Pharmacological targeting of natural killer cells for cancer immunotherapy

Kiho Miyazato | Yoshihiro Hayakawa

Institute of Natural Medicine, University of Toyama, Toyama, Japan

Correspondence
Yoshihiro Hayakawa, Institute of Natural Medicine, University of Toyama, Sugitani 2630, Toyama, Toyama 930-0194, Japan. Email: haya@inm.u-toyama.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 17H06398

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).
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.\textsuperscript{2,14} Cytokines belonging to the common $\gamma$-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 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 $\gamma$-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.\textsuperscript{2,14} Cytokines belonging to the common $\gamma$-chain family, such as IL-2 and IL-15, are essential in the process of NK-cell development and differentiation.\textsuperscript{15} In addition to these common $\gamma$-chain family cytokines, the interaction with stromal cells in bone marrow is equally important for NK-cell development and differentiation.\textsuperscript{16,17} Furthermore, the functional subsets of NK cells can be distinguished using NK-cell maturation markers (ie CD11b, CD27, and DNAM-1)\textsuperscript{9,10,18} and their differentiation process can be controlled by transcription factors (ie E4BP4, T-bet, Eomes, Blimp-1, Aiolos).\textsuperscript{19-23} In this section, we will outline the pharmacological approaches to regulate NK-cell differentiation and maturation processes (Figure 3).
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. 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 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. TGF-β, which is known to suppress the function of NK cells, was also reported to suppress their function through the mTOR pathway. Moreover, NK cells exhibited higher responsiveness to secondary stimulation as memory NK cells in the mouse cytomegalovirus (MCMV) infection model. The regulation of AMP-activated protein kinase (AMPK) by the mTOR pathway was reported to be essential in this process. 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. 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. 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. Conversely, it has also been reported that dasatinib inhibits human T-cell activation and proliferation, and NK-cell cytotoxicity in vitro. 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 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.\(^{59}\) The proteasome inhibitor bortezomib was reported to increase the sensitivity of cancer cells against TRAIL, which is one of the important cytotoxic
Effector molecules of NK cells, and further increase the expression of NKG2D ligand on cancer cells. Histone deacetylase (HDAC) inhibitors were also reported to sensitize cancer cells to TRAIL and increase the expression of NKG2D ligands on cancer cells, although some reduced NK–cell-activating B7-H6 and NKp30 ligand expression.

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. 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. Similar to human MIC, the mouse NKG2D ligand MULT1 is produced as soluble MULT1 (sMULT1). In contrast with the previous report on sMIC, sMULT1 prevents NK cells from becoming unresponsive following the downregulation of NKG2D by its strong binding ability to NKG2D. 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. The pan-KIR2D antibody liliumab (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.

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. 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. In pre-clinical cancer models, PD-1 blockade increased tumor accumulation and antibody-dependent cellular cytotoxicity (ADCC) activity of NK cells. Moreover, Tim-3 was reported to suppress NK-cell activity in melanoma patients, and its expression was correlated with the prognosis. 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). By blocking CD96 or TIGIT using their respective antibodies, NK–cell-dependent anti-tumor immune responses can be elicited. 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.56-68 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.

ACKNOWLEDGMENTS
We are grateful to all members of Hayakawa Laboratory for their support. This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (17H06398) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

CONFICT OF INTEREST
The authors have no conflicts of interest to declare.

ORCID
Yoshihiro Hayakawa https://orcid.org/0000-0002-7921-1171

REFERENCES
1. SunJC, LanierLL. NK cell development, homeostasis and function: parallels with CDB(+)/T cells. Nat Rev Immunol. 2011;11:645-657.
2. De ObaldiaME, BhandoolaA. Transcriptional regulation of innate and adaptive lymphocyte lineages. Annu Rev Immunol. 2015;33:607-642.
3. HerbermanRB, NunnME, HoldenHT, LavrinDH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. Int J Cancer. 1975;16:230-239.
4. HerbermanRB, NunnME, LavrinDH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. Int J Cancer. 1975;16:216-229.
5. SendofJ, AokiT, BoyseEA, BuafocK. Natural occurrence of lymphocytes showing cytotoxic activity to BALB/c radiation-induced leukemia RL male 1 cells. J Natl Cancer Inst. 1975;55:603-609.
6. SmythMJ, HayakawaY, TakedaK, Yagitah. New aspects of natural-killer-cell surveillance and therapy of cancer. Nat Rev Cancer. 2002;2:850-861.
7. Chirossomela, DumasPY, VienneM, VivierE. Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol. 2018;18:671-688.
8. Martinet, SmythMJ. Balancing natural killer cell activation through paired receptors. Nat Rev Immunol. 2015;15:243-254.
9. HayakawaY, SmythMJ. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. J Immunol. 2006;176:1517-1524.
10. HayakawaY, HuntingtonND, NuttSL, SmythMJ. Functional subsets of mouse natural killer cells. Immunol Rev. 2006;214:47-55.
11. O’SullivanTE, SunJC, LanierLL. Natural killer cell memory. Immunity. 2015;43:634-645.
12. HodginsJJ, KhanST, ParkMM, AuerRC, ArdolinoM. Killers 2.0: NK cell therapies at the forefront of cancer control. J Clin Invest. 2019;129:3499-3510.
13. ShimasakiN, Jaina, CampanaD. NK cells for cancer immunotherapy. Nat Rev Drug Discov. 2020;19:200-218.
14. CichockiS, SitnickaE, BrycesonYT. NK cell development and function—plasticity and redundancy unleashed. Semin Immunol. 2014;26:114-126.
15. BecknellB, CaligiuriMA. Interleukin-2, interleukin-15, and their roles in human natural killer cells. Adv Immunol. 2005;86:209-239.
16. RolinkaG, BalcuniunaitG, DemolierEC, CeredigR. The potential involvement of Notch signaling in NK cell development. Immunol Lett. 2006;107:50-57.
17. BeckRC, PadivalM, YehD, RalstonJ, CookeKR, LoweJB. The Notch ligands Jagged2, Delta1, and Delta4 induce differentiation and expansion of functional human NK cells from CD34+ cord blood hematopoietic progenitor cells. Biol Blood Marrow Transplant. 2009;15:1026-1037.
18. MartinetL, Ferrari De AndradeL, GuillereyC et al. DNA-M1 expression marks an alternative program of NK cell maturation. Cell Rep. 2015;11:85-97.
19. GascoyneDM, LongE, Veiga-FernandesH et al. The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. Nat Immunol. 2009;10:1118-1124.
20. Narni-MancinelliE, UgolinIS, VivierE. Tuning the threshold of natural killer cell responses. Curr Opin Immunol. 2013;25:53-58.
21. GordonSM, ChaixJ, RuppJL et al. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. Immunity. 2012;36:55-67.
22. KalliesA, CarottaA, HuntingtonND et al. A role for Blimp1 in the transcriptional network controlling natural killer cell maturation. Blood. 2011;117:1869-1879.
23. HolmesML, HuntingtonND, ThongRP et al. Peripheral natural killer cell maturation depends on the transcription factor Aiolos. EMBO J. 2014;33:2721-2734.
24. ItoS, BollardCM, CarlstenM et al. Ultra-low dose interleukin-2 promotes immune-modulating function of regulatory T cells and natural killer cells in healthy volunteers. Mol Ther. 2014;22:1388-1395.
25. LevinAM, BatesDL, RingAM et al. Exploiting a natural conformational switch to engineer an interleukin-2 ‘superkine’. Nature. 2012;484:529-533.
26. PilletAH, ThezeJ, RoseT. Interleukin (IL)-2 and IL-15 have different effects on human natural killer lymphocytes. Hum Immunol. 2011;72:1013-1017.
27. SchonbergK, RudolphJH, VonnahmehM et al. JAK inhibition impairs NK cell function in myeloproliferative neoplasms. Cancer Res. 2015;75:2187-2199.
28. MarciaSA, Cherifils-Viciniil, ViantC et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol. 2014;15:749-757.
29. BienS, MarciaSA, GuimaraesFS et al. TGF-beta inhibits the activation and functions of NK cells by repressing the mTOR pathway. Sci Signal. 2016;9:ra19.
30. O’SullivanTE, JohnsonLR, KangHH, SunJC. BNI3- and BNI3LP-mediated mitophagy promotes the generation of natural killer cell memory. Immunity. 2015;43:331-342.
31. BriercheckEL, TrottaR, ChenLE et al. PTEN is a negative regulator of NK cell cytolytic function. J Immunol. 2015;194:1832-1840.
32. Ferrari de AndradeL, NgiowSF, Stannardk et al. Natural killer cells are essential for the ability of BRAF inhibitors to control BRAFV600E-mutant metastatic melanoma. Cancer Res. 2014;74:7298-7308.
MIYAZATO AND HAYAKAWA

33. MustjokiS, EckblomM, ArstilaTP et al. Clonal expansion of T/NK cells during tyrosine kinase inhibitor dasatinib therapy. Leukemia. 2009;23:1398-1405.

34. SalihJ, HillpertJ, PlackeT et al. The BCR/ABL-inhibitors imatinib, nilotinib and dasatinib differentially affect NK cell reactivity. Int J Cancer. 2010;127:2119-2128.

35. FraserCK, BlakeSJ, DienerKR et al. Dasatinib inhibits recombinant viral antigen-specific murine CD4+ and CD8+ T-cell responses and NK-cell cytolytic activity in vitro and in vivo. Exp Hematol. 2009;37:256-265.

36. HassoldN, SeystahlK, KempFK et al. Enhancement of natural killer cell effector functions against selected lymphoma and leukemia cell lines by dasatinib. Int J Cancer. 2012;131:E916-E927.

37. CichockiF, ValamehrB, BjordahlR et al. GSK3 inhibition drives maturation of NK cells and enhances their antitumor activity. Cancer Res. 2017;77:5664-5675.

38. ParameswaranR, RamakrishnanP, MoretonSA et al. Repression of GSK3 restores NK cell cytotoxicity in AML patients. Nat Commun. 2016;7:11154.

39. TangPM, ZhouS, MengXM et al. Smad3 promotes cancer progression by inhibiting E4BP4-mediated NK cell development. Nat Commun. 2017;8:14677.

40. PaolinoM, ChoidasA, WallnerS et al. The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. Nature. 2014;507:508-512.

41. SchmiedelBJ, ArelinV, GruenebachF, SchmidtSM, SalihHR. Azacytidine impairs NK cell reactivity while decitabine augments NK cell responsiveness toward stimulation. Int J Cancer. 2011;128:2911-2922.

42. CanyJ, RoevenMWH, Hoogstad-van EvertJS et al. Decitabine enhances targeting of AML cells by CD34(+) progenitor-derived NK cells in NOD/SCID/IL2Rg(null) mice. Blood. 2018;131:202-214.

43. ShorttJ, HsuAK, JohnstoneRW. Thalidomide-analogue biology: immunological, molecular and epigenetic targets in cancer therapy. Oncogene. 2013;32:4191-4202.

44. LuG, MiddletonRE, SunH et al. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science. 2014;343:305-309.

45. GorgunG, CalabreseE, SoydanE et al. Immunomodulatory effects of lenalidomide and pomalidomide on interaction of tumor and bone marrow accessory cells in multiple myeloma. Blood. 2010;116:3227-3237.

46. HayashiT, HideshimaT, AkiyamaM et al. Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: clinical application. Br J Haematol. 2005;128:192-203.

47. Chanan-KhanAA, ChittaK, ErsingN et al. Biological effects and clinical significance of lenalidomide-induced tumour flare reaction in patients with chronic lymphocytic leukaemia: in vivo evidence of immune activation and antitumour response. Br J Haematol. 2011;155:457-467.

48. ZhuD, CorralLG, FlemingYW, SteinB. Immunomodulatory drugs Revlimid (lenalidomide) and CC-4047 induce apoptosis of both hematological and solid tumor cells through NK cell activation. Cancer Immunol Immunother. 2008;57:1849-1859.

49. GasserS, OrsulicS, BrownJE, RaulettDH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature. 2005;436:1186-1190.

50. LundqvistaA, AbramsSI, SchrumpDS et al. Bortezomib and depsipeptide sensitizes tumors to tumor necrosis factor-related apoptosis-inducing ligand: a novel method to potentiate natural killer cell tumor cytotoxicity. Cancer Res. 2006;66:7317-7325.

51. Vales-GomezM, ChisholmSE, Cassady-CainRL, Roda-NavarroP, ReyburnHT. Selective induction of expression of a ligand for the NKG2D receptor by proteasome inhibitors. Cancer Res. 2008;68:1546-1554.

52. SkovS, PedersenMT, AndresenL, StratentPT, WoetmannA, OdumN. Cancer cells become susceptible to natural killer cell killing after exposure to histone deacetylase inhibitors due to glycogen synthase kinase-3-dependent expression of MHC class I-related chain A and B. Cancer Res. 2005;65:11134-11145.

53. FieglerN, TextorS, ArnoldA et al. Downregulation of the activating Nkp30 ligand B7–H6 by HDAC inhibitors impairs tumor cell recognition by NK cells. Blood. 2013;122:684-693.

54. CoudertJD, ScarpellinoL, GrosF, VivierE, HeldW. Sustained NKG2D engagement induces cross-tolerance of multiple distinct NK cell activation pathways. Blood. 2008;111:3571-3578.

55. GrohV, WuJ, YeeC, SpeisT. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature. 2002;419:734-738.

56. DengW, GowenBG, ZhangL et al. Antitumor immunity. A shed NKG2D ligand that promotes natural killer cell activation and tumor rejection. Science. 2015;348:136-139.

57. Sanchez-CorreaA, Lopez-SejasN, DuranE et al. Modulation of NK cells with checkpoint inhibitors in the context of cancer immunotherapy. Cancer Immunol Immunother. 2019;68:861-870.

58. VelardiA, RuggeriL, MancusA, AversaF, ChristiansenFT. Natural killer cell allorecognition of missing self in allogeneic hematopoietic transplantation: a tool for immunotherapy of leukemia. Curr Opin Immunol. 2009;21:525-530.

59. KohrTHE, ThielessA, MarabelleA et al. Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies. Blood. 2014;123:678-686.

60. KeirME, ButteMJ, FreemanGJ, SharpeAH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677-704.

61. BensonDM Jr, BankanCE, MishaR et al. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. Blood. 2010;116:2286-2294.

62. da Silval, GalloisA, Jimenez-BarandaS et al. Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade. Cancer Immunol Res. 2014;2:410-422.

63. ChanCJ, MartinelL, GifillanS et al. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. Nat Immunol. 2014;15:431-438.

64. BlakeSJ, StannardK, LiuJ et al. Suppression of metastases using a new lymphocyte checkpoint target for cancer immunotherapy. Cancer Discov. 2016;6:446-459.

65. Martin-FontechaA, ThomsenLL, BrettS et al. Induced recruitment of mature CD27(high) NK cells with checkpoint inhibitors in the context of cancer immunotherapy. Cancer Immunol Immunother. 2018;6:348-357.

How to cite this article: Miyazato K, Hayakawa Y. Pharmacological targeting of natural killer cells for cancer immunotherapy. Cancer Sci. 2020;111:1869–1875. https://doi.org/10.1111/cas.14418