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REVIEW ARTICLE

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# Pharmacological targeting of natural killer cells for cancer immunotherapy

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## Abstract

Natural killer (NK) cells are innate lymphocytes that rapidly respond to cancer cells without prior sensitization or restriction to the cognate antigen in comparison with tumor antigen-specific T cells. Recent advances in understanding NK-cell biology have elucidated the molecular mechanisms underlying the differentiation and maturation of NK cells, in addition to the control of their effector functions by investigating the receptors and ligands involved in the recognition of cancer cells by NK cells. Such clarification of NK-cell recognition of cancer cells also revealed the mechanism by which cancer cells potentially evade NK–cell-dependent immune surveillance. Furthermore, the recent clinical results of T–cell-targeted cancer immunotherapy have increased the expectations for new immunotherapies by targeting NK cells. However, the potential use of NK cells in cancer immunotherapy is not fully understood. In this review, we discuss the current evidence and future potential of pharmacological targeting of NK cells in cancer immunotherapy.

**KEYWORDS**

antibody, anti-tumor immunity, immunotherapy, NK cell, small molecule

## 1 | INTRODUCTION

Natural killer (NK) cells are innate lymphocytes that play an important role as immune effector cells to protect against virus infection or cancer.[1,2] As their name suggests, NK cells can kill target cells without prior sensitization through the release of cytotoxic granules containing perforin and granzymes, by producing pro-inflammatory cytokines, such as IFN-γ and TNF-α, or by activating death receptors via the expression of TNF-related apoptosis-inducing ligand (TRAIL) or FasL.[3-6] Due to those effector functions, NK cells are considered important effector cells for cancer immune surveillance.[7]

The recent advances in our understanding of NK-cell biology have elucidated the molecular mechanisms understanding the differentiation and maturation of NK cells, in addition to the control of their effector functions (Figure 1). In particular, the mechanisms that regulate the expression of ligands involved in the recognition of cancer cells by NK cells have been investigated extensively over the last several decades. The activation of NK cells is tightly regulated by the balance of signals from activating receptors and inhibitory receptors,[8] therefore it is important to induce the effector function of NK cells that controls different signaling pathways that promote or inhibit NK cell activation. Furthermore, the maturation status of NK cells affects their effector function and/or activation threshold. Therefore, the factors involved in controlling NK-cell differentiation are also essential for maximizing their effector function.[9-11] As the use of NK cells for adoptive cell therapies has been extensively examined and recently reviewed,[12,13] in this review, we discuss the current evidence and future potential of pharmacological targeting of NK cells in cancer immunotherapy (Figure 2).

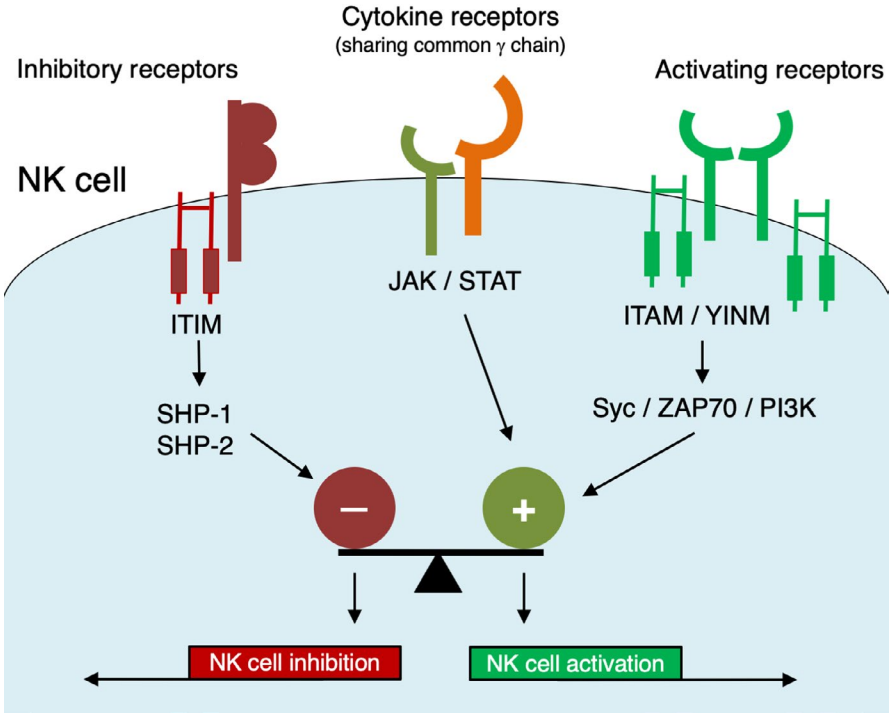


**FIGURE 1** Control of natural killer (NK) cell functions by the balance of activating and inhibitory signals. NK cell function is regulated by signaling through activating and inhibitory receptors, and cytokines belonging to the common γ-chain family play essential roles in the process of NK cell activation. Although immunoreceptor tyrosine-based activation motif (ITAM) and the PI3K-binding motif (YINM motif), and subsequent signaling through Syk/Zap70/PI3K pathways are known as the key downstream NK cell-activating receptors, the NK cell inhibitory receptors contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domains, which recruit intracellular tyrosine phosphatases, such as SHP-1 or SHP-2, to regulate the inhibition of NK cell activation

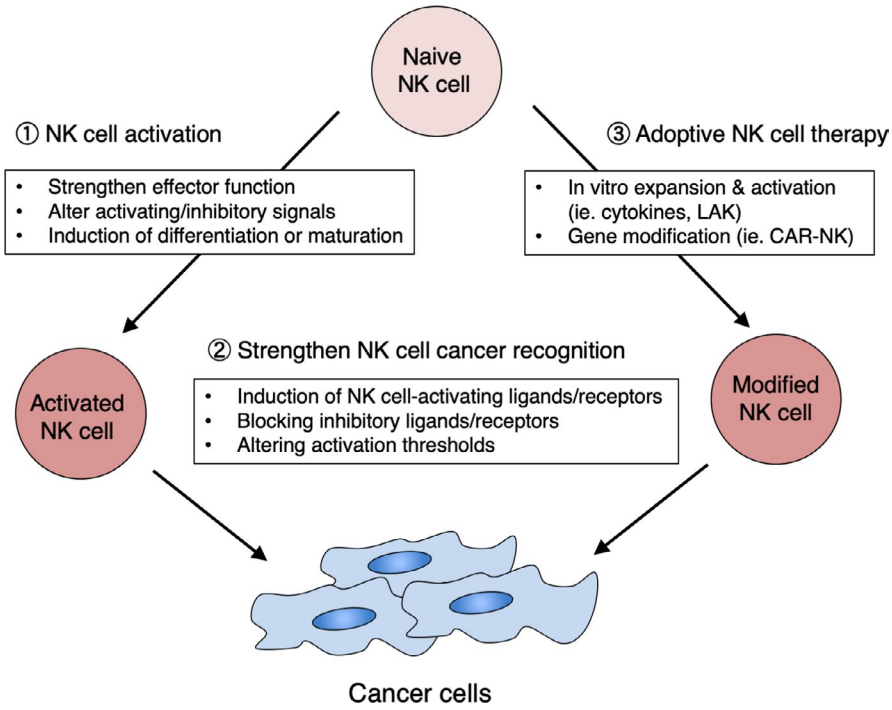


**FIGURE 2** Potential targets of natural killer (NK) cell-based cancer immunotherapy. There are several pharmacological targets that can be used to develop cancer immunotherapies that function by controlling NK cells via their activation or strengthening their cancer cell recognition. The use of NK cells in adoptive cancer immunotherapy is also expected

## 2 | PHARMACOLOGICAL TARGETS OF NK-CELL ACTIVATION

It is becoming more evident that the processes of NK-cell development and differentiation are controlled by a highly complicated mechanism similar to that for other lymphocyte lineage such as T cells and B cells.[2,14] Cytokines belonging to the common γ-chain family, such as IL-2 and IL-15, are essential in the process of NK-cell development and differentiation.[15] In addition to these common γ-chain family cytokines, the interaction with stromal cells in bone marrow is equally important for NK-cell development and differentiation.[16,17] Furthermore, the functional subsets of NK cells can be distinguished using NK-cell maturation markers (ie CD11b, CD27, and DNAM-1)[9,10,18] and their differentiation process can be controlled by transcription factors (ie E4BP4, T-bet, Eomes, Blimp-1, Aiolos).[19-23] In this section, we will outline the pharmacological approaches to regulate NK-cell differentiation and maturation processes (Figure 3).

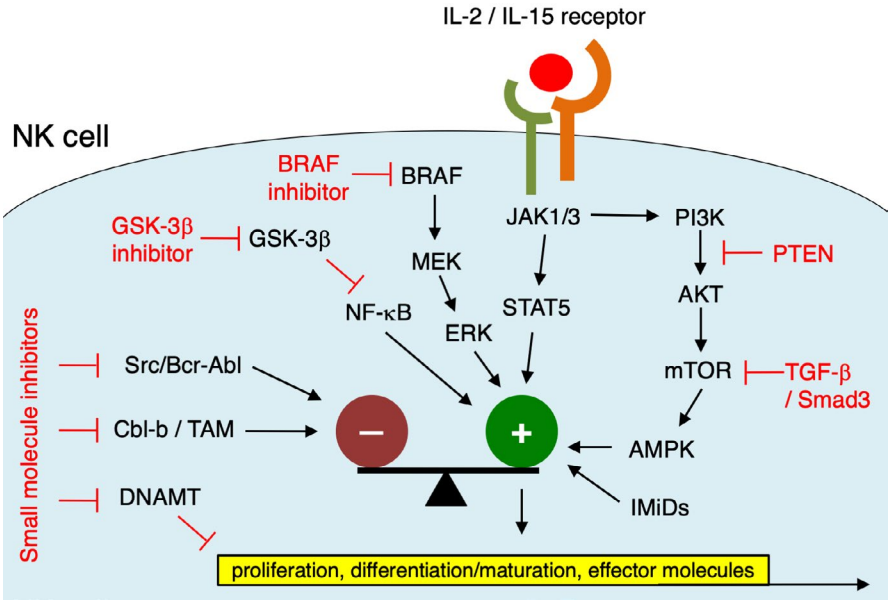


**FIGURE 3** Pharmacological targeting of natural killer (NK) cell activation against cancer cells. Potential pharmacological targets to elicit the anti-tumor effector function of NK cells by regulating molecules involved in their activation, differentiation, and maturation processes

## 2.1 | IL-2 and IL-15 pathways

IL-2 and IL-15 are cytokines that share their receptor subunits (IL-2 receptor β and γ chains), and play an important role in the differentiation, proliferation, and activation of NK cells. Although IL-2 is known to be important for maintaining NK-cell homeostasis, proliferation, and cytotoxic activity, it is also required to maintain regulatory T cells (Treg). Indeed, the clinical administration of IL-2 attenuated the function of NK cells and the proliferation of Treg by suppressing NK cells.[24] To overcome this issue, IL-2 mutants that selectively bind to the IL-2 receptor β chain have been developed. Such IL-2 mutants are expected to have selective activity against NK cells[25] by eliminating the effects on Treg, which often co-express the IL-2 receptor α chain. Unlike IL-2, IL-15 selectively acts on immature NK cells through the activation of STAT5 and the expression of Bcl-2 to induce their differentiation and proliferation.[26] Conversely, JAK kinases are important for downstream signaling of IL-2 and IL-15, and their inhibitor, ruxolitinib, which is clinically used to treat myelofibrosis, suppressed NK-cell function by inhibiting immune synapse formation and their maturation.[27] This result is considered to be clinical evidence demonstrating that the JAK-dependent signaling pathway is important for the maintenance and maturation of NK cells in humans. As the use of IL-2 or IL-15 in cancer treatment has been difficult due to their severe adverse effects, it will be important to assess the utility of IL-2 and IL-15 pathways in NK–cell-targeted cancer immunotherapy in combination with other modalities such as adoptive cell therapy.

## 2.2 | mTOR and PTEN

The importance of the mTOR pathway in NK-cell differentiation has been reported, as it is a downstream molecule in the IL-15 signaling pathway.[28] Activation of STAT5 in NK cells was observed after IL-15 stimulation even at low concentrations, whereas mTOR activation required IL-15 stimulation at higher concentrations. TGF-β, which is known to suppress the function of NK cells, was also reported to suppress their function through the mTOR pathway.[29] Moreover, NK cells exhibited higher responsiveness to secondary stimulation as memory NK cells in the mouse cytomegalovirus (MCMV) infection model.[11] The regulation of AMP-activated protein kinase (AMPK) by the mTOR pathway was reported to be essential in this process.[30] In addition, phosphatase and tensin homolog (*PTEN*), which is a tumor suppressor gene, negatively regulates human NK-cell function through suppression of the downstream AKT, MAPK, and mTOR pathways.[31] In this regard, the *BRAF* mutation is an important driver oncogene in melanoma and, interestingly, the B-RAF inhibitor PLX4720 exhibits NK–cell-dependent anti-tumor effects in association with the activation of ERK molecules.[32] However, the mTOR pathway is generally important for metabolic regulation of many types of immune cells, including NK cells, therefore it is a potential target for pharmacological manipulation of NK-cell activity.

## 2.3 | Src and Bcr-Abl pathway

Src kinases are known to play a major role in inhibiting and activating signaling pathways of NK cells. The small molecule Src/Bcr-Abl tyrosine kinase inhibitor dasatinib, which is approved for the treatment of chronic myeloid leukemia (CML), is known to increase NK-cell effector function against certain lymphoma and leukemia cell lines.[33,34] Conversely, it has also been reported that dasatinib inhibits human T-cell activation and proliferation, and NK-cell cytotoxicity in vitro.[35] Although the mechanism of its controversial effects of dasatinib on NK cells remains unclear, the involvement of Vav phosphorylation

was proposed as a potential mechanism for increased NK-cell activity induced by dasatinib.[34,36]

### 2.4 | Glycogen synthase kinase-3

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine protein kinase involved in the Wnt/β-catenin and NF-κB signaling pathways, and its inhibition accelerates NK-cell maturation and increases their effector function.[37] The use of GSK3 kinase inhibitor greatly increased the expansion of human NK cells with IL-15 in addition to the expression of the late-stage maturation marker CD57. GSK3 inhibition in human NK cells also increased the expression of transcription factors such as T-bet, Zeb2, and Blimp-1, which are associated with NK-cell maturation. Furthermore, the expression of GSK-3β in NK cells was reported to be upregulated in acute myeloid leukemia (AML) patients, which caused NK cells to become dysfunctional.[38] Such dysfunction of NK cells can be reproduced by overexpressing GSK-3 in normal NK cells, whereas genetic or pharmacological GSK3 inactivation increased NK-cell effector function through the induction of LFA-1 expression and the NK-κB signaling pathway.[38]

### 2.5 | Smad3

Smad3 is a well known essential molecule in the canonical TGF-β signaling pathway, and which is known to suppress NK-cell function. The TGF-β/Smad3 signaling pathway directly suppresses E4BP4/NFIL3, which is an upstream molecule of T-bet.[39] In addition to these findings, a Smad3 inhibitor was reported to inhibit tumor progression by increasing NK-cell effector function.

### 2.6 | TAM kinase

Cbl-b, an E3 ubiquitin ligase, is a known inhibitory signal in NK cells and the mechanism by which it controls NK-cell function has been clarified.[40] Cbl-b suppresses NK-cell activation through the ubiquitination of TAM kinases (Tyro-3/Axl/Mer), which are receptor tyrosine kinases essential for homeostatic regulation of the immune system, including NK cells. A small-molecule inhibitor of Tyro3, Axl, and Mertk (TAM) kinases significantly reduced metastasis in a pre-clinical model of melanoma and breast cancer via an NK–cell-dependent mechanism.

### 2.7 | DNA methyltransferase

The DNA methyltransferase inhibitor azacitidine/5-azacytidine is a chemical analog of nucleoside cytidine used to treat AML and myelodysplastic syndromes. Decitabine was reported to increase NK-cell effector function,[41] in addition to their maturation and infiltration into tumor site.[42] The mechanism of action of decitabine on NK cells can be explained by the epigenetic induction of gene expression of cytokines and cytotoxic molecules such as perforin or TRAIL.[42]

### 2.8 | Immunomodulatory drugs (IMiDs)

IMiDs have been used as therapeutic agents for multiple myeloma due to their direct anti-myeloma activity, and anti-angiogenic and immunomodulatory activities.[43] The exact mechanism of the anti-myeloma activity of IMiDs remains unclear, however cereblon was identified as a binding protein of IMiDs to regulate the expression of Ikaros family transcription factors.[44] In its immunomodulatory activity, the importance of NK cells has been extensively reported.[43] In pre-clinical animal models, IMiDs promoted the cytotoxic activity and proliferation of NK cells, in addition to the production of cytokines indirectly through the reduction of SOCS1 in T cells and dendritic cells.[45] It was also reported that IMiDs can directly increase IFN-γ production by NK cells.[46] In clinical practice, IMiDs treatment is associated with an increase in NK-cell number and function, leading to anti-tumor effects.[47] Furthermore, the combination treatment of antibodies and IMiDs in cancer patients has been reported to improve the efficacy of antibodies in an NK–cell-dependent manner.[48] However, the exact molecular mechanism underlying the anti-tumor effects of IMiDs through NK cells is unknown and further studies are still required.

## 3 | PHARMACOLOGICAL TARGETS OF NK-CELL RECOGNITION

Based on the discovery of a number of molecules involved in the target recognition of NK cells, it has become evident that the responsiveness of NK cells to target cells is regulated by the balance between activating and inhibitory receptors.[8] Therefore the tuning of activating and inhibitory signals for NK-cell recognition can be a target for improving NK-cell function. Among the different NK-cell receptors, the known inhibitory NK receptors are Ly-49 family molecules in mouse and killer cell immunoglobulin-like receptor (KIR) family molecules in humans that recognize self-MHC class I molecules. In addition to these self-MHC-recognizing NK-cell inhibitory receptors, other immunosuppressive molecules, which have been applied as immune checkpoint molecules, are also known to suppress NK-cell function. In this section, we discuss the regulatory mechanism of the expression of NK-cell activating ligands and immunosuppressive molecules, and its application to NK–cell-based cancer immunotherapy (Figure 4).

### 3.1 | Targeting of NK–cell-activating ligands

It is widely known that NKG2D is an important activating receptor for NK cells, and the expression of its ligands can be increased by DNA damage and cellular stress, including anti-cancer drug treatment.[49] The proteasome inhibitor bortezomib was reported to increase the sensitivity of cancer cells against TRAIL, which is one of the important cytotoxic

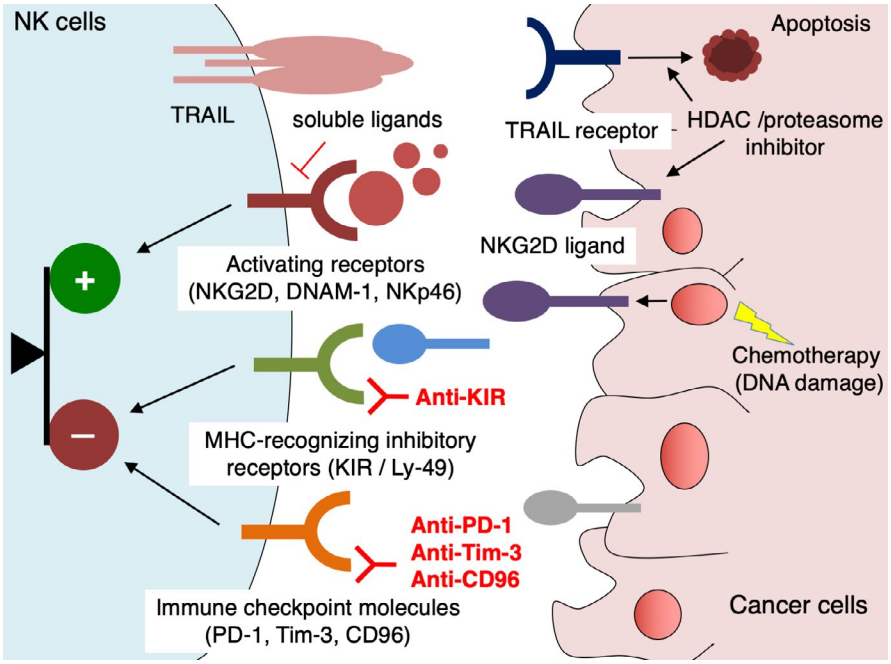


**FIGURE 4** Pharmacological targeting of natural killer (NK) cell recognition of cancer cells. Potential pharmacological targets to increase NK-cell recognition of cancer cells by regulating the expression of NK cell activating or inhibitory receptors and ligands

effector molecules of NK cells,[50] and further increase the expression of NKG2D ligand on cancer cells.[51] Histone deacetylase (HDAC) inhibitors were also reported to sensitize cancer cells to TRAIL and increase the expression of NKG2D ligands on cancer cells,[52] although some reduced NK–cell-activating B7-H6 and NKp30 ligand expression.[53]

In contrast with the importance of NKG2D in NK-cell activation, chronic exposure of NK cells to NKG2D ligands is known to cause cancer cell escape from NK-cell recognition by downregulating NKG2D receptor and inducing unresponsiveness in NK cells.[54] MHC I chain-related molecule (MIC) is one of the NKG2D ligands in humans that can be produced by cancer cells as soluble MIC (sMIC) after shedding by proteases. sMIC is known to downregulate NKG2D expression on NK cells via the same mechanism as chronic exposure to NKG2D ligand on the cell surface, thereby suppressing NK-cell function.[55] Similar to human MIC, the mouse NKG2D ligand MULT1 is produced as soluble MULT1 (sMULT1).[56] In contrast with the previous report on sMIC, sMULT1 prevents NK cells from becoming unresponsiveness following the downregulation of NKG2D by its strong binding ability to NKG2D.[56] This suggests that the pharmacological use of high-affinity soluble NKG2D ligand similar to sMULT1 can increase the responsiveness of human NK cells to cancer cells.

### 3.2 | Targeting NK–cell-suppressing molecules

As the recognition of self-MHC molecules by KIR molecules in humans and Ly-49 molecules in mice is known to limit NK-cell responsiveness, the prevention of such MHC-dependent suppression may increase NK-cell activity. Multiple types of inhibitory KIR are expressed on NK cells and blockade of their function using antibodies can potentiate NK-cell anti-tumor effects.[57,58] The pan-KIR2D antibody lirilumab (IPH2101/BMS-986015), which was designed to block the interaction between KIR2DL-1,-2,-3 inhibitory receptors and their ligands on NK cells to impair their inhibitory signaling, has undergone clinical testing to treat cancer patients by altering NK-cell activity.[59]

In addition to self-MHC-recognizing inhibitory receptors, other types of inhibitory molecules, including known immune checkpoint receptors in T cells, are also expressed on NK cells to suppress their function.[60] Although the exact mechanism by which PD-1 suppresses anti-tumor function of NK cells is unclear, inhibition of PD-1 increased the cytotoxicity of NK cells from myeloma patients in vitro, and the combination of PD-1 antibody or PD-L1 antibody and lenalidomide increased the anti-myeloma activity of NK cells.[61] In pre-clinical cancer models, PD-1 blockade increased tumor accumulation and antibody-dependent cellular cytotoxicity (ADCC) activity of NK cells.[61] Moreover, Tim-3 was reported to suppress NK-cell activity in melanoma patients, and its expression was correlated with the prognosis.[62] In addition to these classical immune checkpoint molecules, CD96 and T-cell immunoreceptor with Ig and ITIM domains (TIGIT) have been also recognized as important negative regulators of NK cells that compete the NK-cell activating receptor CD226 (DNAM-1).[63] By blocking CD96 or TIGIT using their respective antibodies, NK–cell-dependent anti-tumor immune responses can be elicited.[64] Although the roles of TIGIT and CD96 as immune checkpoint receptors in NK-cell biology are just beginning to be explored, accumulating evidence supports the targeting of these NK-cell inhibitory receptors in order to improve their anti-tumor effector functions.

## 4 | CONCLUDING REMARKS

Since the discovery of NK cells as lymphocytes that can eliminate cancer cells without prior sensitization, NK-cell research has been focused on understanding how NK cells distinguish normal healthy cells from cancer cells. Great efforts to clarify the mechanism of NK-cell target recognition have been made, which revealed the receptors and other

related molecules involved in NK-cell recognition. Furthermore, the molecular mechanisms underlying NK-cell differentiation and functional maturation have been elucidated in the last decade. Following the successful application of immune checkpoint inhibitors in clinical practice, their expected use in cancer immunotherapy has been reported. Recent cancer immunotherapy has been mainly focused on T–cell-dependent anti-tumor immunity; however, NK cells are also important anti-tumor effector cells that play predominant roles in early protection against carcinogenesis and/or metastasis. Moreover, NK cells also function not only as direct anti-tumor effector cells, but also as immune regulatory cells via cross-talk with other type of immune cells.[65-68] By altering NK-cell function pharmacologically, their multiple functions can be controlled to elicit anti-tumor immune responses. Although many studies are needed to establish NK–cell-targeted cancer immunotherapy, there are several promising pharmacological targets to activate NK cells. Therefore it is important to evaluate the clinical utility of NK–cell-targeted cancer immunotherapy.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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