EDITORIAL

A key to engineering natural killer cells to attack acute myeloid leukemia

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In this issue of *Hematologica*, Kaito and colleagues report on a way to switch on natural killer (NK) cells so that the activity of these cells against acute myeloid leukemia (AML) is increased.[1] AML is caused by the accumulation of multiple genetic abnormalities in hematopoietic stem and progenitor cells. Despite the development of novel therapies for AML, relapse remains a major problem. Immunotherapy is a promising approach to target diverse clones of AML, but the mechanisms of immune dysregulation in AML and methods to overcome them need to be elucidated.

NK cells are characterized by their cytotoxic capacity against virally infected cells and cancer cells without prior activation or past exposure. Many studies have shown the fundamental role of NK cells in cancer immune surveillance. NK cells are also considered important for disease control in AML based on several observations, including that the anti-leukemic activity of NK cells is inversely correlated with disease progression,[2] and NK-cell function is correlated with prognosis.

As detailed in a recent review,[3] when used as cellular therapy, NK cells are generally safe because of the low risk of allogeneic reactions and cytokine release syndrome. A patient's peripheral blood NK cells are usually infused after *ex vivo* expansion, but the proliferation and functional capacity of autologous cells from previously treated patients are often inadequate, limiting their therapeutic efficacy. While allogeneic NK cells harvested from healthy donors have shown clinical efficacy,[4] various challenges to this strategy remain, including low persistence, lack of optimal protocols for *ex vivo* expansion, and risk of graft-*versus*-host disease mainly due to contaminating T cells. Other sources of NK cells include umbilical cord blood, induced pluripotent stem cells, and the patient-derived NK cell line NK-92, available as an off-the-shelf product. In a phase I clinical trial, NK-92 cells provided responses, without severe toxicity, in some patients.[5] Since the introduction of chimeric antigen receptor (CAR)-T cells, much attention has been focused on the cytolytic potency of chimeric-based technologies. CAR-NK cells are considered a promising option due to their favorable cytotoxicity profile and low manufacturing cost. NK-92 cells have also been modified with CAR and used in clinical trials.[6] However, NK-cell therapies, including CAR-NK cells, still have problems to solve, such as loss of target antigen, a hostile tumor microenvironment, and tumor heterogeneity. One of the biggest problems is the short-term persistence of these cells *in vivo* and, despite many efforts, including cytokine activation and genetic modification,[7] objective responses remain transient.

NK cells express a variety of activating and inhibitory receptors to recognize host cell changes such as malignant transformation or viral infection. In addition to downregulation of major histocompatibility complex class I ligands, the balance of signals from these receptors regulates the cytotoxic function of NK cells. In NK cells from AML patients, downregulation of activating receptors, such as natural cytotoxicity receptors and DNAX accessory molecule-1 (DNAM-1, CD226), and upregulation of inhibitory receptors including CD94 and TIGIT, lead to dysfunction.[8] In AML cells, loss of activating ligands or increased expression of inhibitory ligands has been reported. Among them, the Nectin/Nectin-like family plays an important role in the regulation of NK cells as well as T cells. DNAM-1 is a representative co-stimulatory receptor, while TIGIT, CD96, and PVRIG are inhibitory receptors. Several ligands, including CD155 and NECTIN2 (CD112), interact with these receptors.[9] Ligands and receptors in this family have various binding affinities for each other rather than a one-to-one correspondence (Figure 1). The signaling network mediated by these complex ligand-receptor relationships regulates NK-cell activation precisely in a context-dependent manner. The ratio of CD112 to CD155 expression varies between tumors, and the CD155-TIGIT and CD112-PVRIG axes may play different roles in NK-cell regulation in different tumors. In AML, CD155 and CD112 are reportedly negative prognostic markers,[10] but

their functional significance is not yet clear. NK cells from AML patients also show high TIGIT expression as well as reduced DNAM-1 expression, associated with poor prognosis. However, it has not been clearly understood what kinds of ligand-receptor relationships are important in the pathogenesis of NK-cell abnormalities in AML in terms of therapeutic implications.

In the abovementioned study, Kaito *et al.* showed that CD112 and CD155 are highly expressed in primary AML samples.[1] As was reported previously,[10] cases with high CD112 expression had a poor prognosis. In contrast, DNAM-1 and TIGIT were highly expressed in NK and T cells, but there was no correlation between their expression and clinical characteristics or therapeutic responses. After showing that more than 80% of NK-92 cells expressed TIGIT and 8% expressed DNAM-1, the authors generated NK-92 cells

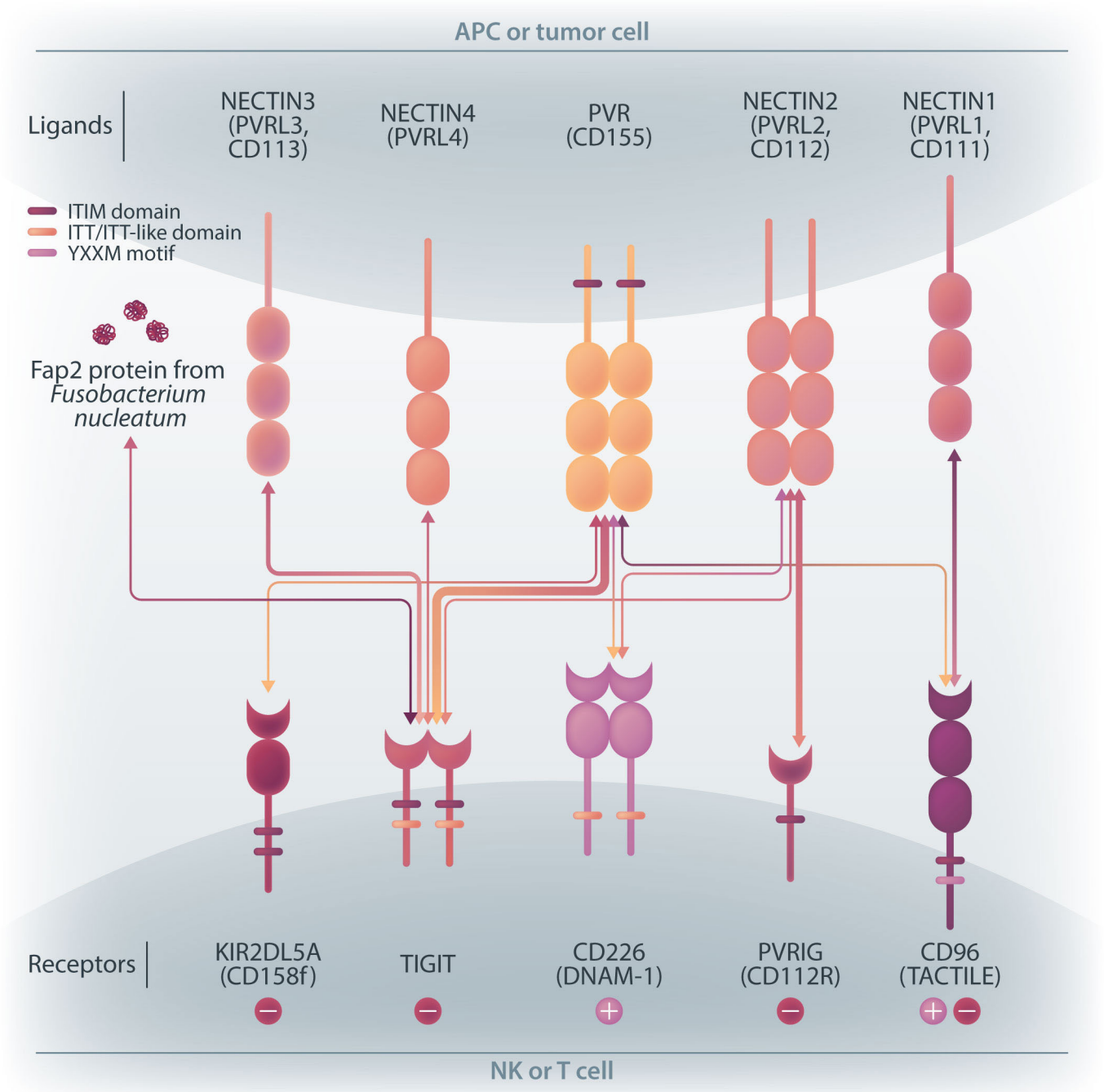

Figure 1. Receptor-ligand interactions of the Nectin/Nectin-like family. The receptors are expressed on natural killer (NK) cells and T cells, while the ligands are present on either antigen-presenting cells or tumor cells. DNAM-1 activates NK cells via interactions with CD155 or CD112, but DNAM-1 has a higher affinity for CD155 than for CD112. The affinity between TIGIT and CD155 is significantly higher than that between DNAM-1 and CD155, so the activating receptor DNAM-1 is competed against the CD155 ligand by the inhibitory receptor TIGIT. The CD112 ligand mediates the co-stimulatory effect via DNAM-1 and the inhibitory effects via interactions with CD112R and TIGIT. CD96 is primarily bound by CD155 and acts as an inhibitory receptor, although it can act as an activating receptor in certain circumstances. In this way, ligands and receptors have various binding affinities for each other rather than a one-to-one correspondence. TIGIT also interacts with Fap2, produced by *Fusobacterium nucleatum* bacteria in the tumor microenvironment, to suppress NK-cell activity. Activating and inhibitory signals induced by the receptor are represented by + and -. Ligand-receptor interactions are illustrated by arrows, with arrow thickness indicating relative affinity. However, a model has recently been reported in which differences in surface expression, rather than differences in intrinsic affinity, are important. APC: antigen-presenting cell; PVRL: poliovirus receptor-related; CD: cluster of differentiation; PVR: poliovirus receptor; ITIM: immunoreceptor tyrosine-based inhibition motif; ITT: immunoglobulin tail tyrosine; Fap2: fibroblast activation protein-2; KIR2DL5A: killer cell immunoglobulin-like receptor, two Ig domains and long cytoplasmic tail 5A; TIGIT: T-cell immunoglobulin and ITIM domain; DNAM-1: DNAX accessory molecule-1; PVRIG: poliovirus receptor-related immunoglobulin domain containing; TACTILE: T cell-activated increased late expression; NK: natural killer.

transfected with DNAM-1 (NK-92 DNAM-1) or lacking TIGIT. In cytotoxicity assays, NK-92 DNAM-1 cells showed enhanced cytotoxicity against AML cells, with increased expression of degranulation markers and intracellular cytokines. In contrast, cells with knockout (KO) of TIGIT did not show these changes nor additive effects with DNAM-1 transfection. NK-92 DNAM-1 cells showed better cytotoxicity than NK-92 cells against CD155 KO AML cells. However, this enhancement was lost against CD112 KO AML cells and, interestingly, the cytotoxicity was rather less against double KO AML cells. When co-cultured with double KO AML cells, the index of activation of NK-92 DNAM-1 cells was attenuated compared to that following co-culture with wild-type AML cells, accompanied by upregulation of inhibitory receptors and signaling molecules, suggesting that the enhanced cytotoxicity of NK-92 DNAM-1 cells is ligand-dependent. These findings were replicated in primary AML blasts and in xenograft models, in which AML cells expressing CD155 and CD112 were subject to enhanced cytotoxicity by NK-92 DNAM-1 cells. While the fact that NK-92 DNAM-1 cells can be suppressed in the absence of an appropriate ligand on AML cells necessitates further analysis of the ligand-receptor relationships before clinical applications, this study demonstrates that DNAM-1 transfection in NK-92 cells enhances cytotoxic activity against AML in a CD112- and CD155-dependent manner.

Further improvements are clearly needed to realize effective NK-cell therapy, including improved tumor targeting, more effective CAR design, and combination with other immunotherapies. Nevertheless, the results presented by Kaito and collleagues are important findings that may cooperate with such improvements.

Disclosures

No conflicts of interest to disclose.

Contributions

Both authors contributed equally.

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