



The NK cell–cancer cycle: advances and new challenges in NK cell–based immunotherapies

Tobias Bald¹, Matthew F. Krummel², Mark J. Smyth^{3,5} and Kevin C. Barry^{4,5}

Natural killer (NK) cells belong to the innate immune system and contribute to protecting the host through killing of infected, foreign, stressed or transformed cells. Additionally, via cellular cross-talk, NK cells orchestrate antitumor immune responses. Hence, significant efforts have been undertaken to exploit the therapeutic properties of NK cells in cancer. Current strategies in preclinical and clinical development include adoptive transfer therapies, direct stimulation, recruitment of NK cells into the tumor microenvironment (TME), blockade of inhibitory receptors that limit NK cell functions, and therapeutic modulation of the TME to enhance antitumor NK cell function. In this Review, we introduce the NK cell–cancer cycle to highlight recent advances in NK cell biology and to discuss the progress and problems of NK cell–based cancer immunotherapies.

Natural killer (NK) cells are effector cells of the innate immune system and belong to the family of innate lymphoid cells (ILCs). By analogy to the classification of T cells, three groups of ILCs have been defined on the basis of cytokine production and expression of transcription factors. Group 1 ILCs include interferon (IFN)- γ -producing NK cells and ILC1s, while ILC2s produce classical T helper (T_H)2 cytokines and ILC3s produce T_H 17 cytokines^{1,2}. NK cells are professional killer cells that recognize and rapidly destroy cells that are dangerous to the host (for example, stressed, foreign, infected or transformed cells) and, as such, contribute to transplantation rejection, viral immunity and cancer immune surveillance (particularly cancer metastasis)^{3,4}. However, besides their ability to kill cells, it is now well established that NK cells also play a critical role in sculpting innate and adaptive immune responses via cellular cross-talk in various disease settings⁵. When compared with T cells in either natural tumor immunity or adoptive cellular therapy (ACT) settings, NK cells display certain advantages and disadvantages (Table 1). In particular, NK cells have a more important role in the elimination of early tumors and metastasis (minimal disease) and are generally found in fewer numbers in established tumors. NK cells have a broader reactivity to tumors (lower specificity, as they do not have a T cell receptor), equivalent effector functions and reduced proliferative capacity and recall response. In an adoptive transfer therapy setting, NK cells have greater off-the-shelf utility and are safer, as they cause fewer immune-related adverse events, but they are more difficult to genetically manipulate.

Similar to myeloid cells, NK cells are a heterogeneous and plastic population, thus they can acquire different phenotypes, depending on the tissue context or signaling cues to which they are exposed^{6,7}. For simplicity, NK cells are defined as CD3⁺CD56⁺ cells in humans and CD3⁺NK1.1⁺NKp46⁺ cells in mice. Furthermore, highly cytotoxic human NK cells are defined as CD56^{dim}CD16^{hi} (hereafter referred to as CD56^{dim}) and are predominantly found in the blood, while immunomodulatory and cytokine-producing NK cells are defined as CD56^{bright}CD16^{lo} (hereafter referred to as CD56^{bright})

and preferentially reside in secondary lymphoid organs, such as lymph nodes (Fig. 1)^{8–10}. A large suite of additional markers can be utilized to further stratify NK cell subsets, for example, CD94/NKG2A, NKp46, CD226, and many more. Functionally similar NK cell subsets have been identified in mice, but with some different markers. For NK cell differentiation in mice, NK cells are marked by tumor necrosis factor receptor superfamily member CD27 and the integrin CD11b/Mac-1. The most cytotoxic NK cells are terminally differentiated and express CD11b but little or no CD27 (that is, CD27[−]CD11b⁺ or ‘CD11b single positive (SP)’), while regulatory NK cells comprise both mature NK cells expressing CD27 (CD27^{hi}) and CD11b and immature NK cells lacking CD11b (that is, CD11b[−]CD27^{hi} or ‘CD27 SP’) (Fig. 1)¹¹. In addition, various subsets of tissue-resident NK cells have been described, which differ from conventional NK cells in their origin, development, and/or function (reviewed in ref. 12).

The NK cell–cancer cycle

By analogy to the cancer–immunity cycle, we here introduce the NK cell–cancer cycle to discuss recent advances in NK cell biology and their importance for cancer immunotherapy (Fig. 2). For productive antitumor immune responses, the human body needs to initiate and orchestrate the activation of multiple immune cell subsets. As discussed by Chen and Mellman, the success of immune checkpoint blockade (ICB) in some cancers shows that targeting single molecules or pathways is promising but is ultimately insufficient across the majority of cancer patients¹³. Thus, our current challenge is to define additional targets in the tumor microenvironment (TME) and secondary lymphatic organs to overcome this limitation.

Step 1. Recruitment of NK cells to the TME. NK cells are commonly found in human tumors; however, they are at low frequency as compared to myeloid and lymphoid cells¹⁴. Increased abundance of NK cells in the TME has been associated with increased overall survival in patients with hepatocellular carcinoma¹⁵, melanoma^{16–20}, pulmonary adenocarcinoma²¹, gastric cancer²², squamous cell lung

¹Oncology and Cellular Immunology Laboratory, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia. ²Department of Pathology, ImmunoX Initiative, and Parker Institute for Cancer Immunotherapy, University of California, San Francisco, San Francisco, CA, USA. ³Immunology of Cancer and Infection Laboratory, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia. ⁴Translational Research Program, Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. ⁵These authors jointly supervised this work: Mark J. Smyth and Kevin C. Barry. ✉e-mail: mark.smyth@qimrberghofer.edu.au; kbarry@fredhutch.org

Table 1 | Advantages and disadvantages of NK cells over T cells in cancer therapy

Feature ^a	NK cells	T cells	Reference
Tumor stage	Elimination and metastasis	Equilibrium and escape	17,718
Number in tumor	+	+++	
Tumor cell recognition	+++ (NKR) ^b	+ (TCR) ^b	179,180
Specificity	+	+++	131,179
Killing capacity	+++	++	181-186
Cytokine release	++	+++	179,183
Proliferative capacity	+	+++	187-189
Recall response	-	+++	179,187,188
Immune-related adverse events	+	+++	190-193
Genetic engineering	+	++	180,194
Off-the-shelf utility	+++	+	195-200

^aFeatures are presented on a scale from low (-) to high (+++). ^bNKR, natural killer cell receptor; TCR, T cell receptor.

cancer²³, non-small cell lung cancer (NSCLC)²⁴, breast cancer²⁵ and renal cell carcinoma²⁶. Recently, NK cells have been further linked to patient responsiveness to anti-PD-1 immunotherapy in metastatic melanoma¹⁸. Here, we will discuss the first step in the NK cell–cancer cycle: NK cell recruitment to the TME (Figs. 2 and 3). The three main factors controlling NK cell recruitment to the tumor are chemoattractants/receptors, immunomodulation of chemokine axes, and physical barriers.

The two main subsets of NK cells, CD56^{bright} and CD56^{dim}, express unique repertoires of chemoattractant receptors, which explains the varying recruitment of NK cell subsets to different tissues (reviewed in ref. 27). Peripheral blood CD56^{bright} NK cells typically express and respond to ligands for CCR2, CCR5, CCR7, CXCR3, CXCR4 and CD62L, while CD56^{dim} NK cells express and respond to ligands to CXCR1, CXCR2, CXCR4, CX3CR1, S1P5 and ChemR23^{14,28–36} (Fig. 1). As described above, mouse CD11b SP and CD27 SP NK cells are the functional equivalents of human NK cell subsets (Fig. 1). Consistent with this relationship, mouse CD11b SP express similar chemokine receptors to those of human CD56^{dim} NK cells, while CD27 SP NK cells have chemokine receptors similar to those of human CD56^{bright} NK cells^{11,37} (Fig. 1).

Recent single-cell RNA sequencing of metastatic melanoma samples has found transcriptional heterogeneity among NK cells in the TME³⁸. Yet even though there may be transcriptional heterogeneity, CD56^{bright} NK cells have been found to be the dominant NK cells in the TME for a number of cancers, including NSCLC and breast cancer^{14,39}. The increased abundance of CD56^{bright} NK cells in the TME of NSCLC and breast cancer is linked to the downregulation of the chemokine CXCL2, which signals through CXCR2, and the concomitant upregulation of the chemokines CXCL9, CXCL10 and CCL19, which signal through CCR7 or CXCR3, in the TME¹⁴. Similarly, in preclinical mouse lymphoma models, tumor cell expression of CXCL9 and CXCL10, which signal through the chemokine receptor CXCR3 on NK cells, is important for the recruitment of NK cells into the TME^{40,41}.

CCL5, the ligand for CCR5, which is uniquely expressed on human CD56^{bright} and mouse CD27 SP NK cells (Fig. 1), has also been implicated in NK cell recruitment to the TME. Other atypical pathways may also control NK recruitment, probably through the CCL5 axis. In one mouse model, tumor-derived progranulin serves to inhibit CCL5 production in an autocrine fashion, leading

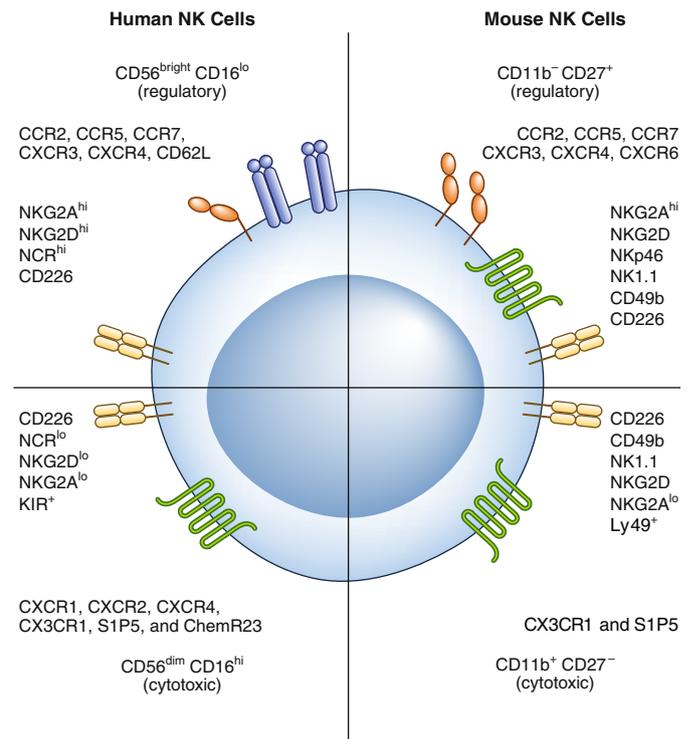


Fig. 1 | Human and mouse NK cells. Simplified human and mouse NK cells can be subdivided into two main subsets on the basis of the expression of CD56 and CD16 in humans and CD11b and CD27 in mice. Important molecules required for NK cell recruitment, activation and effector function in the TME are depicted.

to reduced NK cell infiltration into the TME, loss of tumor control and increased metastasis⁴². Consistent with a role for CCL5 in recruiting NK cells to the TME, in an experimental model of melanoma lung metastasis, IL-33 in the lung TME induces CCL5 production by CD8⁺ T cells and eosinophils, which leads to increased recruitment of NK cells and significantly reduced numbers of lung metastases⁴³. Other studies found that ectopic treatment with or overexpression of IL-33 in transplantable melanoma models leads to increased recruitment and activation of NK cells to the TME^{43,44}, possibly through a mechanism involving CCL5. Another atypical pathway shown to regulate recruitment of NK cells to the TME is controlled by the cytokine IL-17D and the chemokine CCL2. In this pathway, IL-17D produced by tumor cells signals to endothelial cells to induce the production of CCL2, which recruits NK cells to the TME⁴⁵.

CCL27, which signals through CCR10, is another chemokine linked to regulating NK cell recruitment to the TME. Intratumor injection of adenovirus encoding CCL27 increases recruitment of NK cells to the TME in mouse models and also inhibits tumor growth^{46,47}. However, one study found NK cells to be dispensable for tumor growth control, making the importance of CCL27-dependent recruitment of NK cells to the TME less clear⁴⁶. It is interesting to note that endometrial cancer shows a paucity of NK cells in the TME, and this correlates with reduced CCL27, CXCL12 and CCL21 production as compared to adjacent normal tissue⁴⁸. The role of CCL27 in cancer is complicated by the possibility that CCR10 also has tumor-cell-intrinsic functions, as it may enhance the growth and metastasis of melanoma and breast cancer cells^{49,50}. Clearly, more work is needed to fully determine the role of CCL27 in protective immune responses and the progression of cancer.

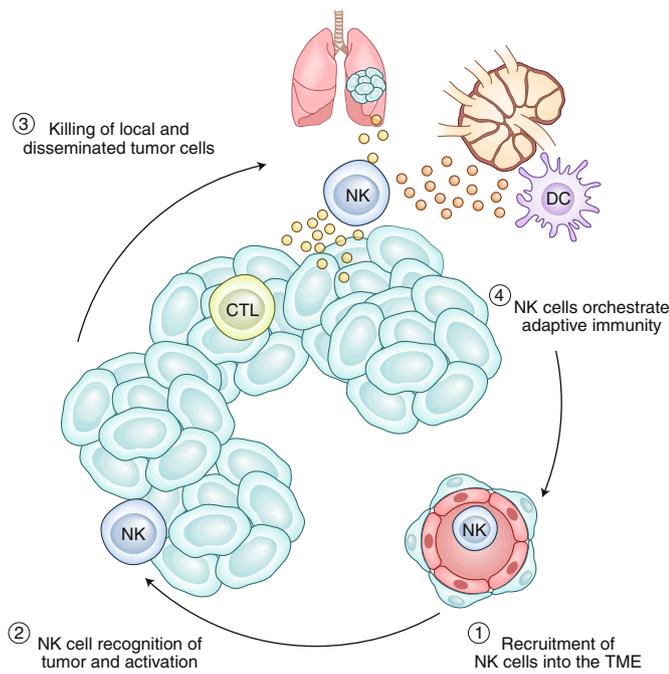


Fig. 2 | The NK cell-cancer cycle. To effectively eliminate cancer cells, the body needs to initiate a self-propagating antitumor immune response. The NK cell-cancer cycle consists of four steps important for initiating and maximizing the efficacy of this innate response. (1) NK cells are recruited into the TME; (2) NK cells recognize cancer cells and undergo full activation; (3) NK cells kill cancer cells locally and systemically; (4) NK cells orchestrate innate and adaptive immunity, for example, by alerting dendritic cells. Finally, due to the elimination of cancer cells locally in the TME or at distant sites, antigens, chemokines and danger-associated molecular patterns are liberated, which further fuel innate adaptive responses and allow the cycle to begin again.

CX3CL1, also known as fractalkine, is the ligand for CX3CR1, the chemokine receptor uniquely expressed on cytotoxic CD56^{dim}/CD11b SP NK cells. High expression of CX3CL1 is a positive prognostic indicator for patient outcome and NK cell infiltration in breast cancer⁵¹, gastric adenocarcinoma⁵², colorectal cancer⁵³, hepatocellular carcinoma⁵⁴ and lung adenocarcinoma⁵⁵. Furthermore, CX3CL1 is downregulated in human breast cancer tissue as compared to adjacent normal tissue, consistent with the finding that there is a high proportion of CD56^{bright} NK cells in the TME of breast cancer¹⁴. It has also been demonstrated that the CX3CL1–CX3CR1 axis is subverted by the tumor through the production of TGF- β ^{56,57}. Subsequent work showed that TGF- β 1 signaling in NK cells induces the expression of microRNA miR-27a-5p, which downregulates the expression of CX3CR1⁵⁸. It was also recently shown that CX3CL1–CX3CR1 signaling plays a role in controlling hepatocellular carcinoma metastasis to the lung⁵⁴. There, it was shown that tumor cells upregulate miR-561-5p, which in turn inhibits the production of CX3CL1 and subsequently reduces NK cell recruitment to the tumor⁵⁴. Taken together, these results suggest an important role for chemokine signaling in regulating the recruitment of NK cells into the TME and provide the rationale for targeting these pathways to increase the number of NK cells in the tumor.

Disruption or modulation of chemokine signaling by two immunomodulatory molecules, HLA-G and CD47, is linked to changes in NK cell recruitment to the TME. HLA-G is a member of the nonclassical HLA-class Ib genes and has strong immune-inhibitory functions. HLA-G is expressed in the TME but not in surrounding normal tissue, and studies have demonstrated that higher

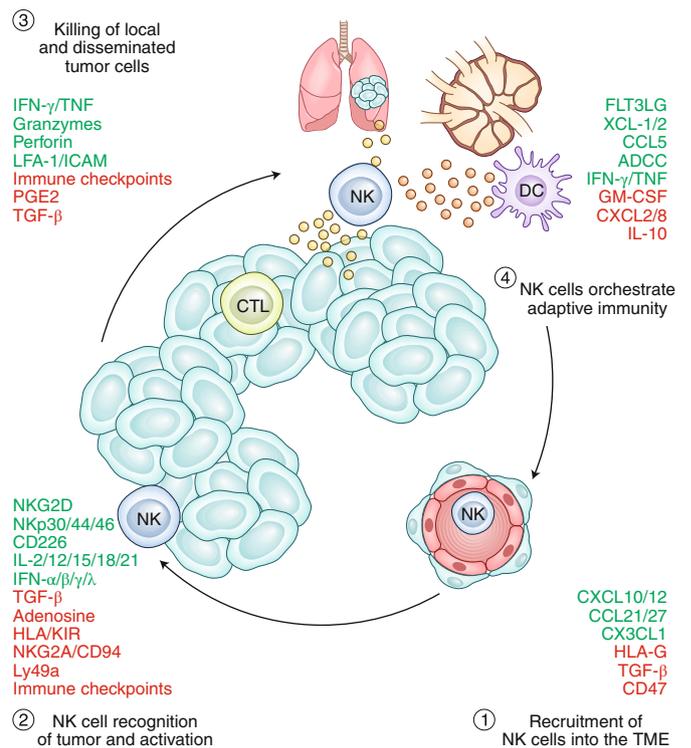


Fig. 3 | Molecules that positively and negatively regulate the NK cell-cancer cycle. Each step in the NK cell-cancer cycle is affected by a variety of stimulatory or inhibitory signals integrated by NK cells. Molecules that positively affect NK cells by promoting their activity are depicted in green, while molecules negatively affecting NK cell activity are shown in red. These negative signals are required to prevent excessive immune responses that lead to exacerbated tissue damage. However, in the TME, these pathways are hijacked by the cancer cells to escape NK cell-mediated immunity and to scotch the initiation of adaptive immunity.

HLA-G expression correlates with increased cancer stage and/or worse patient outcomes (reviewed in ref. ⁵⁹). HLA-G can inhibit NK cell activation, cytokine production and cytotoxicity through the downregulation of STAT3^{60,61}, while soluble HLA-G can reduce the expression of chemokine receptors in human NK cells, including CCR2, CXCR3 and CX3CR1³⁰. These findings suggest that soluble HLA-G found in the serum of cancer patients could impair the recruitment of NK cells to the TME.

The mechanisms by which CD47 regulates NK cell recruitment to the TME remain less clear. In the TME, CD47 has an important role in inhibiting phagocytosis of cancer cells (reviewed in ref. ⁶²). However, CD47 is also expressed on NK cells, and, upon binding its ligand thrombospondin-1 (TSP-1), it can inhibit NK cell activation and proliferation¹⁰. Using an anti-CD47 antibody to block TSP-1 binding to CD47 reversed TSP-1–CD47-mediated inhibition in a human NK cell line, inhibited tumor growth in melanoma-bearing B16 mice, increased NK cell recruitment to the TME and enhanced expression of granzyme B and IFN- γ in NK cells¹⁶. Further studies are needed to explore the mechanisms recruiting NK cells to the TME following anti-CD47 antibody treatment, but these findings suggest that CD47 acts as a NK cell checkpoint and highlight this pathway as a potential therapeutic target to modulate NK cell numbers in the TME.

Stromal barriers may also play a role in regulating NK cell recruitment to tumors (reviewed in ref. ⁵⁷). In tumor regions in which the extracellular matrix proteins collagen type IV and laminin were high, NK cells were not seen to enter the tumor, suggesting these structures around the tumor could prevent NK cell

invasion⁶³. Consistent with this finding, in human NSCLC tissue, NK cells are most commonly found in stromal regions in the tumor, rather than in direct contact with tumor cells³⁹. Furthermore, it has been suggested that, even in tumors where there is high expression of NK cell-attracting chemokines, there is not always a concomitant recruitment of NK cells (reviewed in ref. ⁵⁷). These findings reinforce the need for more research into the physical barriers that limit intratumor NK cells and suggest that emphasis should be placed on studying NK cell localization and its effect on a beneficial immune response.

Step 2. NK cell recognition of tumors and activation. In contrast to T and B lymphocytes, NK cells utilize an array of germ-line encoded activating and inhibitory receptors to identify foreign, stressed, infected or cancerous cells and to exert destruction of the target cell after full activation. Thus, complex signals arising from multiple ligand–receptor interactions need to be integrated, and these form the basis of NK cell recognition and activation. In the following section, we will discuss the second step in the NK cell–cancer cycle: NK cell recognition of tumors and activation by means of cell-contact-dependent and cell-contact-independent mechanisms (Figs. 2 and 3). One, if not the most, important signal for NK cells to identify potential target cells is the loss or aberrant expression of class I major histocompatibility complex (MHC-I) molecules. The recognition and elimination of MHC-I-lacking cells is called ‘missing self-recognition’.

NK cells constitutively express a variety of Ly49-type inhibitory receptors in mice and killer immunoglobulin-like receptors (KIRs) in humans and, in both species, the CD94–NKG2A heterodimer^{64,65}. Inhibitory KIR and Ly49 receptors are critical for the education of NK cells during development, as these receptors recognize classical polymorphic self-MHC-I molecules and thus allow NK cells to distinguish between healthy self tissue and stressed, infected, foreign or transformed cells⁶⁶ (also reviewed in ref. ⁶⁷). To evade adaptive immunity, cancer cells frequently downregulate classical MHC-I molecules, which in turn renders them susceptible to NK cell-mediated control. In addition, CD94–NKG2A recognizes less polymorphic non-classical MHC-I molecules, for example, HLA-E in humans and Q-1 in mice^{68,69}. But along with the activity of inhibitory receptors, NK cells need to receive activating signals to exert their effector function. Below we will discuss contact-dependent NK cell activation in the TME (Figs. 2 and 3).

NK cells are equipped with an armory of activating receptors, which are thought to recognize stress-induced ligands on cancer cells. Natural cytotoxicity receptors (NCRs), namely NKp46 (*NCR1/CD335*), NKp44 (*NCR2/CD336*) and NKp30 (*NCR3/CD337*) belong to the immunoglobulin (Ig) superfamily and are associated with various immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor proteins to recruit and activate downstream kinases (for example, Lck, Fyn, Syk and ZAP-70) to fully activate NK cells (reviewed in ref. ⁷⁰). The identification of cancer cell ligands for NCRs is still a matter of ongoing research. While some ligands for NKp30 have been identified, for example, B7-H6 and Bcl-2-associated athanogene 6 (Bag-6), the ligands for NKp46 remain unknown^{71,72}. NKp80, which has activating properties in NK cells, binds to activation-induced C-type lectin (AICL, encoded by *CLEC2B*), which is upregulated by Toll-like receptor stimulation on myeloid cells⁷³. Recently, Barrow et al. identified platelet-derived growth factor (PDGF)-DD as a ligand for NKp44 using a secretome library screen⁷⁴. NK cells activated with PDGF-DD secreted IFN- γ and tumor necrosis factor (TNF), leading to cell cycle arrest of melanoma, ovarian and breast cancer cells in vitro. Importantly, increased PDGF-DD gene expression correlated with *NCR2* and effector cytokine expression and was associated with a favorable survival in patients with glioblastoma⁷⁵. In line with the idea that NCRs can sense soluble mediators, Nidogen-1, an

extracellular matrix protein, was recently found to bind NKp44⁷⁶. Together, these data suggest that NK cells can be activated or inhibited by secreted molecules engaging with NCRs. This innovative concept opens up a new avenue of research with potential therapeutic value. Beside NCRs, the lectin-like type 2 transmembrane receptor NKG2D plays a crucial role in NK cell-mediated tumor cell killing. NKG2D is expressed on the majority of NK cells in humans and mice and recognizes a variety of MHC-related ligands that are poorly expressed in healthy tissues but strongly expressed in cancer cells^{77,78}. In mice, retinoic acid early inducible-1 (RAE-1), murine UL16-binding protein like transcript-1 (MULT-1) and H60 proteins are ligands for NKG2D, while the ligands for human NKG2D are UL16-binding proteins and MHC class I-chain-related proteins (MICA/MICB)^{79,80}.

Adhesion molecules have also been shown to promote NK cell activation. Lymphocyte function-associated antigen-1 (LFA-1) is expressed on NK cells and interacts with intercellular adhesion molecules (ICAMs) on target cells. Binding of LFA-1 to ICAM-1 can enhance NK cell-mediated cytotoxicity through enhanced polarization of the cytoskeleton machinery, which is required for effective delivery of cytotoxic granules⁸¹. DNAX accessory molecule-1 (CD226/DNAM-1) also contributes to NK cell adhesion, migration and function⁸². Upon binding its ligands CD155 or CD112, both frequently expressed on cancer cells, CD226 promotes NK cell activation and cytotoxicity⁸³.

NK cells are also regulated by many soluble extracellular factors in the TME. It has become increasingly clear that tumor cells and associated myeloid cells and fibroblasts secrete a number of environmental factors, such as cytokines, growth factors, exosomes and microRNAs, which impact the NK cell response. These have been extensively reviewed elsewhere^{84–87}; however, key factors include TGF- β 1 and associated family members, IL-10, extracellular adenosine, prostaglandin E2 and nitric oxide. Additionally, hypoxia and metabolic reprogramming impact NK cell responsiveness^{88–90}. These represent non-receptor immune checkpoints for NK cells that now shape many of the new therapeutic approaches to maintain and boost NK cell effector functions in tumors.

Another important axis of NK cell activation in the TME is driven by proinflammatory cytokines and danger-associated molecular patterns (DAMPs). Cytokines, upon binding their cognate receptor, augment activation, survival, proliferation and maturation of NK cells. Cytokines with NK cell stimulatory capacities are IL-2, IL-12, IL-15, IL-18 and IL-21. While IL-2 and IL-15, either alone or in combination with other cytokines, promote survival and proliferation, IL-12 and IL-18 mainly stimulate IFN- γ production and cytotoxicity in NK cells. IL-21 can enhance NK cell-mediated cytotoxicity by upregulating granzymes and perforin, and the combination of IL-21 and IL-2 exhibited a synergistic effect on NK cell activation⁹¹. Additionally, type I and III interferons are important for NK cell homeostasis and activation^{92,93}. Soluble ligands also have a strong impact on NK cell activation. One example is soluble HLA-G, which is able to activate human NK cells via KIR2DL4, leading to the production of cytokines and chemokines⁹⁴. Soluble NKG2D ligands can inhibit NK cell function by downregulating NKG2D⁹⁵. By contrast, Deng et al. showed that soluble forms of high-affinity NKG2D ligands led to NK cell activation⁹⁶. Thus, it remains unclear what role soluble NKG2D ligands play in NK cell activation.

Step 3. NK cell killing of tumor cells. NK cells are able to kill local and disseminated tumor cells (Figs. 2 and 3, step 3). Furthermore, an eleven-year follow-up study found that reduced NK cell killing capacity in the peripheral blood is correlated with tumor development⁹⁷. Thus, NK cell killing of tumor cells is an important effector function that can help control tumorigenesis. The mechanisms used by NK cells to kill cancer cells have been extensively discussed

Box 1 | NK cell-mediated killing

Cytotoxic granules contain a number of cytotoxic proteins, including the pore-forming proteins perforin and granzysin and effector proteases called granzymes^{201–203}. A recent study using mass cytometry to profile the expression of cytotoxic molecules in peripheral blood mononuclear cells found that human NK cell subsets have differential expression of cytotoxic molecules: CD56^{bright} NK cells showed low GzmB and perforin and high GzmK expression, while CD56^{dim} NK cells showed high GzmB and perforin and low GzmK expression²⁰⁴. These findings are consistent with the different cytotoxic activities of both NK cell subsets. This is also interesting in the context of tumor immunity, given the clear bias toward recruitment of CD56^{bright} NK cells to the tumor (as described in the section “NK cell recruitment”). Granule exocytosis is normally responsible for antibody-mediated cellular cytotoxicity (ADCC), a mechanism through which Fc receptor-bearing NK cells can recognize and kill target cells coated with antibodies bound to tumor-derived surface antigens. This is a particularly relevant consideration for antibody-based therapies.

Death receptor signaling

In addition to NK cell-mediated killing by cytotoxic granules, activated NK cells are capable of inducing apoptosis in target cells through the interaction of Fas ligand (FasL/CD95L) and/or TNF-related apoptosis-inducing ligand (TRAIL) with death receptors on the surface of target cells. FasL and TRAIL have been shown to play important roles in controlling immune responses to cancer²⁰⁵. In particular, these mechanisms appear important in NK cell-mediated control of liver metastasis^{206,207}, NK cell-mediated killing of immature DCs²⁰⁸, and prenatally in the function of fetal NK cells²⁰⁶. In the context of the NK cell–cancer cycle, TNF probably plays an important role in modulating the TME and inducing direct cytotoxicity against tumor cells²⁰⁹. However, studies have shown that TNF alone is weakly cytotoxic or cytostatic and only induces apoptosis when metabolic inhibitors are present (reviewed in ref. ²¹⁰). Thus, it remains unclear whether NK cell-derived TNF controls tumors directly through cytotoxicity or indirectly by acting on the immune microenvironment and tumor vasculature²⁰⁹.

and are summarized in Box 1. NK cell killing and abundance in the tumor correlate with better patient outcomes (reviewed in ^{15–26,98}), even though these cells are found at relatively low levels in tumors^{15–26}. This finding has led many to ponder how this rare cell type can be integral for protecting against cancer. While NK cell killing is clearly protective, NK cells have a number of functions, including cytokine and chemokine production, that shape the immune response to cancer (discussed in Step 5 of the NK cell–cancer cycle), which could amplify their importance. As such, in addition to the obvious antitumor activity of direct cytotoxicity, NK cell-mediated killing of cancer cells also impacts the availability of antigen for presentation, both in the context of normal immune responses and in more clinical settings where monoclonal antibodies are used to induce antibody-dependent cellular cytotoxicity (ADCC) (reviewed in ref. ⁷⁵). NK cell-induced tumor cell death can increase the release of tumor antigens, which in turn can increase the amount of tumor antigen presented to T cells, potentially boosting T cell responses to cancer. Thus, NK cell killing of tumor cells can occur in the primary or disseminated tumor and can lead to a release of tumor antigens to prime an adaptive immune response, but this is unlikely to explain all of the protection afforded by the presence of NK cells in solid tumors.

Step 4. Orchestration of adaptive immune responses by NK cells.

The most common ways that NK cells exert their effect on the adaptive immune response to cancer are through the production of cytokines and by modulating dendritic cell (DC) responses (Figs. 2 and 3, step 4). Activated NK cells produce a variety of cytokines, including IFN- γ , GM-CSF, G-CSF, M-CSF, TNF, IL-5, IL-10, IL-13 and others⁹⁹. IFN- γ is one of the best studied cytokines in the context of antitumor immunity and is a major factor in regulating positive and negative antitumor responses. As the details of the mechanisms by which IFN- γ regulates immune responses have recently been discussed in great detail (reviewed in refs. ^{100,101}), we will focus on how NK cell production of IFN- γ is linked to changes in the adaptive immune response to cancer.

IFN- γ acts directly on a variety of immune cells, including macrophages, DCs, B cells, T cells, and even on NK cells themselves. IFN- γ signaling in macrophages activates these cells, leading to increased inflammatory cytokine production, increased phagocytosis and antigen presentation and enhanced nonspecific cytotoxic activity toward microbial pathogens and tumors¹⁰². Additionally, IFN- γ induces DC maturation, which results in an increase in MHC-I and II expression, upregulation of costimulatory molecules and upregulation of the cellular machinery needed for processing antigens to present to T cells¹⁰³. In addition to upregulating antigen-presentation machinery, IFN- γ -mediated activation of DCs has been shown to induce the expression of IL-12 and IL-15 in DCs, which can play important roles in inducing antitumor responses by CD4⁺ T_H1 and cytotoxic CD8⁺ T cells^{80,104–108}. IFN- γ signaling also affects T cell function directly. IFN- γ signaling in CD4⁺ T cells can stimulate them to develop an antitumor T_H1 phenotype and induces an upregulation of granzyme and IL-2 receptor expression on CD8⁺ T cells, enabling these cells to reach their full cytotoxic potential (reviewed in refs. ^{100,101}). Furthermore, IFN- γ can directly increase antigen presentation in tumor cells, leading to increased tumor immunogenicity^{109–114}.

It is important to note that, while IFN- γ is a strong driver of antitumor immunity, it also contributes to immune evasion through increased expression of immune suppressive molecules such as programmed death-ligand 1 (PD-L1) on tumor and myeloid cells in the TME. Thus, this cytokine can act as a double-edged sword and its function may depend on how it is spatially distributed. More work is required to fully understand the role of IFN- γ in tumor progression and antitumor immunity; however, there is clear evidence that IFN- γ production by NK cells is a major factor that allows NK cells to be integral players in shaping the adaptive immune responses to cancer and disease.

It is well established that there is cross-talk between NK cells and DCs and that the interaction between these two innate immune cell types leads to profound adaptive immune responses to disease and cancer (reviewed in refs. ^{115,116}). DCs are key players in the induction of T cell immune responses and, as antigen presenting cells (APCs), bridge the gap between the innate and adaptive immune systems, making them an important partner in NK cell regulation of adaptive immune responses. Developmental, phenotypical and functional criteria separate DCs into two broad classes, the conventional type 1 DCs (cDC1s) and conventional type 2 DCs (cDC2s), in both humans and mice. cDC1s are classically described as mediators of cellular immunity against intracellular pathogens and cancer, which is at least partially because they specialize in cross-presenting antigens to CD8⁺ T cells, while cDC2s are more heterogeneous and are thought to be more efficient at inducing CD4⁺ T cell responses in cancer (reviewed in ref. ¹¹⁷). There is a rich literature describing the relationship between NK cells and DCs. Initial studies showed that NK cells probably play an important role in shaping DC responses by editing these cells, either by directly killing immature DCs or by inducing their maturation^{106,118–124}. Thus, as is a common theme in immunology, the NK cell–DC interaction may induce proper and

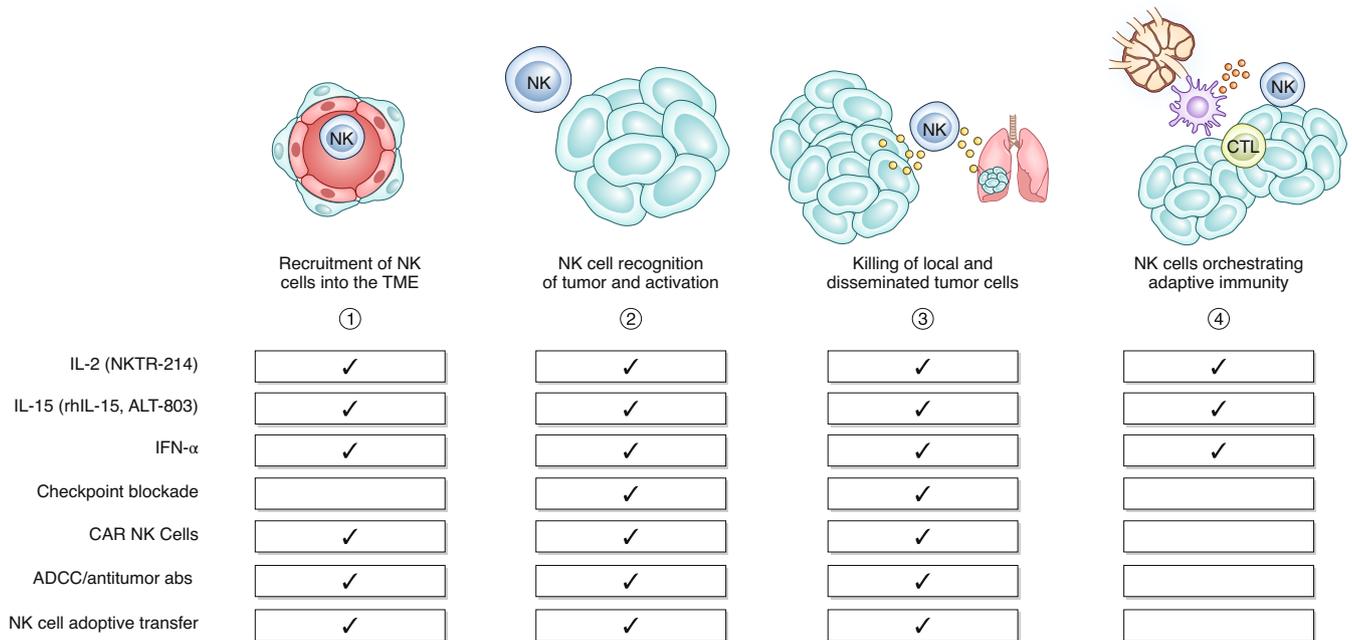


Fig. 4 | Therapies affecting individual steps of the NK cell-cancer cycle.

full DC function but, in certain settings, may also negatively regulate adaptive immune responses. However, these data collectively provide clear evidence of a functional link between NK cell activation and antitumor adaptive immune responses.

In addition to the important role of NK cells in regulating DC maturation, recent studies have found that NK cells act upstream of DC maturation and are also key regulators of DC recruitment, retention, and/or survival in the TME^{18,19}. In a transplantable BRAF^{V600E} mouse model of melanoma, it was shown that NK cells are key in producing the chemokines CCL5 and XCL1/2 to recruit cDC1s into the tumor¹⁹. Importantly, the NK cell-dependent recruitment of cDC1s to the tumor is only seen in the absence of tumor-produced prostaglandin E₂ (PGE₂; *Ptgs1^{-/-}/Ptgs2^{-/-}*), suggesting that NK cell production of CCL5 and XCL1/2 and the recruitment of cDC1s into the tumor are acutely sensitive to immune-suppressive PGE₂. NK cells in the TME also make the cytokine FLT3LG, the formative cytokine for cDC1s^{125,126}, and NK cell levels and *FLT3LG* expression in the tumor correlate with increased cDC1 levels in the TME¹⁸. FLT3LG production by NK cells may regulate cDC1 levels in the TME either by increasing the differentiation of precursor DCs or increasing the survival of cDC1s within the TME^{18,127}. Together, these findings suggest that NK cells, in addition to regulating DC maturation and subsequent T cell priming, are integral in recruiting and supporting cDC1 levels in the TME, an important function given the role TME-resident cDC1s play in supporting protective immune responses to cancer^{18,117,127-130}.

In this step of the NK cell-cancer cycle, it is clear that NK cells directly modulate T cell activity through the production of IFN- γ , and at the same time shape the DC response to cancer through the induction of DC maturation and the recruitment and maintenance of DCs in the TME. The findings presented here suggest that NK cells play an integral role in coordinating and initiating the adaptive immune response to cancer.

Targeting NK cells in cancer—progress and challenges

In the previous sections, we discussed the individual steps required for NK cell recruitment, activation and effector function in the TME. The knowledge gained from basic and preclinical research

over the last 40 years laid the foundation for the development of NK cell-based cancer immunotherapies. In the next section, we will discuss encouraging results and limitations of NK cell-based therapeutic approaches currently under preclinical and clinical evaluation (Fig. 4).

The ideal therapeutic approach should aim to improve NK cells at every step in the NK cell-cancer cycle. A major problem with targeting NK cells in patients with cancer is that many tumors are sparsely infiltrated with NK cells. Thus, a leading approach to boost NK cell-mediated tumor immunity is the adoptive transfer of ex vivo activated autologous (from the same patient) or allogeneic (from a healthy donor) NK cells. While adoptively transferred T cells can cause many severe side effects (for example, cytokine release syndrome, graft-versus-host disease, and so on), the transfer of NK cells is comparatively safe (reviewed in ref. ¹³¹). For example, two clinical trials showed efficacy of adoptively transferred haploidentical NK cells in non-Hodgkin lymphoma and refractory or relapsed myelodysplastic syndrome and in secondary and de novo AML, respectively^{132,133}. Although encouraging results have been achieved in patients with liquid cancers, response rates in patients with solid cancers remain unsatisfying. Genetic engineering of NK cell products is a promising approach to improve the efficacy of NK cell transfer therapies (Box 2). One strategy currently under preclinical evaluation is to overexpress activating molecules, including NKG2D, CXCR2 and membrane-bound IL-15 in NK cells¹³⁴⁻¹³⁷, or alternatively, to reduce the expression of inhibitory receptors like NKG2A¹³⁸. Kamiya et al. elegantly showed that the expression of a single-chain variable fragment recognizing NKG2A fused with an endoplasmic reticulum-retention domain in NK cells prevents the shuttling of NKG2A to the cell surface¹³⁸. NKG2A-modified NK cells showed superior killing of HLA-E-expressing target cells¹³⁸. The discovery and development of CRISPR-Cas9 genome editing technology has opened a new avenue for enhancing NK cell products through modifying the expression levels of activating or inhibitory molecules (reviewed in ref. ¹³⁹).

NK cell expression of chimeric antigen receptors (CARs), directed against surface antigens expressed by tumor cells, is another encouraging approach. For example, NK cells expressing anti-CD19

Box 2 | CAR NK cells harness the power of innate lymphocytes

Expression of chimeric antigen receptors (CAR) in T cells represents an effective strategy to redirect the specificity of effector cells. Adoptive transfer of CAR T cells induces significant and durable responses, averaging around 60–70% across multiple cancer types. Thus, two anti-CD19 CAR T cell products are approved by the Food and Drug Administration and are currently in clinical use. However, besides logistical challenges (for example, challenging and expensive production), the biggest problem with CAR T cells is the severe toxicities observed in patients, including cytokine release syndrome, neurotoxicity and graft-versus-host-disease²¹¹. A recent clinical trial using cord blood-derived HLA-mismatched anti-CD19 CAR NK cells showed a 73% response rate in patients with non-Hodgkin lymphoma and chronic lymphocytic leukemia. Most importantly, the treatment was not associated with adverse immune-related events¹⁴². This very promising study highlights the safety and therapeutic potential of ‘off-the-shelf’ CAR NK cells. There are a number of properties of NK cells that need to be considered in CAR NK cell development. NK cells are more difficult to expand in large numbers than T cells, and their persistence *in vivo* after infusion is limited. NK cells are also educated continually by molecules such as MHC class I²¹², and their functions are highly regulated by a balance between inhibiting and activating signals, meaning that their action may be short-lived. Like T cells, NK cells cryopreserve quite well, but NK cells are more difficult to transfect and genetically engineer than T cells. CARs have a higher affinity for tumor antigens than either endogenous NK cell receptors or TCRs, but synthetic immunology is very effective for NK cells in the context of ADCC. Many questions remain about what the best target would be and whether additional genetic manipulation of NK cells could create superior products. Furthermore, the question of whether CAR NK cells are superior to CAR T cells needs to be addressed in clinical trials.

CARs can efficiently kill autologous acute lymphoblastic leukemia (ALL) cells, which are resistant to CAR-negative NK cell-mediated killing¹⁴⁰. Similarly, Li et al. recently showed that anti-mesothelin CAR-expressing NK cells, derived from induced pluripotent stem cells (iPSCs), significantly impaired the growth of ovarian cancer in a xenograft model¹⁴¹. In this study, not only were they able to generate CAR NK cells from iPSCs, providing another means of creating an ‘off-the-shelf’ NK cell product, but they also designed next-generation NK cell-specific CAR constructs. After screening multiple CAR variants, NK cells expressing a CAR containing the transmembrane domain of NKG2D, the costimulatory domain of 2B4 and the signaling domain of CD3 ζ showed the strongest efficacy¹⁴¹. Importantly, a landmark study by Liu et al. demonstrated that adoptive transfer of allogeneic anti-CD19 CAR NK cells was safe and effective in patients with high-risk CD19⁺ chronic lymphocytic leukemia and non-Hodgkin lymphoma¹⁴². This clinically relevant study is encouraging and highlights the potential for future CAR NK cell-based therapies.

NK cells express or upregulate a variety of inhibitory receptors, such as KIRs, NKG2A/CD94, programmed death-1 (PD-1), T cell immunoglobulin and mucin-domain-containing molecule 3 (TIM-3), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibition motif domains (TIGIT) and CD96 (reviewed in refs. ^{143–146}). Recent reviews have thoroughly discussed our understanding of the relationship between these inhibitory pathways and NK cells in cancer (reviewed in refs. ^{143–146}). In many cases, the underlying biological mechanisms

Box 3 | NKG2A, a promising new target in cancer immunotherapy?

The inhibitory receptor complex NKG2A–CD94 is an attractive and promising target^{213,214}. Currently, six phase 1 and 2 clinical trials using monalizumab as a single agent or in combination are recruiting patients or are in progress for a range of solid and hematological malignancies, including head and neck squamous cell carcinoma and non-small cell lung cancer (NCT02671435, NCT02921685, NCT02643550, NCT03822351, NCT03794544, NCT03088059). Furthermore, two phase 2 trials in patients with microsatellite-stable colorectal cancer and immunotherapy-resistant NSCLC are about to begin (NCT04145193, NCT03833440). Yet despite these clinical efforts, translational research will need to address pressing questions around the NKG2A pathway. Little is known about the expression of NKG2A–CD94 in tumor-infiltrating CD8⁺ and CD4⁺ T cells. Additionally, we need to understand the regulation and expression of HLA-E in healthy and malignant tissues to stratify patients on the basis of the expression of the NKG2A ligand. Studies by van Montfoort et al.¹⁵⁶ and André et al.¹⁵⁷ also raise questions about the contribution of NKG2A to the function of NK and T cells. Overall, blocking or genetic deletion of NKG2A in effector cells seems to be a rational strategy, and future studies will shed more light on the underlying biology and clinical relevance of this molecule.

of these inhibitory pathways, as well as the clinical benefit of targeting the majority of these inhibitory receptors, need to be further explored. Here, we will focus our discussion on inhibitory pathways that have recently been studied in a clinical setting.

The first strategy tested to enhance NK cell function aimed to block HLA–inhibitory receptor interactions. The engagement of inhibitory receptors with HLA molecules is considered a major impediment to NK cell activation. The mAb IPH2101 (1-7F9, human IgG4) binds with high affinity to the inhibitory receptors KIR2DL-1, 2 and 3 and thereby prevents inhibitory signaling mediated by HLA-C molecule allotypes¹⁴⁷. Phase 1 clinical trials showed that IPH2101 enhanced NK cell activation and *ex vivo* cytotoxicity and was safe and well tolerated by patients with cancer^{148,149}. Preclinical studies supporting the combination of KIR blocking with lenalidomide or with rituximab for the treatment of multiple myeloma and lymphoma, respectively, have been previously reviewed¹⁵⁰. Although *in vitro* studies had suggested IPH2101 induced KIR ligand mismatch-mediated tumor killing by NK cells, a phase 2 clinical trial in patients with smoldering MM (KIRMONO) was terminated early due to a lack of clinical efficacy¹⁵¹. Subsequent investigations revealed that KIR2D molecules were removed from the surface of IPH2101-treated NK cells by trogocytosis, with reductions in NK cell function directly correlating with the loss of free KIR2D surface molecules¹⁵². These data raise concerns that unexpected biological events could compromise some antibody-based strategies designed to augment NK cell tumor killing¹⁵³. In spite of its limited clinical benefit as a single agent, future studies will need to assess the potential of IPH2101 in combination therapy.

In addition to KIRs, the heterodimer NKG2A–CD94 has also received great attention. NKG2A–CD94 binds to the nonclassical HLA class I molecule HLA-E (Qa1b in mice), which is often upregulated on cancer cells. Recently, it has been shown that NKG2A inhibits NK activation and target cell killing⁷⁵. Monalizumab (IPH2201), a humanized anti-NKG2A blocking antibody, has been tested in a number of clinical trials as a single agent or in combination with other therapeutics across different cancers; however, many questions remain (Box 3, and extensively reviewed in ref. ¹⁵⁴).

Two recent studies have further shed light on NKG2A as a cancer immunotherapeutic target (reviewed in ref. ¹⁵⁵). Van Montfoort et al. demonstrated that, in preclinical solid tumor models, peptide vaccination combined with antibody blockade of NKG2A on CD8⁺ T cells improved the response rate and survival of mice as compared to peptide vaccination alone¹⁵⁶, while André et al. demonstrated the antitumor activity of NKG2A inhibition in combination with anti-PD-1/PD-L1 blockade in mouse lymphoma models and in human *in vitro* experiments¹⁵⁷. In addition, the interim results of a clinical trial of the combination of monalizumab and cetuximab, a clinically approved anti-EGFR antibody, in patients with head and neck squamous cell carcinoma led to a 30% objective response rate in immunotherapy-refractory patients. Although these results are very promising and provide hope for the future improvement of immunotherapies for cancer patients, many questions remain and need to be addressed in preclinical and clinical studies.

As described in step 3 of the NK cell–cancer cycle, cytokines play an important role in NK cell activation and function (Figs. 2 and 3). Consistent with preclinical studies, clinical work has focused on using cytokines that stimulate NK cells to increase NK cell activity and abundance in the TME, with some of these immunotherapies having beneficial effects on patient survival and disease outcome¹⁵⁸. Here we will focus on three NK cell stimulatory molecules being explored in the clinic: IL-2, IL-15 and IFN- α .

IL-2 activates NK cells by binding to the heterotrimeric IL-2 receptor (IL-2R α (CD25)–IL-2R β (CD122)–IL-2R γ (CD132)). Early studies found that recombinant IL-2 expands effector T cells and NK cells but also induces a robust expansion of regulatory T (T_{reg}) cells in patients. Thus, a number of drugs have been developed that lead to preferential activation and expansion of CD8⁺ T cells and NK cells. The preferential targeting of CD8⁺ T cells and NK cells has been accomplished by skewing binding of IL-2 away from the IL2R α subunit, which is more abundant on T_{reg} cells^{158,159}. One drug that is able to accomplish this is NKTR-214 (bempegaldesleukin), a modified recombinant IL-2 that is well tolerated by patients and is capable of inducing increased numbers and robust activation of CD8⁺ T cells and NK cells within the TME, without changing T_{reg} cell numbers¹⁶⁰. Monotherapy with NKTR-214 in heavily pretreated, non-responsive patients with solid tumors led to 9 of 26 patients showing some level of stable disease, although no objective responses were measured by RECIST (response evaluation criteria in solid tumors)¹⁶⁰. These findings led to the combination of NKTR-214 with anti-PD-1 immunotherapy being used for patients with advanced solid tumors, and preliminary results presented at the 2019 Society for Immunotherapy of Cancer (SITC) Annual Meeting suggest that responses to this combination therapy were durable and increased over time, with evaluable patients showing an objective response rate (ORR) of 53% (20/38), and 34% (13/38) of patients achieving complete responses (CR) at a median follow up time of 18.6 months (NCT02983045). Phase 3 trials are currently underway using NKTR-214 in combination with nivolumab (anti-PD-1) in melanoma (NCT03635983), muscle-invasive bladder cancer (NCT04209114), and renal cell carcinoma (NCT03729245). Other IL-2 combination therapies have been less successful, as bevacizumab, an inhibitor of VEGF and an antibody thought to induce ADCC, showed no benefit over IL-2 therapy alone¹⁶¹.

IL-15 also has stimulatory capacity for NK cells but does not induce T_{reg} cell expansion. Intravenous infusion or subcutaneous injection of recombinant human IL-15 (rhIL-15) led to the expansion and activation of NK cells and CD8⁺ T cells in patients with solid tumors^{162,163}. While there were signs of clinical benefit in patients treated with rhIL-15, no responses were detected on the basis of RECIST criteria^{162,163}. Larger studies will be needed to fully elucidate the clinical benefit of rhIL-15 monotherapy. rhIL-15 has also been combined with haploidentical NK cell infusions in refractory acute myeloid leukemia (AML), and this treatment

led to remission in 35% of patients and better rates of *in vivo* NK cell expansion and remission as compared to previous trials with IL-2¹⁶⁴. While rhIL-15 holds therapeutic promise, animal studies have found that the IL-15 superagonist, IL-15 in complex with its soluble receptor IL-15R α , can lead to a large increase in biological activity and has enhanced activity as a cancer immunotherapeutic¹⁶⁵. ALT-803 is a pharmacological grade IL-15 superagonist that promotes the expansion and activation of NK cells and CD8⁺ T cells in patients with post-relapse hematologic malignancies or solid tumors^{141,166}. ALT-803 has shown some clinical response as a monotherapy in hematologic malignancies and solid tumors. It can also induce signs of clinical response when combined with anti-PD-1 immunotherapy in patients with NSCLC that had refractory or relapsed disease from previous anti-PD-1 treatment^{141,166,167}. These early clinical studies highlight an important role for recombinant IL-15 or IL-15 superagonists in NK cell expansion and have led to the initiation of a number of Phase 2 and 3 studies across different cancer indications and in combination with other therapies, including NCT02989844, NCT03586869, NCT03387098, NCT03329248, NCT03228667, NCT03136406, NCT02523469, NCT02384954, NCT01885897, NCT03022825, NCT02138734.

IFN- α treatment can activate NK cells and T cells to kill cancer cells in acute myeloid leukemia patients¹⁶⁸. A major issue facing patients receiving allogeneic hematopoietic stem cell transplantation (allo-HSCT) to treat acute leukemia is post-transplant relapse. Patients with minimal residual disease following allo-HSCT were found to have lower relapse rates if they were treated with IFN- α ^{169,170}. This finding was attributed to either immunomodulation of NK cells and T cells or direct inhibitory effects of IFN- α on blast cells^{169,170}. Future multicenter clinical studies will be necessary to confirm the efficacy of IFN- α treatment to protect against relapse in patients with leukemia with minimal residual disease.

Clearly, the modulation of NK cells through treatment with cytokines is an important area of clinical research. The potential benefits of cytokine therapies are clear, but these therapies are better understood in hematologic malignancies than in solid tumors. The many ongoing clinical trials in hematologic malignancies and solid tumors are going to lead to exciting findings and new treatments that will undoubtedly target NK cells at every step of the NK cell–cancer cycle.

Bispecific and trispecific killer cell engagers (BiKEs and TriKEs) represent an alternative strategy to efficiently engage CD16 and induce ADCC-like responses (reviewed in refs. ^{150,171–173}). Initial BiKE and TriKE constructs fused a single-chain variable fragment (Fv) against CD16 with, in the case of BiKEs, a single-chain Fv against a tumor antigen, and in the case of TriKEs, two tumor antigens. Strategies using BiKEs and TriKEs are many, but they include single tumor-antigen targeting (for example, CD19, CD20, CD30, CD133, PMSA, BCMA, Her2, CEA, EGFR, and so on), dual tumor-antigen targeting (allowing for avidity-tuned binding to two cancer antigens, which increases the range of targetable tumors), dual TME targeting (allowing for avidity-tuned binding of two antigens on tumor-promoting cells without affecting cells in healthy tissues) and dual targeting of both tumor and TME antigens (combining the prior two approaches). Some molecules, such as PD-L1, CD155, CD47 and CD38, may act as tumor antigens or immunosuppressive immune cell surface antigens when elevated in the TME. The Fc itself, when required, may be afucosylated to enhance ADCC or mutated to silence that function. Nanobodies derived from camelid animals are also emerging as a new force in antibody therapy.

Killer engagers are designed by fusing Fv domains that recognize tumor cell antigens with Fv domains binding CD16 and other largely NK cell-specific surface activation molecules (for example, NKp30, NKp44, NKp46 and NKG2D). There are currently very few other NK cell-specific molecules to choose from, but molecules like NKp30 appear to be stably expressed by all NK cells, and the ligation

of NKp30 can trigger strong degranulation and cytokine release by NK cells. In the absence of cytokines, resting NK cells can be stimulated by combining the activity of a hierarchy of several activating receptors. CD16 is unique in its ability to mediate ADCC and activate significant cytotoxicity and cytokine secretion when triggered alone. Many of the ligands recognized by NK cell receptors represent the body's methods to detect altered or defective cells and support immune activation. The amount of ligand expression on the cell surface can also be modulated by ligand shedding, secretion, or excretion in macrovesicles. The degree to which such ligand regulation affects immune targeting remains to be determined. Recently, Ferrari de Andrade et al. generated an antibody against the human NKG2D ligand MICA/B (major histocompatibility complex class I chain related gene A/B), which prevented proteolytic shedding of the extracellular domains of MICA and MICB and stimulated anti-tumor immunity by activating NKG2D and CD16 on NK cells. In a metastasis model pretreated with human NK cells, this antibody effectively inhibited tumor growth *in vivo*¹⁷⁴.

A number of important factors need to be considered in this area. First, how does the multivalent therapeutic overcome tumor escape from NK cells? Second, is the engager regulated by the TME (for example, by molecules such as TGF- β)? For TriKE constructs, it remains to be seen whether the binding of the NK cell-specific engager (for example, NKp30) is sufficient or whether CD16 signaling is also required. It also remains to be seen whether all or only a subset of NK cell effector functions along with NK cell proliferation are enhanced by these molecular designs. Another important consideration is, if the activation by these therapeutics is too effective, will this lead to NK cell exhaustion? In addition, more work is needed to determine which combination therapies would be good partners for multivalent therapeutics to improve NK cell numbers, as well as their recruitment to and survival in the TME. Lastly, there is a risk that antigen-negative variants of tumors will selectively grow out over time.

Regarding the TME, tailored additional modules (like TGF- β traps) and survival promoting cytokines (like IL-15 and IL-2) remain attractive strategies that could be built into NK cell engagers (for example, BiKEs, TriKEs, and killer engagers). An interesting strategy involving simultaneous blockade of the immune checkpoint ligand PD-L1 and TGF- β , using a bifunctional antibody-ligand trap, was recently reported¹⁷⁵. Nevertheless, these multivalent drugs are currently being investigated in preclinical studies, and safety remains a concern, as they have the potential to trigger cytokine cascades. With these future therapeutics, different constructs have been engineered, and indeed, hundreds of possible formats may be tested preclinically, some of which will reach the clinic in the future. We believe this whittling process should be guided by the principles provided by the NK cell-cancer cycle presented herein.

Outlook

The therapeutic potential of NK cells is incredibly high, and the recent findings that CAR-transduced NK cells have low toxicity and are associated with high response rates drive home the importance of NK cells as potential immunotherapies to treat patients¹⁶⁶. NK cell-based immunotherapies have been more efficacious in hematologic cancers, but progress is also being made to develop these therapies for solid tumors (reviewed in ref. ¹⁷⁶). There is growing excitement about the use of these cells to target cancer, and our understanding of the NK cell-cancer cycle is constantly evolving. While NK cell cytotoxicity is an obvious effector function that plays important roles in controlling cancer, an equally important role of NK cells is to shape the TME and to modulate adaptive immune responses to cancer. Given the diverse roles NK cells play in shaping the immune response to cancer, described here in the context of the NK cell-cancer cycle, it will be important to design and develop new therapies to target pathways that will hit all, or the majority, of

the steps in the NK cell-cancer cycle, with a focus not just on inducing NK cell cytotoxicity but also on harnessing the immunomodulatory effects of NK cells.

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Competing interests

T.B. has research agreements with ENA Therapeutics and Bristol Myers Squibb and is on the scientific advisory board of Oncomyx. M.F.K. is a founder and shareholder of Pionyr Immunotherapeutics and has research agreements with Bristol Myers Squibb, Eli Lilly, Pfizer, Amgen, Abbvie and Genentech. M.J.S. has research agreements with Bristol Myers Squibb and Tizona Therapeutics and is on the scientific advisory board of Tizona Therapeutics and Compass Therapeutics. K.C.B. declares no competing interests.

Additional information

Correspondence should be addressed to M.J.S. or K.C.B.

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