Natural killer cell–based immunotherapy for cancer

Shoubao Ma, Jianhua Yu, Michael A Caligiuri

The Journal of Immunology, 2025, **214**, 1444–1456
https://doi.org/10.1093/jimmun/vkaf036
Advance access publication April 17, 2025
Brief Reviews

Natural killer cell–based immunotherapy for cancer

Shoubao Ma[1,2,3,*], Jianhua Yu[4,5,6,*], and Michael A. Caligiuri[1,2,3,*]

[1]Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, Los Angeles, CA, United States
[2]Hematologic Malignancies Research Institute, City of Hope National Medical Center, Los Angeles, CA, United States
[3]City of Hope Comprehensive Cancer Center, Los Angeles, CA, United States
[4]Division of Hematology and Oncology, Department of Medicine, School of Medicine, University of California, Irvine, CA, United States
[5]Institute for Precision Cancer Therapeutics and Immuno-Oncology, Chao Family Comprehensive Cancer Center, University of California, Irvine, CA, United States
[6]Clemons Family Center for Transformative Cancer Research, University of California, Irvine, Irvine, CA, United States

*Corresponding author. Dr. Shoubao Ma, Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, 1500E Duarte Rad, Los Angeles, CA 91010, United States. Email: shma@coh.org; Dr. Jianhua Yu, Division of Hematology and Oncology, Department of Medicine, School of Medicine, University of California, Irvine, 1001 Health Sciences Road, Irvine, CA 92697, United States. Email: jianhuay@uci.edu and Dr. Michael A Caligiuri, Department of Hematology and Hematopoietic Cell Transplantation, City of Hope National Medical Center, 1500E Duarte Road, Los Angeles, CA 91010, United States. Email: mcaligiuri@coh.org.

Abstract

Natural killer (NK) cells are emerging as a promising tool for cancer immunotherapy due to their innate ability to selectively recognize and eliminate cancer cells. Over the past 3 decades, strategies to harness NK cells have included cytokines, small molecules, antibodies, and the adoptive transfer of autologous or allogeneic NK cells, both unmodified and genetically engineered. Despite favorable safety profiles in clinical trials, challenges such as limited in vivo persistence, exhaustion, and the suppressive tumor microenvironment continue to hinder their efficacy and durability. This review categorizes NK cell–based therapies into 3 major approaches: (i) cellular therapies, including unmodified and chimeric antigen receptor–engineered NK cells; (ii) cytokine-based strategies such as interleukin-2 and interleukin-15 derivatives; and (iii) antibody-based therapies, including immune checkpoint inhibitors and NK cell engagers. We highlight these advancements, discuss current limitations, and propose strategies to optimize NK cell–based therapies for improved cancer treatment outcomes.

Keywords: cancer immunotherapy, CAR NK cells, natural killer cells, NK cell engagers

Introduction

Natural killer (NK) cells were first identified and officially named in 1975 due to their "natural" ability to kill tumor cells without prior sensitization to tumor antigens,[1–4] which is distinct from the mechanism by which T cells recognize and lyse tumor cells. Over the past 5 decades, NK cells have been thoroughly characterized and have now been recognized as part of the family of innate lymphoid cells.[5] NK cells arise from bone marrow progenitor cells and are mainly present in bone marrow, liver, and peripheral blood, where they comprise around 10% of the total peripheral lymphocytes in healthy individuals at a steady state. Human NK cells are generally divided into 2 subsets based on the relative surface density of CD56 and CD16 (also known as FcγRIIIa): $CD56^{bright}CD16^{-}$ NK cells and $CD56^{dim}CD16^{+}$ NK cells. $CD56^{bright}$ NK cells have predominantly immunoregulatory properties mediated by a potent cytokine-producing capacity, while $CD56^{dim}$ NK cells have a marked cytotoxic function.[6,7] More recently, 3 major NK cell populations, named NK1, NK2, and NK3, have been identified in healthy human blood through single-cell RNA-sequencing analyses.[8] NK1 comprises $CD56^{dim}$ NK cells, NK2 corresponds to $CD56^{bright}$ cells, and NK3 resembles $CD57^{+}$ adaptive NK cells.[8] This classification provides new insights into the heterogeneity within the NK cell population.

NK cells are often regarded as innate counterparts of $CD8^{+}$ cytotoxic T cells, both being key mediators of cellular cytotoxicity. In contrast to T cells that use their surface receptor, known as the T cell receptor, to recognize peptides presented by major histocompatibility complex (MHC) molecules, NK cell recognition of target cells and activation is regulated by a balance between signals mediated through activating and inhibitory receptors. NK cells sense MHC class I (MHC-I) surface expression on cells via a set of MHC-I–specific inhibitory receptors, including killer cell immunoglobulin-like receptors (KIRs) and the receptor complex CD94-NKG2A, to maintain self-tolerance.[9–11] However, NK cells can recognize and eliminate tumor or infected cells that lose expression of some or all self MHC-I molecules, a phenomenon referred to as "missing-self" recognition.[12] Moreover, NK cells can recognize self-molecules that are induced or upregulated on the surface of stressed cells through activating receptors, such as NKG2D.[13–15] With inputs from both activating and inhibitory signals, NK cells distinguish between normal and distressed cells and eliminate the latter directly or indirectly. Upon target recognition, NK cells can directly destroy stressed cells by releasing a pore-forming protein called perforin and several cytotoxic proteases known as granzymes, or by engaging death receptors such as TRAIL (TNF-related apoptosis-inducing ligand) or Fas ligand, which initiate a caspase cascade that induces apoptosis in target cells. Activated NK cells can also produce a number of effector cytokines, including interferon γ (IFN-γ), tumor necrosis factor α, granulocyte-macrophage colony-stimulating factor, FLT-3L, and several chemokines including MIP-1α and MIP-1β, XCL1,

Received: December 12, 2024. **Accepted:** February 18, 2025

and CCL5, which can further elicit adaptive immune responses. Through these multifunctional activities, NK cells contribute to immune surveillance, helping to eliminate susceptible targets and amplify inflammatory responses against viruses and cancers.

The unique properties of NK cells, which distinguish them from T cells, make them a promising tool for immunotherapy in cancer and other diseases. Over the past 3 decades, various strategies have been developed to harness NK cells in cancer therapy, including the use of cytokines, small molecules, antibodies, and the adoptive transfer of autologous or allogeneic NK cells, either unmodified or genetically engineered. While many clinical trials have demonstrated favorable safety profiles for NK cell therapies, proof of their efficacy and duration remains insufficient. The remaining challenges include NK cell–specific issues such as limited survival and persistence in vivo and shared issues with T cell therapy, such as cell exhaustion and immune suppression with the tumor microenvironment. In this brief review, we provide an overview of current NK cell–based immunotherapy strategies, which are categorized into 3 types: (i) NK cell–based cellular therapies, including the adoptive transfer of NK cell lines, autologous or allogeneic primary NK cells, either unmodified or genetically manipulated with chimeric antigen receptors (CARs), cytokines, or other genes; (ii) cytokine-based endogenous NK cell–stimulating therapies, such as interleukin (IL)-2, IL-15, and their derivatives; and (iii) antibody-based approaches unleashing endogenous NK cell activity, including immune checkpoint inhibitors (ICIs) and NK cell engagers (NKCEs). We conclude with a discussion of current limitations and challenges in NK cell immunotherapy and a perspective on future directions in the field.

Current strategies for NK cell–based immunotherapies

NK cell–based cellular therapies

NK cell lines

Human NK cell lines, such as NK-92, NKL, NKG, KHYG-1, HANK-1, and NK-YS, represent an attractive source for adoptive immunotherapy. Among them, NK-92 is the most widely used and has already entered clinical trials.[16–19] NK-92 cells are easier to expand, and they do not express inhibitory KIRs that would otherwise restrict NK effector functions.[20] However, their cancerous origin raises safety concerns, and they must be irradiated before infusion,[21] which limits their long-term in vivo persistence and overall therapeutic potential. Furthermore, the lack of CD16 expression reduces their ability to mediate cell killing via antibody-dependent cellular cytotoxicity (ADCC).[22] However, several genetically engineered NK-92 variants have been developed to enhance cytotoxicity and ADCC, including high-affinity NK, which expresses a high-affinity CD16 Fc receptor,[23] as well as CAR-engineered NK-92 cells targeting ErbB2,[24] CD19,[25] CD20,[25] CD33,[26] HER2,[27] CS1,[28] GD2,[29] epidermal growth factor receptor (EGFR),[30] and FLT3.[31] According to multiple clinical trials, administering a large quantity of CAR NK-92 cells has been demonstrated to be safe, with studies showing no major adverse effects, even at high cell doses.[26,32] NK-92 cells and their engineered target-specific variants are well positioned to serve as off-the-shelf NK cell therapies for cancer patients. Moreover, genetic modifications have enabled the creation of NK-92 variants that simultaneously express an Fc receptor, cytokines, CARs, or other receptors, opening new avenues for targeted cancer therapy.[33]

Autologous NK cells

Stimulation of NK cells with cytokines such as IL-2, IL-12, IL-15, IL-18, and type I IFN results in highly activated NK cells, which can lyse tumor cells that express self-MHC molecules. The therapeutic feasibility of NK cells was first explored in the 1980s in the form of lymphokine-activated killer (LAK) cells, an autologous mixture of T and NK cells expanded ex vivo with high-dose IL-2 (Fig. 1A). While LAK cells efficiently lyse autologous tumor cells in vitro, only limited clinical efficacy was observed in patients treated with autologous LAK cells.[34–36] Although the adoptively transferred autologous NK cells seemed to persist in vivo for weeks to months,[35] they lost the killing activity in vivo, possibly due to their inhibitory receptors recognizing self-MHC antigens.[35] For this reason, few studies currently rely on autologous NK cells but shift their focus to allogeneic NK cells to treat cancer. However, autologous NK cells may still be specifically useful in particular clinical situations. For example, autologous NK cells allow infusion without needing lymphodepletion. In addition, by arming the NK cells with an antibody binding to the activating NK cell receptor CD16, a bispecific antibody recognizing an activating NK receptor and a tumor-associated antigen, or a CAR, autologous NK cells can be redirected to target cancer cells based solely on the NK cell recognition of a tumor-associated antigen, overriding its own inhibitory signal following recognition of self-MHC antigens.[37]

Allogeneic NK cells

The lack of expression or mismatch of the self-HLA class I allele, as can occur with donor NK cells infused into an allogeneic HLA class I–mismatched recipient, prevents inhibitory KIRs from recognizing self, often leading to NK alloreactivity. This can be further potentiated when recipient cells express stress ligands or other ligands recognized by activating NK cell receptors.[38] Based on the concept originally proposed by Ruggeri et al.,[38] the feasibility and safety of the adoptive transfer of allogeneic NK cells into patients were first demonstrated by Miller et al.[39] in 2005. Their clinical trials showed that adoptively transferred human NK cells derived from haploidentical-related donors could persist in vivo and mediate antitumor effects against malignant blasts in patients with acute myeloid leukemia (AML).[39] Since then, numerous clinical studies have confirmed the safety of infused allogeneic NK cells and minimal risk of graft-versus-host disease, cytokine release syndrome, or immune effector cell–associated neurotoxicity syndrome in both hematologic malignancies and solid tumors.[40–49] While adoptive allogeneic NK cell therapy has shown promising efficacy in treating hematological malignancies,[40–45,50] its effectiveness in clinical studies of solid tumors remains limited.[46–48] Nevertheless, these studies have highlighted the potential of allogeneic NK cells as a therapeutic option. The favorable safety profile of allogeneic NK cell infusions has further fueled the development of therapeutic NK cell products.

Commonly used allogeneic NK cells are collected from haploidentical or unrelated donor peripheral blood mononuclear cells or umbilical cord blood (UCB). These relatively mature NK cells are expanded ex vivo using irradiated feeder cells and cytokines (e.g., IL-2, IL-15) until a sufficient number of cells for infusion is obtained (Fig. 1B). The feeder cells are typically

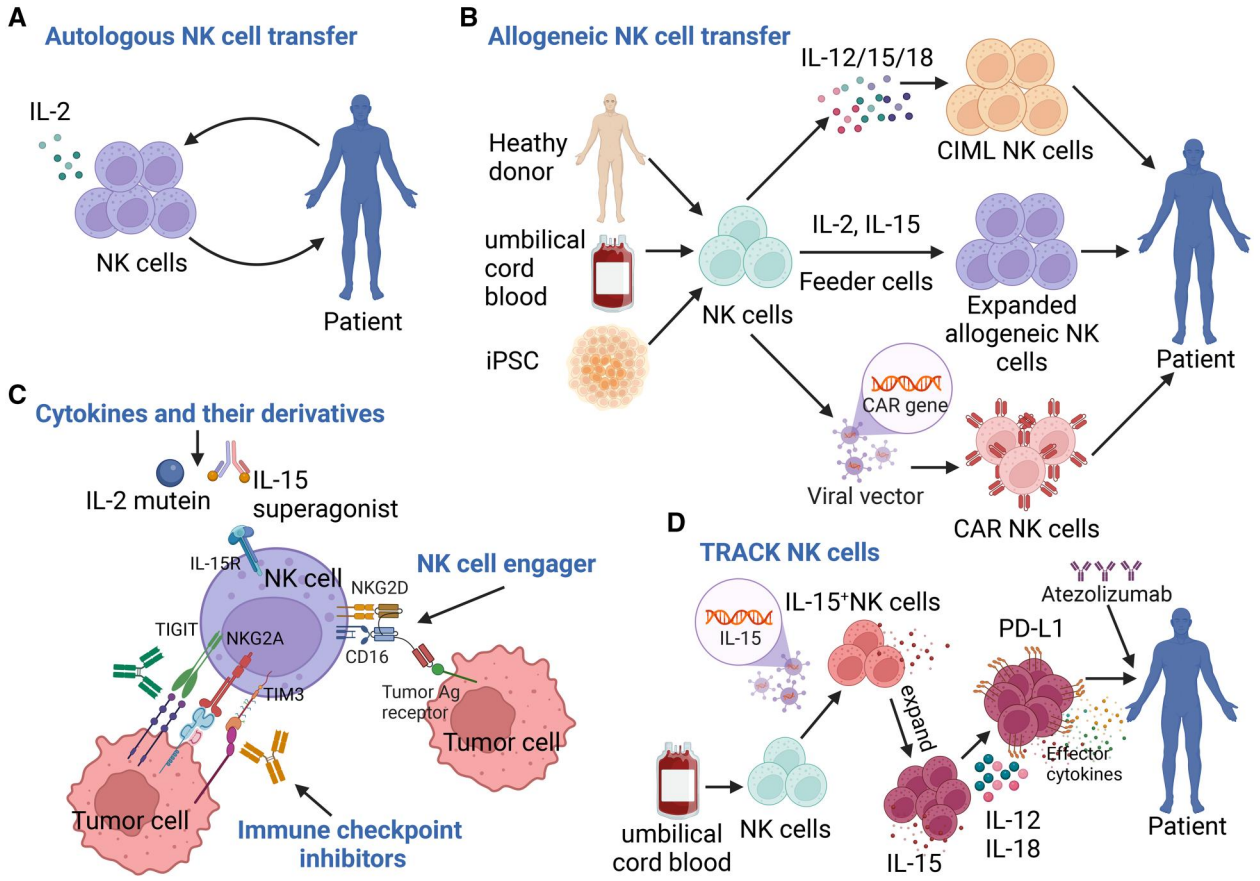

Figure 1. Current strategies for NK cell–based immunotherapies. (A) Autologous NK cell transfer. (B) Allogeneic NK cell transfer, including nonmodified ex vivo expanded NK cells, cytokine-induced memory-like (CIML) NK cells, and CAR NK cells. (C) NK cell therapies based on cytokines, immune checkpoint inhibitors, and NK cell engagers. (D) Workflow for producing TRACK NK cells. Created with Biorender.com.

derived from the erythroleukemic cell line K562, which is transduced to express various costimulatory molecules, such as 4-1BBL, and membrane-bound cytokines like IL-15 or IL-21.[51,52] Additionally, feeder cell–free expansion systems have been developed, including the use of K562-derived membrane particles that retain the expression of 4-1BBL and IL-21.[53] NK cells can also be generated from UCB stem cells or induced pluripotent stem cells (iPSCs).[54,55] Unmodified UCB-derived NK cells have been evaluated in clinical settings, showing a favorable safety profile.[56,57] iPSCs, in particular, allow for precise genetic modifications and the generation of an unlimited number of uniform, off-the-shelf NK cells, overcoming donor-dependent variability. For instance, preclinical studies show that iPSC-derived NK cells with *CISH* knockout or CAR modifications demonstrate improved metabolic fitness and enhanced antitumor activity.[58,59]

Current allogeneic NK cell therapy has infused up to $\sim 1 \times 10^8$ cells/kg/dose and multiple infusions of ex vivo expanded NK cells. However, these cells exhibit limited persistence and suboptimal antitumor activity in vivo. Strategies to prolong NK cell persistence and enhance their activity are under development. One promising approach involves cytokine-induced memory-like (CIML) NK cells, which are generated through overnight ex vivo activation with IL-12, IL-15, and IL-18 (Fig. 1B).[55,60] CIML NK cells demonstrate enhanced responsiveness to cytokines (IL-12 + IL-15), tumor targets, or activating receptor restimulation (Ly49H or NK1.1 in mice and CD16 in humans), and the ability to functionally persist long-term in vivo.[61–63] A recent study revealed that CIML NK cells exhibit distinct transcriptional and epigenetic profiles compared with conventional effector NK cells.[64] CIML NK cells can be further refined into 2 subsets, named enriched memory-like 1 (eML-1) and eML-2 NK cells, which originate from $CD56^{bright}$ and $CD56^{dim}$ NK cells, respectively.[64] Both eML-1 and eML-2 NK cells can persist in patients following adoptive CIML NK cell therapy.[64] Adoptively transferred nonengineered CIML NK cells have shown to be safe, capable of expanding and persisting in vivo, and resistant to irradiation in clinical studies.[44,45,65] Furthermore, preclinical studies demonstrated that CIML NK cells synergize with CAR engineering, significantly enhancing CAR NK cell antitumor responses across several cancer types.[66–70]

Beyond CIML NK cells, human adaptive-like "memory" $NKG2C^+CD56^{dim}CD16^+$ NK cells in peripheral blood from cytomegalovirus-seropositive individuals have been extensively studied in recent years and are currently explored as a treatment strategy for hematological cancers.[8,71–76] This adaptive NK cell subset is often characterized by the upregulated expression of NKG2C, CD16, CD57, and CD2, and restricted expression of NKG2A, Syk, FcεRγ, and KIR.[73,74,77,78] These cells exhibit high proliferative capacity, longer-term persistence, and increased cytotoxicity.[73,74,77,78] Recent studies have demonstrated that expanded $NKG2C^+$ adaptive NK cells have potent antitumor activity against multiple myeloma (MM), acute lymphoblastic leukemia, and AML.[79–81] In addition, in vitro "tumor-primed" NK cells also display memory-like properties.[78,82–84] Tumor-primed, memory-like NK cells have shown both safety and promising efficacy when infused into AML patients.[85,86] A

clinical trial is currently investigating the in vivo tumor priming of endogenous NK cells using a replication-incompetent tumor cell line in patients with advanced myelodysplastic syndromes or multiply relapsed AML (NCT05933070). The clinical application of NK cells with memory-like properties holds great promise in fulfilling the long-sought breakthrough potential of NK cell–based cancer immunotherapy.

In summary, allogeneic NK cells are safe and have shown clinical benefits against a number of cancers, particularly against AML. However, the use of allogeneic NK cells to treat hematologic malignancies and solid tumors still faces various challenges, which mostly fall on the NK cells, including ineffective trafficking, ineffective recognition of tumors, and poor NK cell activity and persistence in vivo. To address these challenges, genetic manipulation of NK cells, such as the transduction of a CAR, has potential for improving NK cell adoptive transfer therapies and is being actively explored in preclinical and clinical studies, as discussed in the following section.

Genetically engineered NK cells

The antitumor efficacy of NK cells is often limited by the lack of sufficient endogenous cytokines to sustain the survival of exogenously administered NK cells in vivo, immunosuppressive cytokines in the tumor microenvironment (TME) such as transforming growth factor β (TGF-β), as well as by immunosuppressive myeloid cells that impair NK cell function and survival. Indeed, targeting the TGF-β pathway can enhance NK cell effector function in a TGF-β–rich environment. For example, UCB NK cells transduced with a dominant-negative TGF-β receptor II (DNRII), which blocks TGF-β signaling by disrupting the assembly of the functional signaling complex of TGF-β and its type I and II receptors, exhibit enhanced cytotoxic activity against neuroblastoma in vitro under TGF-β–rich conditions and superior antitumor activity in vivo in a mouse neuroblastoma model.[87,88] Similarly, B7H3 CAR NK cells cotransduced with DNRII maintain their cytolytic function against glioblastoma multiforme even in the presence of exogenous TGF-β.[89] Moreover, iPSC-derived CAR NK cells engineered to either knock out the TGF-β receptor 2 or express DNRII are resistant to TGF-β inhibition, demonstrating improved antitumor activity against hepatocellular carcinoma.[90] In addition to targeting TGF-β pathways, the cytokine-inducible SH2-containing protein, encoded by the *CISH* gene, has been identified as a key inhibitory checkpoint of IL-15 signaling in NK cells.[91] Ablating *CISH* enhances the metabolic fitness and antitumor activity of armored IL-15–secreting UCB-derived CAR NK cells.[92] Furthermore, the simultaneous deletion of *CISH* and *TGFβR2* produces robust antitumor effects in both mouse and iPSC-derived NK cells.[93,94] We previously found that Cbl-b is also a negative regulator in NK cells, and knockdown of Cbl-b resulted in increased effector functions.[95] Another promising approach to boost NK cell activity against solid tumors involves genetically engineering NK cells to express chemokine receptors that enhance migration to tumor sites, as chemokines play an important role in regulating NK cell tracking into tumors.[96] Chemokine receptors such as CXCR1,[97] CXCR2,[98] variant of CXCR4,[99] CCR4, and CCR2B[100] have shown potential in improving NK cell trafficking toward tumors. These preclinical studies collectively highlight the potential of genetic engineering to overcome tumor-associated challenges and enhance NK cell efficacy in cancer immunotherapy.

Cytokine engineering has also emerged as a powerful strategy to sustain the in vivo persistence and activity of NK cells. Among cytokines, IL-15 has garnered the most attention in translational and clinical research due to its ability to enhance NK cell cytotoxicity, proliferation, and persistence.[101–105] Numerous preclinical studies have demonstrated that systemically administered IL-15 or its analogs, as well as genetically modified NK or CAR NK cells engineered to produce membrane-bound or secretory IL-15, can enhance NK cell in vivo persistence and antitumor activity.[31,69,70,106–108] Moreover, incorporating IL-15 into NK cells has thus far been safe and without serious adverse events. A clinical study utilizing IL-15/CD19 CAR NK cells secreting soluble IL-15 highlighted that a high-dose infusion of IL-15/CD19-CAR NK cells (1×10^7 cells per kilogram of body weight) does not result in any grade 3 or 4 infusion-related toxicities, cytokine release syndrome, or neurotoxicity.[109]

CAR NK cells

The development of CAR T cell therapies has revolutionized the field of cellular therapy for the treatment of cancer.[110] NK cells have emerged as a safe and effective cell type to introduce a platform for CAR NK cell engineering. The feasibility of CAR NK cells was established by engineering the NK-92 cell line with a CAR.[24,26,28,30] Early clinical studies safely using fresh allogeneic, mismatched CAR NK cells and more recent preclinical work using cryopreserved allogeneic, mismatched CAR NK cells suggest that CAR NK cells could be developed as a universal, off-the-shelf product, making them more accessible and scalable for clinical applications.[70,109,111] As of November 2024, ClinicalTrials.gov lists 89 CAR NK cell clinical trials targeting hematopoietic malignancies and solid tumors in various stages: 2 completed, 40 actively recruiting or enrolling by invitation, 5 active but not recruiting, 14 not yet recruiting, 24 with unknown status, and 4 suspended/terminated/withdrawn. Most trials are in phase 1 (61) or phase 2 (25). Notably, the number of CAR NK clinical trials has surged from just 19 in December 2020,[112] reflecting rapid advancements in the field over the past 2 years. Anti-CD19 CAR NK cells have demonstrated promising therapeutic efficacy in B cell malignancies in clinical settings.[109,111] The recent findings from a phase 1/2 trial of IL-15/CD19 CAR NK cells in 37 heavily pretreated patients with relapsed or refractory B cell malignancies revealed encouraging results. Both the day 30 and day 100 overall response (OR) rates were 48.6%.[111] Additionally, the 1-yr overall survival (OS) and progression-free survival (PFS) were 68% and 32%, respectively.[111] Of note, patients who achieved OR had higher levels and longer persistence of CAR NK cells compared with nonresponders.[111] In addition to B cell malignancies, CAR NK cells have shown significant antitumor activity in preclinical studies targeting other hematologic cancers and solid tumors. For example, CAR NK cells targeting CS1 or CD70 for MM,[28,113] CD5 for T cell malignancies,[114] EGFR for glioblastoma multiforme (GBM)[30,115] and breast cancer brain metastases,[116] prostate stem cell antigen for pancreatic cancer,[70] and FLT3 for AML.[31] These compelling preclinical results have driven the initiation of clinical trials to further explore and validate the therapeutic potential of CAR NK cells in diverse cancer types.

Cytokine-based endogenous NK cell–stimulating therapies

Cytokines, including IL-2, IL-12, IL-15, IL-18, IL-21, and type I IFNs, play critical roles in the development, maturation, activation, and survival of NK cells. Among these, IL-2 has been one of the earliest and most extensively studied in clinical settings due to its ability to stimulate the proliferation of NK cells and $CD8^+$ T cells and induce tumor regression. Early clinical trials demonstrated that IL-2, whether used alone or in combination with LAK therapy, produced significant clinical responses in cancer patients.[117–119] In fact, recombinant human IL-2 (aldesleukin) became the first immunotherapy approved by the U.S. Food and Drug Administration for treating metastatic renal cell carcinoma in 1992 and metastatic melanoma in 1998. However, the clinical use of IL-2 is limited by dose-dependent toxicity and concerns about its tendency to promote T regulatory (Treg) cell expansion,[120] which can inhibit the activation and function of other effector T cells.[121] To overcome these issues and improve clinical outcomes, strategies in IL-2 mutein design aim to either increase IL-2 receptor β (IL-2Rβ) binding affinity or decrease IL-2Rα binding affinity (Fig. 1C). For instance, IL-2 muteins created by substituting residues R38, F42, Y45, and E62 with alanines have demonstrated antitumor efficacy similar to wild-type IL-2 but exhibit lower toxicity and reduced activity in inducing Treg cells.[122] Another strategy involves fusing IL-2 with the extracellular domain of IL-2Rα to block the IL-2Rα-binding site, thereby promoting an IL-2Rβ bias.[123] Additionally, a mutant form of IL-2, known as "super-2," has been developed to enhance IL-2Rβ binding affinity, avoiding Treg cell interference and leading to superior expansion and enhanced anticancer activity of cytotoxic T cells.[124] A further innovative approach is the use of an orthogonal (ortho) IL-2 and IL-2 receptor system to selectively deliver the ortho-IL-2 to ortho-IL-2R-engineered T cells.[125] While most IL-2–based muteins currently focus on enhancing T cell activity, it remains to be seen whether these modifications can enhance NK cell activity or persistence in vivo in clinical settings. Designing and testing an NK cell–biased IL-2 mutein for cancer treatment is an area of ongoing interest.

IL-15 is widely regarded as a promising cytokine for NK cell–based cancer therapy. Similar to IL-2, IL-15 is a pleiotropic cytokine that promotes the survival, proliferation, and cytotoxicity of both NK and $CD8^+$ T cells.[101–103] However, unlike IL-2, IL-15 does not induce activation-induced cell death or stimulate the proliferation of Treg cells, possibly making it more suitable for cancer immunotherapy. Since its discovery, more than 200 clinical trials have investigated various forms of IL-15 in cancer treatment.[101] Although IL-15 monotherapy has shown safety and a notable increase in circulating NK and $CD8^+$ T cells, it has produced only limited objective responses and dose-dependent toxicity as a single-agent therapy.[126–128] To overcome this limitation, efforts have been made to develop IL-15 derivatives, which have been evaluated in clinical trials as monotherapies or in combination with chemotherapy or immune checkpoint inhibitors to enhance their therapeutic potential (NCT02384954, NCT04136756, NCT03388632, NCT04616196, NCT05676749). Of note, in 2024, the U.S. Food and Drug Administration approved N-803 (Anktiva; ImmunityBio), a first-in-class IL-15 superagonist that combines an IL-15 mutant (IL-15N72D) with an IL-15Rα/IgG1 Fc fusion protein (Fig. 1C), in combination with Bacillus Calmette–Guérin for patients with Bacillus Calmette–Guérin–unresponsive non–muscle-invasive bladder cancer. N803 has also been proven to be safe and effectively induces proliferation, expansion, and activation of peripheral blood NK cells and $CD8^+$ T cells when administered subcutaneously in combination with PD-1 blockade (e.g., nivolumab) or tumor-targeting monoclonal antibodies (e.g., rituximab) for the treatment of metastatic non-small cell lung cancer (NSCLC) and non-Hodgkin lymphoma,[127,129] further expanding its broad implications for the field of cancer immunotherapy. Moreover, CAR NK cells are now regularly engineered to produce IL-15 for preclinical studies and clinical applications to enhance NK cell in vivo persistence and cytotoxic activity.[31,69,70,109,111] Importantly, as described previously, thus far IL-15 is safe and does not cause serious adverse events in clinical studies when transduced as a secretable protein into CAR NK cells.[109,111]

Antibody-based endogenous approaches unleashing NK cell activity

Immune checkpoint inhibitors

In the TME, NK cells frequently exhibit an exhausted status, which impairs their immunosurveillance function and contributes to successful tumor immune evasion.[130] In addition to the classical NK cell inhibitory receptors KIRs and NKG2A, several other immune checkpoints have also been shown to cause dysfunction of NK cells in various cancers and chronic infections, including PD-1/PD-L1, TIGIT, TIM-3, LAG3, and the Siglec family (Siglec-7/9).[131–133] Inhibition of specific NK cell checkpoint receptors has the potential to reverse NK cell dysfunction in tumors and unleash the activity of NK cells in both preclinical studies and clinical trials. Here, we briefly review targeting 5 representative checkpoint receptors (Fig. 1C), including NKG2A, PD-1/PD-L1, TIM-3, and TIGIT, for unleashing NK cell antitumor activity from the previously mentioned clinical trials.

NKG2A

NKG2A is covalently associated with CD94 on the surface of NK cells and $CD8^+$ T cells and recognizes the nonclassical MHC-I molecule, HLA-E in humans, and Qa-1 in mice.[134] It is widely recognized as a key immune checkpoint for NK cells. The interaction between NKG2A and HLA-E plays a crucial role in tumor immune escape, and NKG2A-mediated mechanisms are being actively explored to develop potential antitumor therapeutic strategies. Blocking NKG2A has been shown to significantly enhance tumor immunity by promoting the effector functions of both NK and $CD8^+$ T cells in mice and humans.[135] An antibody targeting CD94/NKG2A (monalizumab), developed by Innate Pharma, has been evaluated in multiple clinical trials as a monotherapy or in combination with other checkpoint inhibitors.[136–139] While monalizumab monotherapy has demonstrated limited efficacy, its combination with other antibodies has shown promising response rates.[136–139] Currently, 2 phase 3 trials are ongoing to assess the efficacy and safety of monalizumab in combination with cetuximab (NCT04590963) or durvalumab (NCT05221840) in patients with recurrent or metastatic head and neck cancer or stage III unresectable NSCLC, respectively. If successful, monalizumab would validate the strategy of targeting NK cell checkpoints in immunotherapy and provide an alternative or complementary approach to current PD-1/PD-L1 inhibitors. Alternatively, using CRISPR/Cas9 to disrupt the NKG2A-encoding gene *KLRC1* in NK

cells represents another promising approach, as preclinical evidence indicates that *KLRC1* deletion significantly enhances NK cell responses to HLA-E$^+$ tumor targets.[44]

PD-1/PD-l1

Whether NK cells express PD-1 is still in the debate. Some studies show minimal to no PD-1 expression on NK cells,[140] while others report detectable levels, particularly in the context of cancer patients in which NK cells might upregulate PD-1 expression upon encountering tumor cell.[141,142] However, blocking the PD-1/PD-L1 axis has been shown to enhance NK cell–antitumor response in both preclinical and clinical settings. Our group has demonstrated that PD-1 blockade can enhance NK cell function against MM tumor cell targets.[142] A recent randomized clinical trial of 109 patients with advanced NSCLC compared the combination of the first line anti-PD-1 antibody pembrolizumab with single or multiple infusions of allogeneic NK cells with KIR mismatch.[143] The results showed that combined therapy of pembrolizumab plus NK cells had a higher OR rate (ORR) (36.4% vs. 18.5%), improved OS (15.5 mo vs. 13.3 mo), and PFS (6.5 mo vs. 3.3 mo) compared with pembrolizumab monotherapy. Further, a subset analysis showed that those patients who received pembrolizumab plus multiple infusions of allogeneic NK cells fared better than those who had received a single infusion of allogeneic NK cells.[143] A separate but analogous study demonstrated that pembrolizumab plus autologous ex vivo expanded NK cell infusion showed better efficacy when combined with compared with pembrolizumab monotherapy in advanced NSCLC.[144] The estimated 2-yr survival rate was 58.3% versus 16.7% (pembrolizumab plus SNK01 vs. pembrolizumab monotherapy).[144]

Our group and others have reported that human NK cells express high levels of PD-L1 when exposed to solid tumors or leukemia cells, and this expression is inversely proportional to the surface levels of MHC-I on the tumor cells.[145,146] Notably, PD-L1$^+$ NK cells exhibit an activated phenotype with enhanced effector functions compared with their PD-L1$^-$ counterparts.[145] The anti-PD-L1 monoclonal antibody atezolizumab directly binds to PD-L1 on the NK cell and further enhances the functionality of PD-L1$^+$ NK cells in terms of their cytotoxicity and cytokine secretion.[145] Thus, in this case, the atezolizumab functions not only as a checkpoint inhibitor to block the PD-1–PD-L1 interaction between tumor and T cells, but also to directly activate PD-L1$^+$ tumor-reactive or activated by cytokine (TRACK) NK cells. Building on these findings, our team recently initiated a clinical trial (NCT05334329) to evaluate genetically engineered NK cells without or with atezolizumab, for patients with refractory or relapsed NSCLC.[147] These NK cells, named TRACK NK cells, are engineered to secrete soluble IL-15 and are pretreated with IL-12 and IL-18 to induce high levels of PD-L1 expression prior to infusion into patients (Fig. 1D). Our preclinical studies demonstrated that the administration of frozen, off-the-shelf, allogeneic TRACK NK cells is safe in preclinical models of human NSCLC and exhibits potent antitumor activity with improved survival, both alone and in combination with atezolizumab.[69] The clinical study in NSCLC patients (NCT05334329) is ongoing.

TIM-3

TIM-3 is a well-recognized exhaustion marker in T cells. While its expression is minimal on resting T cells and only upregulated following chronic stimulation, TIM-3 is constitutively expressed at significantly higher levels on resting NK cells.[148] In patients with advanced melanoma, NK cells exhibit elevated TIM-3 expression, and these TIM-3$^+$ NK cells are functionally impaired or exhausted.[149] Importantly, TIM-3 blockade has been shown to enhance NK cell cytotoxicity in healthy donors and reverse the exhaustion of NK cells in cancer patients in preclinical settings.[148–150] Consequently, TIM-3 is considered a promising target for developing antibody-based and/or NK cell–based immunotherapeutic strategies for cancer. To date, over 110 clinical trials have investigated anti-TIM-3 antibodies, exploring both monotherapy and combination treatments, including bispecific antibodies—most notably anti-TIM-3/anti-PD-1 constructs.[151] Clinical studies have demonstrated that simultaneously targeting TIM-3 and PD-1 is more effective than targeting either pathway alone.[152,153] A number of ongoing phase 1 and 2 clinical trials are evaluating combination therapies and bispecific antibodies targeting TIM-3 and PD-1 (e.g., NCT04641871, NCT03680508, NCT03708328, NCT04931654, NCT05357651).

TIGIT

Exhausted NK cells usually have increased expression of TIGIT,[154,155] which inhibits their cytotoxic function upon binding to its ligand CD155.[156] TIGIT$^+$ NK cells have been shown to have reduced IFN-γ, TNF-α, and CD107a expression, and TIGIT blockade has been shown to restore NK cell function and enhance antitumor effects in murine models.[154] However, its efficacy as a checkpoint target in humans has been inconsistent. Studies in both T cells and NK cells suggest TIGIT requires costimulation with cytokines or combined blockade of additional checkpoint receptors, such as PD-1, for optimal results.[157,158] Interestingly, recent findings challenge the notion of TIGIT as a marker of NK cell dysfunction. For example, TIGIT$^+$ NK cells in GBM exhibit hyperfunctionality,[159] while TIGIT$^+$ NK cells in AML patients show enhanced cytotoxicity, cytokine production, and granzyme B expression.[160] Despite these complexities, there are up to 90 clinical trials evaluating 23 different anti-TIGIT antibodies, including 16 phase 3 trials designed to assess various strategies targeting TIGIT. From the limited published clinical data,[161–163] anti-TIGIT antibodies have shown minimal activity as monotherapy in advanced solid tumors.[162,163] However, their combination with anti-PD-1 therapies has demonstrated enhanced efficacy.[161] Despite this potential, results from 3 phase 3 trials for tiragolumab, an anti-TIGIT antibody, have been disappointing. The SKYSCRAPER-02 trial (NCT04256421) in SCLC showed no significant improvement in PFS or OS.[164] Similarly, the SKYSCRAPER-01 trial (NCT04294810) in PD-L1–high NSCLC failed to meet its PFS endpoint.[165] Additionally, the SKYSCRAPER-06 trial (NCT04619797), which evaluated tiragolumab in combination with the PD-L1 inhibitor atezolizumab and chemotherapy in metastatic nonsquamous lung cancer, also did not meet its PFS endpoint.[166] Even with these high-profile disappointments, TIGIT is being pursued for anticancer therapy, especially in combination with other immunotherapeutic approaches, such as ICIs targeting the PD-1/PD-L1 pathway.

NK cell engager

NKCEs are a class of bioengineered molecules designed to redirect endogenous NK cells to tumor cells while

simultaneously activating their cytotoxic function (Fig. 1C). NKCEs are relatively easy to manufacture, less expensive than CAR NK cell therapy, and have extended retention times in the body, making them a more cost-effective strategy. Additionally, NKCEs can be combined with adoptive NK cell transfer by forming complexes with allogeneic NK cells.[167] NKCEs use an Fc domain of human IgG to bind CD16 on NK cells, and a single-chain variable fragment (scFv) to recognize tumor antigens such as CD19,[168] CD30,[169] CD33,[170] and CD123.[171] Beyond CD16, NKCEs can also target other activating NK cell receptors, including NKG2D,[172] NKp30,[168] NKp46,[168] and NKG2C.[173] Some NKCEs also incorporate cytokines like IL-15 and IL-2 to enhance NK cell survival and antitumor activity.[173,174] Additionally, NKCEs can be designed to include immune checkpoint receptor blockade, further enhancing antitumor function and reducing NK cell exhaustion.[175]

The tetraspecific NK cell engager represents a significant advancement over trifunctional NKCEs. A representative tetraspecific NKCE consists of an IgG1 Fc domain engaging CD16, 2 scFvs targeting the NKp46 activating receptor and a specific tumor-associated antigen (TAA), and an IL-2 variant (IL-2v). The IL-2v is engineered with a point mutation that enables selective binding to the low-affinity βγIL-2 receptor, expressed by NK cells at steady state, while avoiding the high-affinity IL-2Rαβγ constitutively expressed by Tregs cells. For instance, an IL-2v/NKp46/IgG1-Fc/CD20 tetraspecific NKCE has been shown to preferentially induce NK cell proliferation and activation across mouse, nonhuman primate, and human models, and exhibit robust antitumor effects in mouse and xenograft models of B cell lymphoma.[174,176] A growing number of NKCEs are currently in development, with some already progressing to clinical trials.[169,171] However, most NKCEs are primarily tested in hematological malignancies, with limited applications in solid tumors to date. Moreover, the efficacy of NKCE monotherapy will likely require further optimization, as demonstrated by the latest phase 2 study of AFM13, a bispecific CD30/CD16 antibody. The study reported an ORR of only 32.4% (95% confidence interval, 23.7%–42.1%) and a complete response rate of 10.2% (95% confidence interval, 5.2%–17.5%), despite exhibiting a favorable safety profile.[177] The addition of allogeneic NK cells may improve NKCE activity in some types of cancer, and both preclinical and clinical evidence has shown that NKCE combined with allogeneic NK cells results in significant improvements,[167,178] as discussed subsequently.

NK cell–based immunotherapy in the future

NK cell–based immunotherapy has emerged as a promising new approach to cancer treatment, with the potential to overcome some limitations of T cell-based therapies. However, NK cell–based therapy still faces several challenges and limitations in both ex vivo and in vivo settings. A detailed discussion of these challenges has been extensively reviewed elsewhere.[112,130,179–181] Here we briefly summarize the key obstacles specific to NK cell therapy based on our experiences. In addition to common challenges shared with other immune therapies—such as immunosuppression TME, poor tumor infiltration, and on-target, off-tumor toxicity—NK cell therapy faces 2 primary limitations. The first is poor in vivo persistence, as unlike antigen-specific T cells or CAR T cells, which undergo clonal expansion upon encountering their target antigen, allogeneic CAR or non–CAR NK cells that are expanded ex vivo with supraphysiological cytokine exposure often exhibit limited persistence once infused into patients. The second is NK cell dysfunction or exhaustion, as prolonged ex vivo expansion processes can lead to NK cell exhaustion. Similarly, cytokine or NKCE-based therapies, which rely on the presence of healthy NK cells, may induce NK cell exhaustion with continuous stimulation in vivo. Additionally, unlike the clinical successes seen with T cell ICIs targeting PD-1 and CTLA-4, ICIs for NK cells have thus far not yielded impressive results. For instance, clinical trials targeting NK cell checkpoints, such as TIGIT antibodies, have faced repeated challenges as discussed above. Despite these setbacks, the insights gained from these studies provide a critical foundation for future clinical research. Subsequently we discuss some potential strategies and advancements that could improve the efficacy and duration of NK cell–based immunotherapies.

Optimizing NK cell manufacturing

Current NK cell products are typically expanded ex vivo using K562 feeder cells engineered to express membrane-bound IL-15 or IL-21 and 4-1BBL, along with cytokine IL-2. This method enables over 2,000-fold expansion of NK cells from UCB within 14 d. It has become a standard approach for clinical manufacturing of off-the-shelf, nonengineered and engineered NK cell therapies.[109,111] However, prolonged exposure to cytokines during expansion protocols can induce exhaustion in mature peripheral blood NK and UCB NK cells. To address this limitation, NK cell generation from UCB-derived $CD34^+$ progenitor cells has been developed.[54,182] These progenitor-derived NK cells exhibit a highly active phenotype and demonstrate potent cytotoxicity against acute AML in vitro and in vivo.[182] Moreover, they have been shown to be safe and clinically applicable.[56]. Engineering these $CD34^+$ progenitor–derived NK cells with higher-affinity CD16a further enhances antibody-dependent cellular cytotoxicity.[183] Future studies are needed to compare the in vivo persistence and antitumor efficacy of NK cells expanded directly from UCB versus those derived from $CD34^+$ progenitor cells. Another strategy to enhance NK cell fitness and activity involves optimizing culture conditions, including feeder cells, serum replacements, cytokines, and other supplements, as reviewed by Lamers-Kok et al.[184] For example, adding nicotinamide, a form of vitamin B3, to the culture medium has been shown to improve both the in vitro expansion and in vivo persistence of NK cells.[185] Infusion of nicotinamide-expanded allogeneic NK cells demonstrated promising results in a phase 1 clinical trial for treating non-Hodgkin lymphoma.[186]

Improving cell cryopreservation

Cryopreservation is another critical step in producing final off-the-shelf NK cell products. Freezing and thawing processes can damage cells, with a recent study showing that cryopreserved NK cells undergo apoptosis due to granzyme B leakage from cytotoxic vesicles.[187] Pretreating NK cells with a combination of IL-15 and IL-18 prior to cryopreservation has been shown to improve recovery rates to ∼90% to 100%.[187] The quality of UCB is also vital for successful NK cell manufacturing. UCB samples frozen shortly after collection (within 12 h) and with lower numbers of nucleated red blood cells yield substantially better outcomes compared with those frozen after longer delays (24–48 h).[111] Therefore,

optimizing UCB collection, NK cell expansion, and final product cryopreservation is crucial for enhancing therapeutic efficacy. These efforts require further research and refinement to improve clinical outcomes.

Combining allogeneic off-the-shelf NK cells with NKCEs

Combining allogeneic NK cells with NKCEs is a promising strategy that leverages complementary mechanisms to enhance the effectiveness of each approach. NKCEs can help overcome the limited specificity of allogeneic NK cells by targeting tumor antigens, while NK cells serve as potent effectors for NKCE-mediated ADCC. Additionally, NKCEs incorporated with cytokines can maintain NK cell persistence and activation during the therapeutic window by continually engaging them with tumor targets. This combination also has the potential to counteract the suppressive effects of the TME and the poor infiltration by incorporating ICIs and chemokines into NKCEs. For example, UCB-derived CIML NK cells, when complexed with AFM13 demonstrated enhanced killing of $CD30^+$ tumor cells, resulting in a CAR-like response.[167] A completed phase 1 and 2 trial (NCT04074746) demonstrated that AFM13 combined with allogeneic cord blood–derived NK cells achieved a 92.8% ORR and a 66.7% complete response rate in patients with refractory CD30-positive lymphomas.[178] Moving forward, more robust clinical trials are needed to evaluate the safety, efficacy, and optimal dosing of allogeneic NK cells and NKCE combinations across diverse cancer types, with a particular focus on solid tumors, improving tumor infiltration, and reducing NK cell exhaustion.

Developing in vivo CAR NK cells

Current ex vivo CAR T and CAR NK cell therapies require a complicated manufacturing process involving cell isolation, genetic modification, and expansion outside the body. This process is intricate, time-consuming, and can take several weeks. Furthermore, patients must undergo chemotherapy beforehand to prepare their bodies for the infusion of these engineered immune cells. Recent advancements have introduced in vivo CAR cell therapies, in which immune cells are engineered directly within the patient using delivery vehicles that carry the CAR gene. This approach can significantly reduce manufacturing costs, shorten turnaround times, and eliminate the need for preconditioning chemotherapy, making it a more patient-friendly alternative.[188] Excitingly, in vivo strategies allow the creation of both CAR T cells and CAR NK cells in a single patient using a single delivery vector.[189] Various delivery systems are currently under exploration for in vivo CAR therapy, including viral vectors such as lentivirus, retrovirus, and adeno-associated virus, as well as nanocarriers like polymers, lipid nanoparticles, and exosomes.[188] While challenges such as limited in vivo transduction efficiency and off-target effects remain, preclinical studies have demonstrated the safety and efficacy of in vivo CAR T cell generation using lentiviral vectors or lipid nanoparticles.[190–192] Several in vivo CAR immune cell therapies are now in clinical trials (NCT05969041, NCT06478693, NCT06528301, NCT06539338). While most current efforts focus on CAR T cell therapy, in vivo engineering has significant potential for NK cell–based therapies as well. This approach could potentially enhance endogenous NK cell function in the setting of an immunosuppressive tumor microenvironment, improve their persistence, and increase tumor susceptibility to NK cell–mediated cytotoxicity. In vivo CAR NK therapy represents an exciting frontier in cancer immunotherapy, offering the potential to expand the therapeutic arsenal against a variety of malignancies.

Conclusions

NK cell–based immunotherapy represents an exciting and rapidly evolving field of research in oncology. The unique properties of NK cells, including their innate ability to target tumor cells and modulate immune responses, have laid a strong foundation for innovative therapeutic strategies. Advances in genetic engineering, cytokine therapies, cryopreservation, and delivery systems, such as in vivo CAR NK cell technologies, highlight significant progress in overcoming challenges like limited cell persistence and the immunosuppressive tumor environment. Looking forward, a deeper understanding of NK cell biology, coupled with technological innovations, will likely drive transformative improvements in both efficacy and accessibility. Beyond cancer, expanding the scope of NK cell–based treatments to autoimmune diseases and infectious diseases opens avenues for broader therapeutic applications. As research advances, NK cell–based immunotherapy is poised to become a keystone of precision medicine, shaping the future of oncology and beyond.

Author contributions

S.M., J.Y., and M.A.C. conceived and designed the review. S. M., J.Y., and M.A.C. wrote, reviewed and/or revised the paper. J.Y. and M.A.C. acquired funding. All authors discussed and approved the manuscript.

Funding

The work was supported by grants from the National Institutes of Health (CA210087, CA265095, and CA163205 to M.A.C.; NS106170, AI129582, CA247550, CA264512, CA266457, and CA223400 to J.Y.), the Leukemia and Lymphoma Society (1364-19 to J.Y.), and the California Institute for Regenerative Medicine (TRAN1-14716 to MAC; TRAN1-14003 and DISC2-14190 to J.Y.).

Conflicts of interest

M.A.C. and J.Y. hold a provisional patent on TRACK NK cells. S.M. has no declaration of any potential conflict.

References

1. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol. 1975;5:112–117.
2. Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol. 1975;5:117–121.
3. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. Int J Cancer. 1975;16:216–229.
4. Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and

allogeneic tumors. II. Characterization of effector cells. Int J Cancer. 1975;16:230–239.
5. Spits H et al. Innate lymphoid cells—a proposal for uniform nomenclature. Nat Rev Immunol. 2013;13:145–149.
6. Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. J Immunol. 1986;136:4480–4486.
7. Cooper MA et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. Blood. 2001; 97:3146–3151.
8. Rebuffet L et al. High-dimensional single-cell analysis of human natural killer cell heterogeneity. Nat Immunol. 2024; 25:1474–1488.
9. Brooks AG, Posch PE, Scorzelli CJ, Borrego F, Coligan JE. NKG2A complexed with CD94 defines a novel inhibitory natural killer cell receptor. J Exp Med. 1997;185:795–800.
10. Colonna M, Samaridis J. Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. Science. 1995;268:405–408.
11. D'Andrea A et al. Molecular cloning of NKB1. A natural killer cell receptor for HLA-B allotypes. J Immunol. 1995; 155:2306–2310.
12. Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today. 1990; 11:237–244.
13. Diefenbach A, Jensen ER, Jamieson AM, Raulet DH. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. Nature. 2001;413:165–171.
14. Cerwenka A, Baron JL, Lanier LL. Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor in vivo. Proc Natl Acad Sci U S A. 2001;98:11521–11526.
15. Groh V et al. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. Nat Immunol. 2001;2:255–260.
16. Arai S et al. Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial. Cytotherapy. 2008;10:625–632.
17. Tonn T et al. Treatment of patients with advanced cancer with the natural killer cell line NK-92. Cytotherapy. 2013; 15:1563–1570.
18. Williams BA et al. A phase I trial of NK-92 cells for refractory hematological malignancies relapsing after autologous hematopoietic cell transplantation shows safety and evidence of efficacy. Oncotarget. 2017;8:89256–89268.
19. Boyiadzis M et al. Phase 1 clinical trial of adoptive immunotherapy using "off-the-shelf" activated natural killer cells in patients with refractory and relapsed acute myeloid leukemia. Cytotherapy. 2017;19:1225–1232.
20. Gong JH, Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. Leukemia. 1994; 8:652–658.
21. Navarrete-Galvan L et al. Optimizing NK-92 serial killers: gamma irradiation, CD95/Fas-ligation, and NK or LAK attack limit cytotoxic efficacy. J Transl Med. 2022;20:151.
22. Maki G, Klingemann HG, Martinson JA, Tam YK. Factors regulating the cytotoxic activity of the human natural killer cell line, NK-92. J Hematother Stem Cell Res. 2001;10:369–383.
23. Jochems C 2nd, et al. An NK cell line (haNK) expressing high levels of granzyme and engineered to express the high affinity CD16 allele. Oncotarget. 2016;7:86359–86373.
24. Uherek C et al. Retargeting of natural killer-cell cytolytic activity to ErbB2-expressing cancer cells results in efficient and selective tumor cell destruction. Blood. 2002;100:1265–1273.
25. Boissel L et al. Retargeting NK-92 cells by means of CD19- and CD20-specific chimeric antigen receptors compares favorably with antibody-dependent cellular cytotoxicity. Oncoimmunology. 2013;2:e26527.
26. Tang X et al. First-in-man clinical trial of CAR NK-92 cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia. Am J Cancer Res. 2018; 8:1899–1089.
27. Zhang C et al. ErbB2/HER2-specific NK cells for targeted therapy of glioblastoma. J Natl Cancer Inst. 2016;108:
28. Chu J et al. CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma. Leukemia. 2014; 28:917–927.
29. Esser R et al. NK cells engineered to express a GD2 -specific antigen receptor display built-in ADCC-like activity against tumour cells of neuroectodermal origin. J Cell Mol Med. 2012; 16:569–581.
30. Han J et al. CAR-engineered NK cells targeting wild-type EGFR and EGFRvIII enhance killing of glioblastoma and patient-derived glioblastoma stem cells. Sci Rep. 2015;5:11483.
31. Mansour AG et al. Off-the-shelf CAR-engineered natural killer cells targeting FLT3 enhance killing of acute myeloid leukemia. Blood Adv. 2023;7:6225–6239.
32. Li Q et al. Abstract A014: phase I clinical trial with PD-1/MUC1 CAR-pNK92 immunotherapy. Cancer Immunol Res. 2019; 7:A014.
33. Schomer NT et al. Providing a homing receptor for CAR engineered NK cells—improving cellular immunotherapy for B-cell lymphoma. Blood. 2018;132:4547.
34. Burns LJ et al. IL-2-based immunotherapy after autologous transplantation for lymphoma and breast cancer induces immune activation and cytokine release: a phase I/II trial. Bone Marrow Transplant. 2003;32:177–186.
35. Parkhurst MR, Riley JP, Dudley ME, Rosenberg SA. Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumor regression. Clin Cancer Res. 2011;17:6287–6297.
36. Krause SW et al. Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: a clinical phase i trial. Clin Cancer Res. 2004; 10:3699–3707.
37. Lanier LL. Face off—the interplay between activating and inhibitory immune receptors. Curr Opin Immunol. 2001;13:326–331.
38. Ruggeri L et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002; 295:2097–2100.
39. Miller JS et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105:3051–3057.
40. Bachanova V et al. Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. Blood. 2014;123:3855–3863.
41. Rubnitz JE et al. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. J Clin Oncol. 2010; 28:955–959.
42. Curti A et al. Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients. Blood. 2011; 118:3273–3279.
43. Romee R et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. Sci Transl Med. 2016;8:357ra123.
44. Berrien-Elliott MM et al. Multidimensional analyses of donor memory-like nk cells reveal new associations with response after adoptive immunotherapy for leukemia. Cancer Discov. 2020; 10:1854–1871.
45. Berrien-Elliott MM et al. Hematopoietic cell transplantation donor-derived memory-like NK cells functionally persist after transfer into patients with leukemia. Sci Transl Med. 2022; 14:eabm1375.
46. Yang Y et al. Phase I study of random healthy donor-derived allogeneic natural killer cell therapy in patients with malignant

lymphoma or advanced solid tumors. Cancer Immunol Res. 2016;4:215–224.
47. Perez-Martinez A et al. A phase I/II trial of interleukin-15—stimulated natural killer cell infusion after haplo-identical stem cell transplantation for pediatric refractory solid tumors. Cytotherapy. 2015;17:1594–1603.
48. Geller MA et al. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy. 2011;13:98–107.
49. Iliopoulou EG et al. A phase I trial of adoptive transfer of allogeneic natural killer cells in patients with advanced non-small cell lung cancer. Cancer Immunol Immunother. 2010;59:1781–1789.
50. Heuser M et al. A prospective phase I/IIa trial to evaluate the safety and efficacy of GTA002, an off-the-shelf, ex vivo-cultured allogeneic NK cell preparation in patients with acute myeloid leukemia in complete morphological remission who have measurable residual disease. Journal of Clinical Oncology. 2021; 39:TPS7053.
51. Denman CJ et al. Membrane-bound IL-21 promotes sustained ex vivo proliferation of human natural killer cells. PLoS One. 2012; 7:e30264.
52. Lu T et al. Hijacking TYRO3 from tumor cells via trogocytosis enhances NK-cell effector functions and proliferation. Cancer Immunol Res. 2021;9:1229–1241.
53. Oyer JL et al. Natural killer cells stimulated with PM21 particles expand and biodistribute in vivo: Clinical implications for cancer treatment. Cytotherapy. 2016;18:653–663.
54. Spanholtz J et al. High log-scale expansion of functional human natural killer cells from umbilical cord blood CD34-positive cells for adoptive cancer immunotherapy. PLoS One. 2010;5:e9221.
55. Knorr DA et al. Clinical-scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy. Stem Cells Transl Med. 2013;2:274–283.
56. Dolstra H et al. 20174107–4118. Successful transfer of umbilical cord blood CD34(+) hematopoietic stem and progenitor-derived NK cells in older acute myeloid leukemia patients. Clin Cancer Res 23:
57. Shah N et al. Phase I study of cord blood-derived natural killer cells combined with autologous stem cell transplantation in multiple myeloma. Br J Haematol. 2017;177:457–466.
58. Zhu H et al. Metabolic reprograming via deletion of CISH in human iPSC-derived NK cells promotes in vivo persistence and enhances anti-tumor activity. Cell Stem Cell. 2020;27: 224–237.e6.
59. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-derived natural killer cells engineered with chimeric antigen receptors enhance anti-tumor activity. Cell Stem Cell. 2018;23: 181–192.e5.
60. Romee R et al. Cytokine activation induces human memory-like NK cells. Blood. 2012;120:4751–4760.
61. Gang M, Wong P, Berrien-Elliott MM, Fehniger TA. Memory-like natural killer cells for cancer immunotherapy. Semin Hematol. 2020;57:185–193.
62. Cooper MA et al. Cytokine-induced memory-like natural killer cells. Proc Natl Acad Sci U S A. 2009;106:1915–1919.
63. Ni J, Miller M, Stojanovic A, Garbi N, Cerwenka A. Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. J Exp Med. 2012;209:2351–2365.
64. Foltz JA et al. Cytokines drive the formation of memory-like NK cell subsets via epigenetic rewiring and transcriptional regulation. Sci Immunol. 2024;9:eadk4893.
65. Bednarski JJ et al. Donor memory-like NK cells persist and induce remissions in pediatric patients with relapsed AML after transplant. Blood. 2022;139:1670–1683.
66. Gang M et al. CAR-modified memory-like NK cells exhibit potent responses to NK-resistant lymphomas. Blood. 2020; 136:2308–2318.
67. Tarannum M et al. CAR memory-like NK cells targeting the membrane proximal domain of mesothelin demonstrate promising activity in ovarian cancer. Sci Adv. 2024;10:eadn0881.
68. Dong H et al. Memory-like NK cells armed with a neoepitope-specific CAR exhibit potent activity against NPM1 mutated acute myeloid leukemia. Proc Natl Acad Sci U S A. 2022;119: e2122379119.
69. Lu T et al. Preclinical evaluation of off-the-shelf PD-L1+ human natural killer cells secreting IL15 to treat non-small cell lung cancer. Cancer Immunol Res. 2024;12:731–743.
70. Teng KY et al. Off-the-shelf prostate stem cell antigen-directed chimeric antigen receptor natural killer cell therapy to treat pancreatic cancer. Gastroenterology. 2022;162:1319–1333.
71. Guma M et al. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. Blood. 2004;104:3664–3671.
72. Lopez-Verges S et al. Expansion of a unique CD57(+)NKG2Chi natural killer cell subset during acute human cytomegalovirus infection. Proc Natl Acad Sci U S A. 2011;108:14725–14732.
73. Marquardt N et al. Cutting edge: identification and characterization of human intrahepatic CD49a+ NK cells. J Immunol. 2015; 194:2467–2471.
74. Schlums H et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity. 2015;42:443–456.
75. Lee J et al. Epigenetic modification and antibody-dependent expansion of memory-like NK cells in human cytomegalovirus-infected individuals. Immunity. 2015;42:431–442.
76. Brownlie D et al. Expansions of adaptive-like NK cells with a tissue-resident phenotype in human lung and blood. Proc Natl Acad Sci U S A. 2021;118:e2016580118.
77. Liu LL et al. Critical role of CD2 co-stimulation in adaptive natural killer cell responses revealed in NKG2C-deficient humans. Cell Rep. 2016;15:1088–1099.
78. Arellano-Ballestero H et al. Proteomic and phenotypic characteristics of memory-like natural killer cells for cancer immunotherapy. J Immunother Cancer. 2024;12:e008717.
79. Liu LL et al. Ex vivo expanded adaptive NK cells effectively kill primary acute lymphoblastic leukemia cells. Cancer Immunol Res. 2017;5:654–665.
80. Haroun-Izquierdo A et al. Adaptive single-KIR^{+}NKG2C^{+} NK cells expanded from select superdonors show potent missing-self reactivity and efficiently control HLA-mismatched acute myeloid leukemia. J ImmunoTher Cancer. 2022;10:e005577.
81. Cho H et al. Adaptive natural killer cells facilitate effector functions of daratumumab in multiple myeloma. Clin Cancer Res. 2021;27:2947–2958.
82. North J et al. Tumor-primed human natural killer cells lyse NK-resistant tumor targets: evidence of a two-stage process in resting NK cell activation. J Immunol. 2007;178:85–94.
83. Sabry M et al. Leukemic priming of resting NK cells is killer Ig-like receptor independent but requires CD15-mediated CD2 ligation and natural cytotoxicity receptors. J Immunol. 2011; 187:6227–6234.
84. Sabry M et al. Tumor- and cytokine-primed human natural killer cells exhibit distinct phenotypic and transcriptional signatures. PLoS One. 2019;14:e0218674.
85. Kottaridis PD et al. Two-stage priming of allogeneic natural killer cells for the treatment of patients with acute myeloid leukemia: a phase I trial. PLoS One. 2015;10:e0123416.
86. Fehniger TA et al. A phase 1 trial of CNDO-109-activated natural killer cells in patients with high-risk acute myeloid leukemia. Biol Blood Marrow Transplant. 2018;24:1581–1589.
87. Yvon ES et al. Cord blood natural killer cells expressing a dominant negative TGF-beta receptor: Implications for adoptive immunotherapy for glioblastoma. Cytotherapy. 2017;19:408–418.
88. Burga RA et al. Engineering the TGFbeta receptor to enhance the therapeutic potential of natural killer cells as an immunotherapy for neuroblastoma. Clin Cancer Res. 2019;25:4400–4412.
89. Chaudhry K et al. Co-transducing B7H3 CAR-NK cells with the DNR preserves their cytolytic function against GBM in the presence of exogenous TGF-beta. Mol Ther Methods Clin Dev. 2022; 27:415–430.

90. Thangaraj JL, Coffey M, Lopez E, Kaufman DS. Disruption of TGF-beta signaling pathway is required to mediate effective killing of hepatocellular carcinoma by human iPSC-derived NK cells. Cell Stem Cell. 2024;31:1327–1343.e5.
91. Delconte RB et al. CIS is a potent checkpoint in NK cell–mediated tumor immunity. Nat Immunol. 2016;17:816–824.
92. Daher M et al. Targeting a cytokine checkpoint enhances the fitness of armored cord blood CAR-NK cells. Blood. 2021; 137:624–636.
93. Souza-Fonseca-Guimaraes F et al. TGFbeta and CIS inhibition overcomes NK-cell suppression to restore antitumor immunity. Cancer Immunol Res. 2022;10:1047–1054.
94. Gerew A et al. Deletion of CISH and TGFβR2 in iPSC-derived NK cells promotes high cytotoxicity and enhances in vivo tumor killing. Blood. 2021;138:2780–2780.
95. Lu T et al. Cbl-b is upregulated and plays a negative role in activated human NK cells. J Immunol. 2021;206:677–685.
96. Ran GH et al. Natural killer cell homing and trafficking in tissues and tumors: from biology to application. Signal Transduct Target Ther. 2022;7:205.
97. Ng YY, Tay JCK, Wang S. CXCR1 expression to improve anticancer efficacy of intravenously injected CAR-NK cells in mice with peritoneal xenografts. Mol Ther Oncolytics. 2020; 16:75–85.
98. Kremer V et al. Genetic engineering of human NK cells to express CXCR2 improves migration to renal cell carcinoma. J Immunother Cancer. 2017;5:73.
99. Levy E et al. Enhanced bone marrow homing of natural killer cells following mRNA transfection with gain-of-function variant CXCR4(R334X). Front Immunol. 2019;10:1262.
100. Feigl FF et al. Efficient redirection of NK cells by genetic modification with chemokine receptors CCR4 and CCR2B. Int J Mol Sci. 2023;24:
101. Ma S, Caligiuri MA, Yu J. Harnessing IL-15 signaling to potentiate NK cell–mediated cancer immunotherapy. Trends Immunol. 2022;43:833–847.
102. Carson WE et al. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J Exp Med. 1994;180:1395–1403.
103. Carson WE et al. A potential role for interleukin-15 in the regulation of human natural killer cell survival. J Clin Invest. 1997; 99:937–943.
104. Ma S et al. An XBP1s-PIM-2 positive feedback loop controls IL-15-mediated survival of natural killer cells. Sci Immunol. 2023; 8:eabn7993.
105. Wang Y et al. The IL-15-AKT-XBP1s signaling pathway contributes to effector functions and survival in human NK cells. Nat Immunol. 2019;20:10–17.
106. Imamura M et al. Autonomous growth and increased cytotoxicity of natural killer cells expressing membrane-bound interleukin-15. Blood. 2014;124:1081–1088.
107. Liu E et al. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. Leukemia. 2018;32:520–531.
108. Lu T et al. Off-the-shelf CAR natural killer cells secreting IL-15 target spike in treating COVID-19. Nat Commun. 2022; 13:2576.
109. Liu E et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med. 2020;382:545–553.
110. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018;359:1361–1365.
111. Marin D et al. Safety, efficacy and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19(+) B cell tumors: a phase 1/2 trial. Nat Med. 2024;30:772–784.
112. Yilmaz A, Cui H, Caligiuri MA, Yu J. Chimeric antigen receptor-engineered natural killer cells for cancer immunotherapy. J Hematol Oncol. 2020;13:168.
113. Lin P et al. CD70 CAR NK cells in the treatment of multiple myeloma. Blood. 2023;142:3463–3463.
114. Zu Y et al. Targeting CD5 chimeric antigen receptor-engineered natural killer cells against T-cell malignancies. Exp Hematol Oncol. 2024;13:104.
115. Ma R et al. An oncolytic virus expressing IL15/IL15Ralpha combined with off-the-shelf EGFR-CAR NK cells targets glioblastoma. Cancer Res. 2021;81:3635–3648.
116. Chen X et al. A combinational therapy of EGFR-CAR NK cells and oncolytic herpes simplex virus 1 for breast cancer brain metastases. Oncotarget. 2016;7:27764–27777.
117. Rosenberg SA et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med. 1985;313:1485–1492. and
118. Lotze MT et al. High-dose recombinant interleukin 2 in the treatment of patients with disseminated cancer. Responses, treatment-related morbidity, and histologic findings. JAMA. 1986; 256:3117–3124.
119. Rosenberg SA et al. Prospective randomized trial of high-dose interleukin-2 alone or in conjunction with lymphokine-activated killer cells for the treatment of patients with advanced cancer. J Natl Cancer Inst. 1993;85:622–632. and
120. Shah MH Jr., et al. A phase I study of ultra low dose interleukin-2 and stem cell factor in patients with HIV infection or HIV and cancer. Clin Cancer Res. 2006;12:3993–3996.
121. Koreth J 3rd, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. N Engl J Med. 2011;365:2055–2066.
122. Carmenate T et al. Human IL-2 mutein with higher antitumor efficacy than wild type IL-2. J Immunol. 2013;190:6230–6238.
123. Lopes JE et al. ALKS 4230: a novel engineered IL-2 fusion protein with an improved cellular selectivity profile for cancer immunotherapy. J Immunother Cancer. 2020;8:e000673.
124. Levin AM et al. Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'. Nature. 2012;484:529–533.
125. Sockolosky JT et al. Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes. Science. 2018; 359:1037–1042.
126. Miller JS et al. A first-in-human phase I study of subcutaneous outpatient recombinant human IL15 (rhIL15) in adults with advanced solid tumors. Clin Cancer Res. 2018;24:1525–1535.
127. Wrangle JM et al. ALT-803, an IL-15 superagonist, in combination with nivolumab in patients with metastatic non-small cell lung cancer: a non-randomised, open-label, phase 1b trial. Lancet Oncol. 2018;19:694–704.
128. Conlon KC et al. IL15 by continuous intravenous infusion to adult patients with solid tumors in a phase I trial induced dramatic NK-cell subset expansion. Clin Cancer Res. 2019; 25:4945–4954.
129. Foltz JA et al. Phase I Trial of N-803, an IL15 receptor agonist, with rituximab in patients with indolent non-hodgkin lymphoma. Clin Cancer Res. 2021;27:3339–3350.
130. Ma S, Caligiuri MA, Yu J. Harnessing natural killer cells for lung cancer therapy. Cancer Res. 2023;83:3327–3339.
131. Falco M et al. Identification and molecular cloning of p75/AIRM1, a novel member of the sialoadhesin family that functions as an inhibitory receptor in human natural killer cells. J Exp Med. 1999;190:793–802.
132. Nicoll G et al. Identification and characterization of a novel siglec, siglec-7, expressed by human natural killer cells and monocytes. J Biol Chem. 1999;274:34089–34095.
133. Zhang JQ, Nicoll G, Jones C, Crocker PR. Siglec-9, a novel sialic acid binding member of the immunoglobulin superfamily expressed broadly on human blood leukocytes. J Biol Chem. 2000;275:22121–22126.
134. Lee N et al. HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. Proc Natl Acad Sci U S A. 1998; 95:5199–5204.
135. Andre P et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. Cell. 2018;175:1731–1743.e13.

136. Tinker AV Jr., et al. Dose-ranging and cohort-expansion study of monalizumab (IPH2201) in patients with advanced gynecologic malignancies: a trial of the Canadian Cancer Trials Group (CCTG): IND221. Clin Cancer Res. 2019;25:6052–6060.
137. Galot R et al. A phase II study of monalizumab in patients with recurrent/metastatic squamous cell carcinoma of the head and neck: the I1 cohort of the EORTC-HNCG-1559 UPSTREAM trial. Eur J Cancer. 2021;158:17–26.
138. Herbst RS et al. COAST: an open-label, phase II, multidrug platform study of durvalumab alone or in combination with oleclumab or monalizumab in patients with unresectable, stage III non-small-cell lung cancer. J Clin Oncol. 2022;40:3383–3393.
139. Patel SP et al. Phase 1/2 study of monalizumab plus durvalumab in patients with advanced solid tumors. J Immunother Cancer. 2024;12:e007340.
140. Judge SJ et al. Minimal PD-1 expression in mouse and human NK cells under diverse conditions. J Clin Invest. 2020; 130:3051–3068.
141. Pesce S et al. Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. J Allergy Clin Immunol. 2017; 139:335–346.e3.
142. Benson DM Jr., 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.
143. Lin M et al. Pembrolizumab plus allogeneic NK cells in advanced non-small cell lung cancer patients. J Clin Invest. 2020; 130:2560–2569.
144. Park HJ et al. Two-year efficacy of SNK01 plus pembrolizumab for non-small cell lung cancer: expanded observations from a phase I/IIa randomized controlled trial. Thorac Cancer. 2022; 13:2050–2056.
145. Dong W et al. The mechanism of anti-PD-L1 antibody efficacy against PD-L1-negative tumors identifies NK cells expressing PD-L1 as a cytolytic effector. Cancer Discov. 2019;9:1422–1437.
146. Sierra JM et al. Tumor-experienced human NK cells express high levels of PD-L1 and inhibit CD8(+) T cell proliferation. Front Immunol 2021;12:745939.
147. Villalona-Calero MA et al. First-in-human trial of engineered NK cells in lung cancer refractory to immune checkpoint inhibitors. JCI Insight. Published online February 4, 2025.
148. Ndhlovu LC et al. Tim-3 marks human natural killer cell maturation and suppresses cell-mediated cytotoxicity. Blood. 2012; 119:3734–3743.
149. da Silva IP et al. Reversal of NK-cell exhaustion in advanced melanoma by Tim-3 blockade. Cancer Immunol Res. 2014; 2:410–422.
150. Jiang W et al. Tim-3 blockade elicits potent anti-multiple myeloma immunity of natural killer cells. Front Oncol. 2022; 12:739976.
151. Sauer N et al. TIM-3 as a promising target for cancer immunotherapy in a wide range of tumors. Cancer Immunol Immunother. 2023;72:3405–3425.
152. Curigliano G et al. Phase I/Ib clinical trial of sabatolimab, an anti-TIM-3 antibody, alone and in combination with spartalizumab, an anti-PD-1 antibody, in advanced solid tumors. Clin Cancer Res. 2021;27:3620–3629.
153. Hellmann MD et al. Safety and immunogenicity of LY3415244, a bispecific antibody against TIM-3 and PD-L1, in patients with advanced solid tumors. Clin Cancer Res. 2021;27:2773–2781.
154. Zhang Q et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. Nat Immunol. 2018;19:723–732.
155. Jackson Z et al. Sequential single-cell transcriptional and protein marker profiling reveals TIGIT as a marker of CD19 CAR-T cell dysfunction in patients with non-Hodgkin lymphoma. Cancer Discov. 2022;12:1886–1903.
156. Liu S et al. Recruitment of Grb2 and SHIP1 by the ITT-like motif of TIGIT suppresses granule polarization and cytotoxicity of NK cells. Cell Death Differ. 2013;20:456–464.
157. Chauvin JM et al. TIGIT and PD-1 impair tumor antigen-specific CD8(+) T cells in melanoma patients. J Clin Invest. 2015; 125:2046–2058.
158. Chauvin JM et al. IL15 stimulation with TIGIT blockade reverses CD155-mediated NK-cell dysfunction in melanoma. Clin Cancer Res. 2020;26:5520–5533.
159. Lupo KB et al. TIGIT contributes to the regulation of 4-1BB and does not define NK cell dysfunction in glioblastoma. iScience. 2023;26:108353.
160. Jia B et al. TIGIT expression positively associates with NK cell function in AML patients. Blood. 2018;132:5250–5250.
161. Cho BC et al. Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study. Lancet Oncol. 2022;23:781–792.
162. Niu J et al. First-in-human phase 1 study of the anti-TIGIT antibody vibostolimab as monotherapy or with pembrolizumab for advanced solid tumors, including non-small-cell lung cancer(⋆). Ann Oncol. 2022;33:169–180.
163. Mettu NB et al. A phase 1a/b open-label, dose-escalation study of etigilimab alone or in combination with nivolumab in patients with locally advanced or metastatic solid tumors. Clin Cancer Res. 2022;28:882–892.
164. Rudin CM et al. SKYSCRAPER-02: Primary results of a phase III, randomized, double-blind, placebo-controlled study of atezolizumab (atezo) + carboplatin + etoposide (CE) with or without tiragolumab (tira) in patients (pts) with untreated extensive-stage small cell lung cancer (ES-SCLC). J Clin Oncol. 2022; 40:LBA8507.
165. Genentech provides update on phase III skyscraper-01 study in PD-L1-high metastatic non-small cell lung cancer. Accessed Nov 25, 2024. https://www.businesswire.com/news/home/20230822987765/en/Genentech-Provides-Update-on-Phase-III-SKYSCRAPER-01-Study-in-PD-L1-High-Metastatic-Non-Small-Cell-Lung-Cancer.
166. Genentech provides update on phase II/III skyscraper-06 study in metastatic non-squamous non-small cell lung cancer. Accessed Jul 3, 2024. https://www.businesswire.com/news/home/20240703379884/en/Genentech-Provides-Update-on-Phase-IIIII-SKSCYRAPER-06-Study-in-Metastatic-Non-Squamous-Non-Small-Cell-Lung-Cancer.
167. Kerbauy LN et al. Combining AFM13, a bispecific CD30/CD16 antibody, with cytokine-activated blood and cord blood-derived NK cells facilitates CAR-like responses against CD30(+) malignancies. Clin Cancer Res. 2021;27:3744–3756.
168. Colomar-Carando N et al. Exploiting natural killer cell engagers to control pediatric B-cell precursor acute lymphoblastic leukemia. Cancer Immunol Res. 2022;10:291–302.
169. Rothe A et al. A phase 1 study of the bispecific anti-CD30/CD16A antibody construct AFM13 in patients with relapsed or refractory Hodgkin lymphoma. Blood. 2015;125: 4024–4031.
170. Gleason MK et al. CD16xCD33 bispecific killer cell engager (BiKE) activates NK cells against primary MDS and MDSC CD33+ targets. Blood. 2014;123:3016–3026.
171. Stein AS et al. A first-in-human study of CD123 NK cell engager SAR443579 in relapsed or refractory acute myeloid leukemia, B-cell acute lymphoblastic leukemia, or high-risk myelodysplasia. J Clin Oncol. 2023;41:7005.
172. Chan WK Jr., et al. A CS1-NKG2D bispecific antibody collectively activates cytolytic immune cells against multiple myeloma. Cancer Immunol Res. 2018;6:776–787.
173. Chiu E et al. Anti-NKG2C/IL-15/anti-CD33 killer engager directs primary and iPSC-derived NKG2C(+) NK cells to target myeloid leukemia. Mol Ther. 2021;29:3410–3421.

174. Demaria O et al. Antitumor immunity induced by antibody-based natural killer cell engager therapeutics armed with not-alpha IL-2 variant. Cell Rep Med. 2022;3:100783.
175. Bogen JP et al. Design of a trispecific checkpoint inhibitor and natural killer cell engager based on a 2 + 1 common light chain antibody architecture. Front Immunol. 2021;12:669496.
176. Demaria O et al. A tetraspecific engager armed with a non-alpha IL-2 variant harnesses natural killer cells against B cell non-Hodgkin lymphoma. Sci Immunol. 2024;9:eadp3720.
177. Kim WS et al. A Phase 2 study of acimtamig (AFM13) in patients with CD30-positive, relapsed or refractory peripheral T-cell lymphomas. Clin Cancer Res. 2025;31:65–73.
178. Nieto Y et al. Innate Cell Engager (ICE®) AFM13 combined with preactivated and expanded (P+E) cord blood (CB)-derived natural killer (NK) cells for patients with refractory CD30-positive lymphomas: final results. Blood. 2023;142:774–774.
179. Vivier E et al. Natural killer cell therapies. Nature. 2024; 626:727–736.
180. Laskowski TJ, Biederstadt A, Rezvani K. Natural killer cells in antitumour adoptive cell immunotherapy. Nat Rev Cancer. 2022;22:557–575.
181. Sayegh M, Ma S, Yu J. Application of natural killer immunotherapy in blood cancers and solid tumors. Curr Opin Oncol. 2023;35:446–452.
182. de Jonge P et al. Good manufacturing practice production of CD34(+) progenitor-derived NK cells for adoptive immunotherapy in acute myeloid leukemia. Cancer Immunol Immunother. 2023;72:3323–3335.
183. van Hauten PMM et al. Engineering of CD34+ progenitor-derived natural killer cells with higher-affinity CD16a for enhanced antibody-dependent cellular cytotoxicity. Cytotherapy. 2024;26:252–260.
184. Lamers-Kok N et al. Natural killer cells in clinical development as non-engineered, engineered, and combination therapies. J Hematol Oncol. 2022;15:164.
185. Peled T et al. Enhanced in vivo persistence and proliferation of NK cells expanded in culture with the small molecule nicotinamide: development of a clinical-applicable method for NK expansion. Blood. 2017;130:657–657.
186. Cichocki F et al. Nicotinamide enhances natural killer cell function and yields remissions in patients with non-Hodgkin lymphoma. Sci Transl Med. 2023;15:eade3341.
187. Berjis A et al. Pretreatment with IL-15 and IL-18 rescues natural killer cells from granzyme B-mediated apoptosis after cryopreservation. Nat Commun. 2024;15:3937.
188. Bui TA, Mei H, Sang R, Ortega DG, Deng W. Advancements and challenges in developing in vivo CAR T cell therapies for cancer treatment. EBioMedicine. 2024;106:105266.
189. Andorko JI et al. Targeted in vivo generation of CAR T and NK cells utilizing an engineered lentiviral vector platform. Blood. 2023;142:763–763.
190. Nicolai CJ et al. In vivo CAR T-cell generation in nonhuman primates using lentiviral vectors displaying a multidomain fusion ligand. Blood. 2024;144:977–987.
191. Pfeiffer A et al. In vivo generation of human CD19-CAR T cells results in B-cell depletion and signs of cytokine release syndrome. EMBO Mol Med. 2018;10e9158.
192. Rurik JG et al. CAR T cells produced in vivo to treat cardiac injury. Science. 2022;375:91–96.

The Journal of Immunology, 2025, 214, 1444–1456
https://doi.org/10.1093/jimmun/vkaf036
Brief Reviews