with irradiated EBV-infected cell lines^{27,28}. The exact mechanism for how some EBV lines activate and expand NK cells unknown, but this approach may be more appealing than the above studies since large numbers of stimulator cells can be created, tested and

cryopreserved for future use; perhaps leading to improved quality control and easier methods that are compliant with good manufacturing practice (GMP) regulations.

Regulatory T cells: Development and Function

Tregs make up ~5% of the peripheral blood T cell compartment and play a critical role in peripheral tolerance. Phenotypically, they are CD4⁺ T cells that lack expression of the IL-7 receptor (CD127) and constitutively express the high affinity IL-2 receptor, CD25 (i.e., CD4⁺CD127⁻CD25⁺). Since activated conventional T cells (Tcon) can also express CD25, Tregs are best defined by the expression of the master regulatory gene forkhead box p3 (Foxp3). This transcription factor drives a gene expression profile that endows Tregs with profound immunoregulatory capacity that suppresses immune activation and cell proliferation, leading to immune tolerance. Proof of the necessity of Tregs in the induction of peripheral tolerance is best evidenced by *scruffy* mice or in humans with IPEX syndrome (immunodysregulation polyendocrinopathy and enteropathy, X-linked), where the lack functional Foxp3 leads to severe and uncontrolled lymphoproliferation and autoimmunity²⁹. Treg ontogeny occurs through two distinct processes including a thymic selection pathway resulting in so called, “natural” or nTregs, as well as the peripheral conversion of Tcon to Tregs, resulting in “induced” or iTregs (reviewed in³⁰). The thymic pathway of Treg development involves selection steps which are similar to, but distinct from the developmental events that occur during thymic selection for Tcon. Regarding the development of iTregs, recent studies show that in the presence of TGF- β and IL-2, naïve T cells can be converted to iTregs, but other conditions have also been described³⁰.

Tregs control both the antigen priming and effector stages of Tcon activation, but exactly how they mediate their immune suppressive properties is not yet clearly defined. Tregs functionally suppress a wide range of cell types including T cells, NK cells, B cells and antigen presenting cells (APCs) in an MHC-unrestricted manner which occurs only when the cells are in close physical proximity (contact) (reviewed in^{31,32}). While studies have suggested that Treg activation is required for suppression³¹, more recently, studies in humans question this since resting Tregs from patients with cancer could suppress NK cell functions^{33,34}. Whether these observations are due to differences in the assays used, target cell types tested or prior *in vivo* activation is not known.

A number of potential mechanisms of Treg mediated suppression have been put forth using *in vitro* T cell assays and knock-out mice, but no single pathway has been shown to be clearly implicated over another, perhaps suggesting that Tregs function through multiple mechanisms depending on the context or the cell type being suppressed (reviewed in^{31,35}). For instance, Tregs may directly contact APCs, resulting in the attenuation of APC function through interactions between CTLA-4 and LAG-3 (on Tregs) binding to CD80/CD86 and MHC class II (on APCs). Alternative methods of Treg-induced suppression may be through neuropilin-1 (NRP-1) which prevents access of APCs to effector cells. Alternatively, Tregs can mount an immunological attack on a number of different cell types through a granzyme/perforin cytotoxicity pathway. Other mechanisms by which Tregs may mediate suppression are through soluble factors, such as counter-regulatory cytokines such as TGF- β , IL-10, IL-27, IL-35 or the production of adenosine by cell associated ectoenzymes (CD39 and CD71).

Lastly, through expression of the high affinity IL-2 receptor (CD25), Tregs may act as “cytokine sinks” and effectively outcompete other cells for the available IL-2, which is essential for immune activation.

Tregs and GVHD

Preclinical murine transplant models have convincingly established that Tregs have the capacity to prevent alloreactive T cell responses and experimental GVHD (reviewed in³⁶). While the early data on human Tregs and allo-HCT was mixed, the majority of recent studies support a role for Tregs in the protection from both acute and chronic GVHD³⁶⁻³⁸. For instance, stem cell grafts with a higher content of Tregs have been correlated with less acute GVHD^{39,40}. Likewise more rapid Treg reconstitution is associated with less acute GVHD, while patients with delayed Treg recovery have a higher likelihood of GVHD^{41,42}. As described above, Tregs can be generated through thymic differentiation and Matsuoka and colleagues investigated the Tregs reconstituting early after allo-HCT. They show that the majority had an activated/memory phenotype, consistent with homeostatic proliferation. Treg numbers normalized at ~9 months after transplant, but prolonged CD4 lymphocytopenia was associated with Treg apoptosis and an increased risk of chronic GVHD (cGVHD)⁴³. Thus, Tcons may provide IL-2 to Tregs to facilitate their survival early after allo-HCT. These observations may; thereby link successful early Tcon immune recovery to reduced aGVHD via IL-2 and Tregs. Further studies from this same group demonstrate that in the setting of cGVHD low dose IL-2 ($0.3-3 \times 10^6$ IU) could be safely administered and resulted in *in vivo* Treg expansion and clinical improvements in some patients⁴⁴. Considering the important role of Tregs in preventing alloreactivity, Treg depleted donor lymphocyte infusion (DLI) has also been tested in patients with relapsed leukemia or lymphoma following allo-HCT. In a study by Maury and coworker, patients were eligible Treg depleted DLI if they did not experience aGVHD or remission following conventional, unmanipulated DLI. Of the 17 patients treated patients, 6 developed aGVHD after one or two doses. The development of aGVHD led to partial or complete remissions that were associated with significantly improved survival⁴⁵.

Adoptive Therapy with Tregs

Investigators are currently piloting a variety of different clinical grade Treg isolation techniques including magnetic bead selection and high speed cell sorting based on surface phenotype (CD3+CD25^{high} or CD3+CD25^{high}CD45RA+). However, due to the relative paucity of Tregs in the peripheral blood and the high ratios of Treg:Tcon that are potentially needed to suppress GVHD, it is anticipated that Treg expansion will be needed^{35,38}. Methods have been published to expand Tregs with IL-2 and either CD3 $\times$ CD28 paramagnetic beads or aAPCs that express costimulatory molecules, such as CD80 or CD86 and Fc receptors (CD32 and/or CD64) that can bind antibodies such as CD3^{46,47}. Alternative approaches have been to generate iTreg by culturing naïve T cells with TGF- β , IL-2 and rapamycin⁴⁶.

Early results of the clinical adoptive transfer experiments have demonstrated safety and efficacy of Treg infusions. In the setting of double umbilical cord blood transplantation,

investigators at the University of Minnesota performed a dose escalation study using UCB-derived Treg at doses of 1, 3 and 30×10^5 Treg/kg at D+1 after UCB infusion. In an additional cohort, a second dose (30×10^5 Treg/kg) was given at D+15 as well. Tregs were isolated from a partially matched 3rd UCB unit (4-6/6 matched with the recipient) and then expanded using CD3 x CD28 magnetic beads and IL-2. In this study, Treg recipients had significantly lower rates of aGVHD compared to historical controls, while there was no obvious impact on either infectious complications or relapse rates⁴⁸. In an alternative approach, Di Ianni tested adoptive transfer of donor-derived, haploidentical Tregs which were infused during the period of chemotherapy-induced lymphocytopenia (day -4). The rationale was that the Tregs would expand *in vivo* and prevent Tcon induced aGVHD. At day 0, patients received megadose CD34+ selected hematopoietic stem cells and Tcons at doses which would otherwise be expected to cause GVHD. This was a dose escalation study where a fixed number of Tregs were delivered (2×10^6 /kg) and the dose of Tcons was escalated (0.5 to 4×10^6 /kg). Strikingly, only 2/26 patients developed significant aGVHD (>grade2) and there was no significant increase in relapse. Moreover, the functional recovery of T and B cells directed against pathogens was dramatically better compared to historical controls and there was no impact on the recovery of alloreactive NK cells⁴⁹.

NK and Treg Interactions

Studies from mice and humans clearly demonstrate bidirectional interactions between NK cells and Tregs. For instance, the depletion of Tregs in mice resulted in NK cell proliferation and augmented cytotoxicity, providing evidence that Tregs suppress NK cells *in vivo*^{34,50}. Conversely, numerous human studies show that high numbers of Tregs within solid tumors are associated with inferior outcomes (reviewed in^{33,34}), perhaps suggesting an impairment in NK function. More direct proof of a Treg:NK interaction is provided by *in vitro* studies using coculture, where human Tregs prevented NK cytotoxicity at ratios of 1 Treg to 5 NK cells³³. In the same study, Tregs also inhibited IFN- γ production by NK cells. This suppression was maintained even with formalin-fixed Tregs and could be blocked by antibodies against membrane bound TGF- β , strongly implicating this counter-regulatory cytokine in the inhibition of NK function. Treg induced suppression of NK cytotoxicity and IFN- γ production could be overcome by signaling through γ -chain cytokines IL-2, -4 and -7. Mechanistically, Tregs suppress NK cytotoxicity through the reduction of NK cell activating receptors, including NKG2D^{33,51} and NKp30⁵¹, both of which play key roles in recognition of malignant targets. Similarly, Tregs prevent IL-2 induced NKp44 expression⁵² which is also used by activated NK cells to recognize malignant cells. In other studies, human plasmacytoid dendritic cells (pDCs) could drive NK proliferation and this was further increased by the IL-2 produced by CD4+ Tcons. The addition of Tregs did not directly impair pDC-driven NK cell proliferation, but they did prevent Tcon augmented NK cell proliferation, suggesting that Tregs competed for IL-2 in this system⁵³. Murine studies further support a role of Tregs as a “cytokine sink” of available IL-2. For instance, in a diabetic model Treg depletion led to increased NK cell proliferation and augmentation of diabetes. Importantly these findings could be blocked by IL-2 specific antibodies⁵⁴. Complimentary studies by Gasteiger show that Tregs prevented the IL-2 dependent NK maturation and activation in a tumor and viral infection model⁵⁵. Moreover, upon depletion

of Tregs, NK cells were more responsive to targets that were missing self-MHC, a situation analogous to KIR ligand mismatch in humans⁵⁶. Still further proof of this, is from our own preliminary studies which show that Tregs can impair NK cell function when IL-2 is present in the culture media, but not when IL-15 is present (unpublished, Bachanova, Verneris, Miller).

While the vast majority of data support a Treg mediated suppression of NK cells, there is some data to suggest NK:Treg crosstalk is bidirectional and that NK cells can impair Treg expansion and function. For instance, in a Tuberculosis model, NK cells cultured with mycobacterium exposed APCs impaired Treg proliferation and could even kill expanded Tregs in an NKG2D-dependent manner⁵⁷. These results suggest that under steady state Tregs negatively modulate NK cells, but during infectious challenges, the opposite occurs, perhaps as a method to respond to such challenges. Other studies have documented that NK derived IFN- γ can prevent the expression of Foxp3 and the peripheral conversion of Tcons to Tregs⁵⁸.

Potential Opportunities

Tregs play an important role in allo-HCT by taming allo-reactivity and quelling GVHD, but they also may impair NK (or T cell)-mediated GVL. While the early human studies of Treg adoptive transfer do not necessarily support this claim^{48,49}, the numbers of treated patients are small and the diseases heterogeneous. Moreover, the dose of Tregs infused is relatively small, leaving the impact of Tregs on NK cell function and relapse an open question. Understanding the interactions between Tregs and NK cells presents opportunities to possibly modulate either GVL or GVHD. As described above, Treg depletion in the setting of DLI improved GVL responses⁴⁵ and a similar approach (of Treg depletion) could be used to modify hematopoietic stem cell grafts. Other strategies to augment GVL responses may involve transient Treg eradication using of CD25 directed antibodies (dclizamab) or immune toxins conjugated IL-2 (denileukin diftitox). In fact, our recent studies suggest that that denileukin diftitox prior to haploidentical NK cell infusions led to NK cell expansion in the blood of individuals 14 days after infusion, a marker of disease response (Bachanova, Verneris and Miller, unpublished). Still other approaches to augment GVL reactions after allo-HCT may use drugs that selectively impair Tregs. Recently, lenalidomide or pomalidomide have been shown to impair Treg expansion and function *in vitro*. While the mechanism of the suppression is not entirely clear, *in vitro* studies show these immune modulatory drugs (IMiDs) impaired Foxp3 expression, but not TGF- β production⁵⁹. Lenalidomide has recently been tested in the post-transplant period in a group of myeloma patients undergoing allo-HCT. Briefly, lenalidomide was started at D100-180 after transplant in this dose escalation study. The maximum tolerated dose of lenalidomide was 5 mg/d $\times$ 21 days for an anticipated 4 cycles. aGVHD occurred in 38% of patients, mostly during the first two cycles. Some patients showed changes in the immunological parameters, including NK and Tcon activation and a reduction in Tregs. Patients who showed these changes had superior survival⁶⁰.

In summary, donor NK cells have the capacity to recognize and kill leukemia cells. The rapid recovery of NK cells early after allo-HCT is implicated in GVL responses.

Conversely, Tregs restrain immune responses and mediate tolerance. Murine studies and human *in vitro* experiments support the assertion that Tregs antagonize NK responses. Whether this occurs in humans following adoptive transfer or allo-HCT is an open question. While some studies support an augmented NK cell response in the absence of Tregs, the clinical trials are just now being performed. Regardless, manipulation of these two cell types using the methods described above have the potential to not only reduce aGVHD, but also augment GVL. We look forward to clinical studies aimed at accomplishing this task.

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Table 1

NK associated Activation and Inhibitor Receptors and Their Ligands

Inhibitory Receptors		Activating Receptors	
NK	Target	NK	Target
KIR2DL1	HLA-C2	KIR2DS1	HLA-C2
KIR2DL2/3	HLA-C1	KIR2DS2	?
KIR2DL4	HLA-G	KIR2DS3, S4 and S5	?
KIR3DL1	HLA-Bw4	CD16	IgG
KIR3DL2	HLA-A3, -A11	NKp30	B7-H6 (BAT3)
NKG2A/CD94	HLA-E	NKp46	Viral hemagglutinins, ??
SIGLEC 3, 7, 9	Sialic acid	NKp44	Viral hemagglutinins, ??
KLRG1	Cadherins	CD244 (2B4)	CD48
NKR-P1A	LLT-1	CD226 (DNAM-1)	CD112 (Nectin-2), CD155 (PVR)
LILRB1 (CD85j, ILT2)	HLA class I	CD94-NKG2C	HLA-E
		CD94-NKG2E	HLA-E
		NKG2D	ULBP 1-6, MICA, MICB
		NTB-A	NTB-A
		CD96 (Tactile)	CD155 (PVR)
		NKp80	AICL
		CD160 (BY55)	HLA-C