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

Tobias Bald[1], Matthew F. Krummel[2], 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_H17 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 settings[5]. 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 patients[13]. 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 cells[14]. Increased abundance of NK cells in the TME has been associated with increased overall survival in patients with hepatocellular carcinoma[15], melanoma[16–20], pulmonary adenocarcinoma[21], gastric cancer[22], squamous cell lung

[1]Oncology and Cellular Immunology Laboratory, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia. [2]Department of Pathology, ImmunoX Initiative, and Parker Institute for Cancer Immunotherapy, University of California, San Francisco, San Francisco, CA, USA. [3]Immunology of Cancer and Infection Laboratory, QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia. [4]Translational Research Program, Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA. [5]These 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	177,178
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

[a]Features are presented on a scale from low (–) to high (+++). [b]NKR, natural killer cell receptor; TCR, T cell receptor.

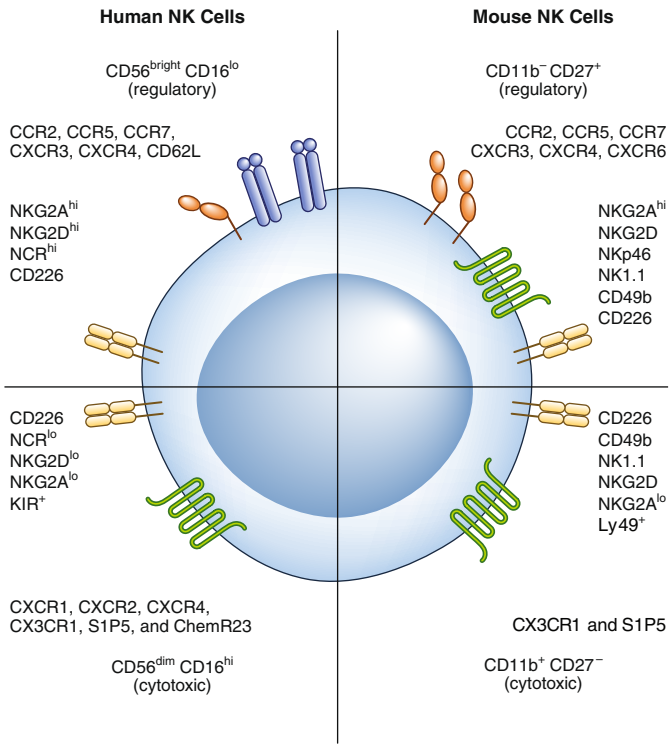

Fig. 1 | Human and mouse NK cells. Simplified human and mouse NK cells can be subdivided into three main subsets on the basis of the expression of CD56 and CD16 in humans and CD11b and CD27 in mice. Important molecules required for NK cell recruitment, activation and effector function in the TME are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

cancer[23], non-small cell lung cancer (NSCLC)[24], breast cancer[25] and renal cell carcinoma[26]. Recently, NK cells have been further linked to patient responsiveness to anti-PD-1 immunotherapy in metastatic melanoma[18]. 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 TME[38]. 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 TME[14]. 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 to reduced NK cell infiltration into the TME, loss of tumor control and increased metastasis[42]. 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 metastases[43]. 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 TME[45].

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 clear[46]. 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 tissue[48]. 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.

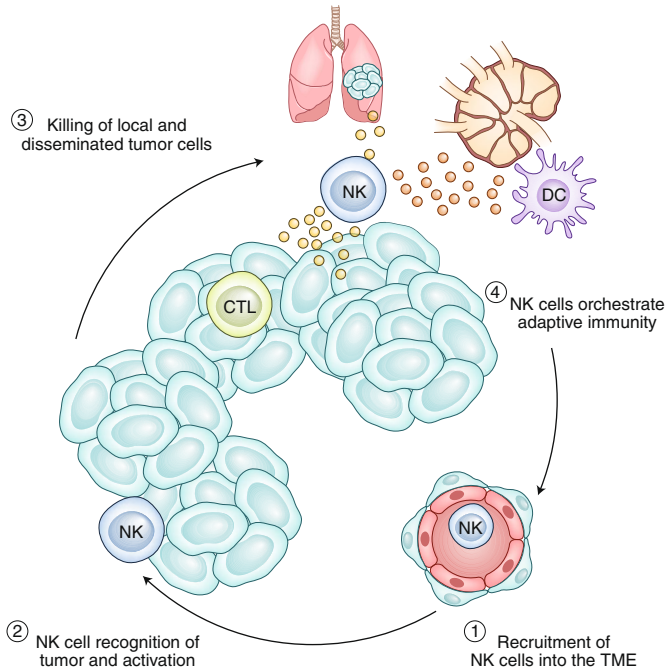

Fig. 2 | The NK cell–cancer cycle. To effectively eliminate cancer cells, the body needs to initiate a self-propagating antitumor immune response. The NK cell–cancer cycle consists of four steps important for initiating and maximizing the efficacy of this innate response. (1) NK cells are recruited into 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.

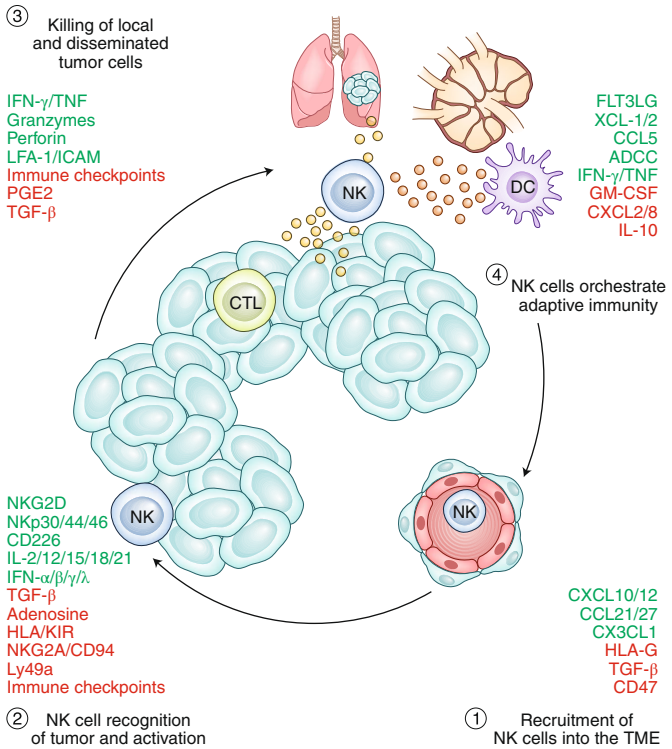

Fig. 3 | Molecules that positively and negatively regulate the NK cell–cancer cycle. Each step in the NK cell–cancer cycle is affected by a variety of stimulatory or inhibitory signals integrated by NK cells. Molecules that positively affect NK cells by promoting their activity are depicted in green, 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.

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 cancer[51], gastric adenocarcinoma[52], colorectal cancer[53], hepatocellular carcinoma[54] and lung adenocarcinoma[55]. 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 cancer[14]. 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-β1 signaling in NK cells induces the expression of microRNA miR-27a-5p, which downregulates the expression of CX3CR1[58]. It was also recently shown that CX3CL1–CX3CR1 signaling plays a role in controlling hepatocellular carcinoma metastasis to the lung[54]. 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 tumor[54]. 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 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 CX3CR1[30]. 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 proliferation[40]. 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 cells[16]. 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

invasion[63]. 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 cells[39]. 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 cells[66] (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 cells[73]. Recently, Barrow et al. identified platelet-derived growth factor (PDGF)-DD as a ligand for NKp44 using a secretome library screen[74]. 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 glioblastoma[75]. In line with the idea that NCRs can sense soluble mediators, Nidogen-1, an extracellular matrix protein, was recently found to bind NKp44[76]. 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 granules[81]. DNAX accessory molecule-1 (CD226/DNAM-1) also contributes to NK cell adhesion, migration and function[82]. Upon binding its ligands CD155 or CD112, both frequently expressed on cancer cells, CD226 promotes NK cell activation and cytotoxicity[83].

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-β1 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 activation[91]. 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 chemokines[94]. Soluble NKG2D ligands can inhibit NK cell function by downregulating NKG2D[95]. By contrast, Deng et al. showed that soluble forms of high-affinity NKG2D ligands led to NK cell activation[96]. 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 development[97]. 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 granulysin 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 expression[204]. 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 cancer[205]. In particular, these mechanisms appear important in NK cell–mediated control of liver metastasis[206,207], NK cell–mediated killing of immature DCs[208], and prenatally in the function of fetal NK cells[206]. 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 cells[209]. 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 vasculature[209].

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 others[99]. 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 cytocidal activity toward microbial pathogens and tumors[102]. 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 cells[103]. 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

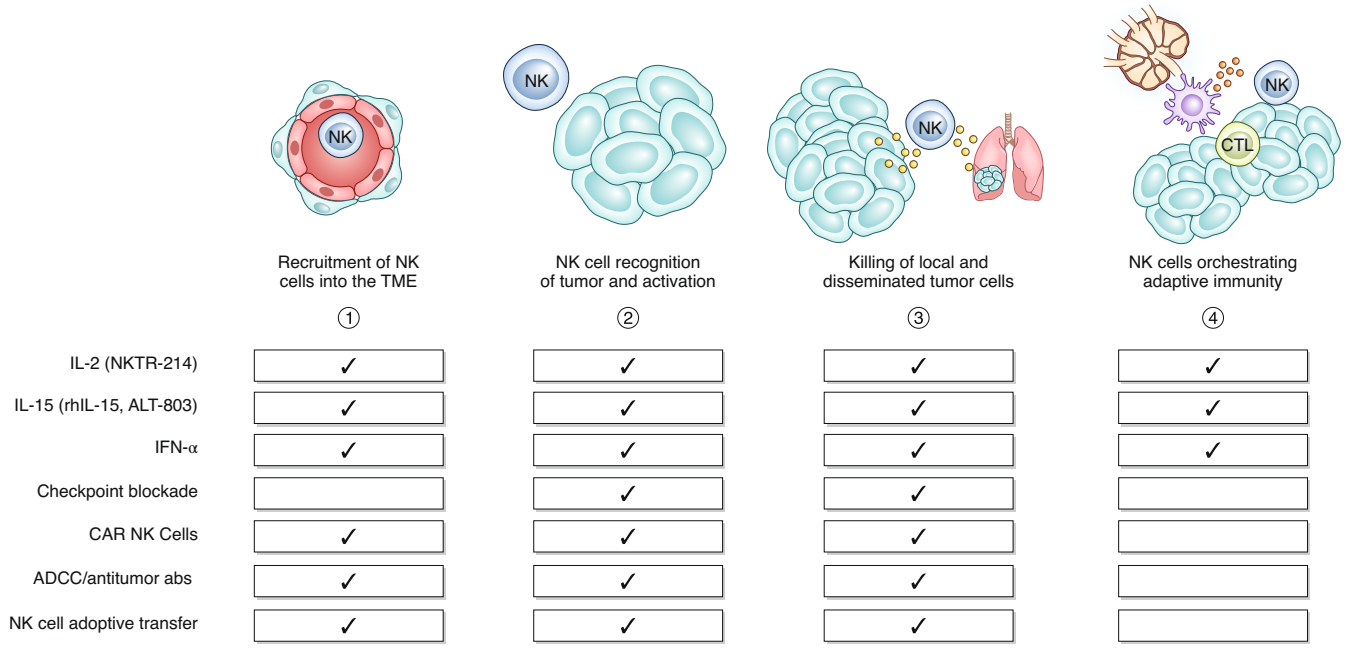

Fig. 4 | Therapies affecting individual steps of the NK cell–cancer cycle.

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 tumor[19]. Importantly, the NK cell–dependent recruitment of cDC1s to the tumor is only seen in the absence of tumor-produced prostaglandin E_2 (PGE_2; *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 PGE_2. 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 TME[18]. 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 cells[134–137], or alternatively, to reduce the expression of inhibitory receptors like NKG2A[138]. 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 surface[138]. NKG2A-modified NK cells showed superior killing of HLA-E-expressing target cells[138]. 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-disease[211]. 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 events[142]. 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 I[212], 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.

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

The inhibitory receptor complex NKG24–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.

CARs can efficiently kill autologous acute lymphoblastic leukemia (ALL) cells, which are resistant to CAR-negative NK cell–mediated killing[140]. 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 model[141]. 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 CD3ζ showed the strongest efficacy[141]. Importantly, a landmark study by Liu et al. demonstrated that adoptive transfer of allogenic anti-CD19 CAR NK cells was safe and effective in patients with high-risk $CD19^+$ chronic lymphocytic leukemia and non-Hodgkin lymphoma[142]. 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 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 allotypes[147]. 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 reviewed[150]. 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 efficacy[151]. 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 molecules[152]. These data raise concerns that unexpected biological events could compromise some antibody-based strategies designed to augment NK cell tumor killing[153]. 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 killing[75]. 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 alone[156], 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 experiments[157]. 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 outcome[158]. 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-2Rα (CD25)–IL-2Rβ (CD122)–IL-2Rγ (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 IL2Rα 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 numbers[160]. 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 alone[161].

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-2[164]. While rhIL-15 holds therapeutic promise, animal studies have found that the IL-15 superagonist, IL-15 in complex with its soluble receptor IL-15Rα, can lead to a large increase in biological activity and has enhanced activity as a cancer immunotherapeutic[165]. 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 patients[168]. 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 antitumor 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 enagers). An interesting strategy involving simultaneous blockade of the immune checkpoint ligand PD-L1 and TGF-β, using a bifunctional antibody–ligand trap, was recently reported[175]. 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 patients[166]. 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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References

1. Vivier, E. et al. Innate lymphoid cells: 10 years on. *Cell* **174**, 1054–1066 (2018).
2. Colonna, M. Innate lymphoid cells: diversity, plasticity, and unique functions in immunity. *Immunity* **48**, 1104–1117 (2018).
3. Chiossone, L., Dumas, P.-Y., Vienne, M. & Vivier, E. Natural killer cells and other innate lymphoid cells in cancer. *Nat. Rev. Immunol.* **18**, 671–688 (2018).
4. López-Soto, A., Gonzalez, S., Smyth, M. J. & Galluzzi, L. Control of metastasis by NK cells. *Cancer Cell* **32**, 135–154 (2017).
5. Gasteiger, G. & Rudensky, A. Y. Interactions between innate and adaptive lymphocytes. *Nat. Rev. Immunol.* **14**, 631–639 (2014).
6. Crinier, A. et al. High-dimensional single-cell analysis identifies organ-specific signatures and conserved NK cell subsets in humans and mice. *Immunity* **49**, 971–986.e5 (2018).
7. Collins, P. L. et al. Gene regulatory programs conferring phenotypic identities to human NK cells. *Cell* **176**, 348–360.e12 (2019).
8. Frey, M. et al. Differential expression and function of L-selectin on $CD56^{bright}$ and $CD56^{dim}$ natural killer cell subsets. *J. Immunol.* **161**, 400–408 (1998).
9. Sedlmayr, P. et al. Differential phenotypic properties of human peripheral blood $CD56^{dim+}$ and $CD56^{bright+}$ natural killer cell subpopulations. *Int. Arch. Allergy Immunol.* **110**, 308–313 (1996).
10. Hayakawa, Y., Huntington, N. D., Nutt, S. L. & Smyth, M. J. Functional subsets of mouse natural killer cells. *Immunol. Rev.* **214**, 47–55 (2006).
11. Hayakawa, Y. & Smyth, M. J. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. *J. Immunol.* **176**, 1517–1524 (2006).
12. Peng, H. & Tian, Z. Diversity of tissue-resident NK cells. *Semin. Immunol.* **31**, 3–10 (2017).
13. Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1–10 (2013).
14. Carrega, P. et al. $CD56^{bright}perforin^{low}$ noncytotoxic human NK cells are abundant in both healthy and neoplastic solid tissues and recirculate to secondary lymphoid organs via afferent lymph. *J. Immunol.* **192**, 3805–3815 (2014).
15. Wu, M., Mei, F., Liu, W. & Jiang, J. Comprehensive characterization of tumor infiltrating natural killer cells and clinical significance in hepatocellular carcinoma based on gene expression profiles. *Biomed. Pharmacother.* **121**, 109637 (2020).
16. Nath, P. R. et al. Natural killer cell recruitment and activation are regulated by CD47 expression in the tumor microenvironment. *Cancer Immunol. Res.* **7**, 1547–1561 (2019).
17. Cursons, J. et al. A gene signature predicting natural killer cell infiltration and improved survival in melanoma patients. *Cancer Immunol. Res.* **7**, 1162–1174 (2019).
18. Barry, K. C. et al. A natural killer–dendritic cell axis defines checkpoint therapy–responsive tumor microenvironments. *Nat. Med.* **24**, 1178–1191 (2018).
19. Böttcher, J. P. et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* **172**, 1022–1037.e14 (2018).
20. Ali, T. H. et al. Enrichment of $CD56^{dim}KIR^{+}CD57^{+}$ highly cytotoxic NK cells in tumour-infiltrated lymph nodes of melanoma patients. *Nat. Commun.* **5**, 5639 (2014).
21. Takanami, I., Takeuchi, K. & Giga, M. The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma. *J. Thorac. Cardiovasc. Surg.* **121**, 1058–1063 (2001).
22. Ishigami, S. et al. Prognostic value of intratumoral natural killer cells in gastric carcinoma. *Cancer* **88**, 577–583 (2000).
23. Villegas, F. R. et al. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. *Lung Cancer* **35**, 23–28 (2002).
24. Soo, R. A. et al. Prognostic significance of immune cells in non-small cell lung cancer: meta-analysis. *Oncotarget* **9**, 24801–24820 (2018).
25. Muntasell, A. et al. NK cell infiltrates and HLA class I expression in primary $HER2^{+}$ breast cancer predict and uncouple pathological response and disease-free survival. *Clin. Cancer Res.* **25**, 1535–1545 (2019).
26. Remark, R. et al. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. *Clin. Cancer Res.* **19**, 4079–4091 (2013).

27. Melsen, J. E., Lugthart, G., Lankester, A. C. & Schilham, M. W. Human circulating and tissue-resident CD56^{bright} natural killer cell populations. *Front. Immunol.* **7**, 262 (2016).
28. Bernardini, G., Antonangeli, F., Bonanni, V. & Santoni, A. Dysregulation of chemokine/chemokine receptor axes and NK cell tissue localization during diseases. *Front. Immunol.* **7**, 402 (2016).
29. Lima, M. et al. Chemokine receptor expression on normal blood CD56^{+} NK-cells elucidates cell partners that comigrate during the innate and adaptive immune responses and identifies a transitional NK-cell population. *J. Immunol. Res.* **2015**, 839684 (2015).
30. Morandi, F. et al. Soluble HLA-G dampens CD94/NKG2A expression and function and differentially modulates chemotaxis and cytokine and chemokine secretion in CD56^{bright} and CD56^{dim} NK cells. *Blood* **118**, 5840–5850 (2011).
31. Grégoire, C. et al. The trafficking of natural killer cells. *Immunol. Rev.* **220**, 169–182 (2007).
32. Parolini, S. et al. The role of chemerin in the colocalization of NK and dendritic cell subsets into inflamed tissues. *Blood* **109**, 3625–3632 (2007).
33. Campbell, J. J. et al. Unique subpopulations of CD56^{+} NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J. Immunol.* **166**, 6477–6482 (2001).
34. Polentarutti, N. et al. IL-2-regulated expression of the monocyte chemotactic protein-1 receptor (CCR2) in human NK cells: characterization of a predominant 3.4-kilobase transcript containing CCR2B and CCR2A sequences. *J. Immunol.* **158**, 2689–2694 (1997).
35. Allavena, P. et al. Induction of natural killer cell migration by monocyte chemotactic protein-1, -2 and -3. *Eur. J. Immunol.* **24**, 3233–3236 (1994).
36. Ponzetta, A. et al. Multiple myeloma impairs bone marrow localization of effector natural killer cells by altering the chemokine microenvironment. *Cancer Res.* **75**, 4766–4777 (2015).
37. Marquardt, N., Wilk, E., Pokoyski, C., Schmidt, R. E. & Jacobs, R. Murine CXCR3^{+}CD27^{bright} NK cells resemble the human CD56^{bright} NK-cell population. *Eur. J. Immunol.* **40**, 1428–1439 (2010).
38. de Andrade, L. F. et al. Discovery of specialized NK cell populations infiltrating human melanoma metastases. *JCI Insight* **4**, e133103 (2019).
39. Carrega, P. et al. Natural killer cells infiltrating human nonsmall-cell lung cancer are enriched in CD56^{bright}CD16^{-} cells and display an impaired capability to kill tumor cells. *Cancer* **112**, 863–875 (2008).
40. Przewoznik, M. et al. Recruitment of natural killer cells in advanced stages of endogenously arising B-cell lymphoma: implications for therapeutic cell transfer. *J. Immunother.* **35**, 217–222 (2012).
41. Wendel, M., Galani, I. E., Suri-Payer, E. & Cerwenka, A. Natural killer cell accumulation in tumors is dependent on IFN-γ and CXCR3 ligands. *Cancer Res.* **68**, 8437–8445 (2008).
42. Voshtani, R. et al. Progranulin promotes melanoma progression by inhibiting natural killer cell recruitment to the tumor microenvironment. *Cancer Lett.* **465**, 24–35 (2019).
43. Qi, L. et al. Interleukin-33 activates and recruits natural killer cells to inhibit pulmonary metastatic cancer development. *Int. J. Cancer* **146**, 1421–1434 (2020).
44. Gao, X. et al. Tumoral expression of IL-33 inhibits tumor growth and modifies the tumor microenvironment through CD8^{+} T and NK cells. *J. Immunol.* **194**, 438–445 (2015).
45. O'Sullivan, T. et al. Interleukin-17D mediates tumor rejection through recruitment of natural killer cells. *Cell Rep.* **7**, 989–998 (2014).
46. Gao, J.-Q. et al. Antitumor effect by interleukin-11 receptor α-locus chemokine/CCL27, introduced into tumor cells through a recombinant adenovirus vector. *Cancer Res.* **63**, 4420–4425 (2003).
47. Gao, J.-Q. et al. NK cells are migrated and indispensable in the antitumor activity induced by CCL27 gene therapy. *Cancer Immunol. Immunother.* **58**, 291–299 (2009).
48. Degos, C. et al. Endometrial tumor microenvironment alters human NK cell recruitment, and resident NK cell phenotype and function. *Front. Immunol.* **10**, 877 (2019).
49. Lin, H.-Y. et al. CCR10 activation stimulates the invasion and migration of breast cancer cells through the ERK1/2/MMP-7 signaling pathway. *Int. Immunopharmacol.* **51**, 124–130 (2017).
50. Simonetti, O. et al. Potential role of CCL27 and CCR10 expression in melanoma progression and immune escape. *Eur. J. Cancer* **42**, 1181–1187 (2006).
51. Park, M. H., Lee, J. S. & Yoon, J. H. High expression of CX3CL1 by tumor cells correlates with a good prognosis and increased tumor-infiltrating CD8^{+} T cells, natural killer cells, and dendritic cells in breast carcinoma. *J. Surg. Oncol.* **106**, 386–392 (2012).
52. Hyakudomi, M. et al. Increased expression of Fractalkine is correlated with a better prognosis and an increased number of both CD8^{+} T cells and natural killer cells in gastric adenocarcinoma. *Ann. Surg. Oncol.* **15**, 1775–1782 (2008).
53. Ohta, M. et al. The high expression of Fractalkine results in a better prognosis for colorectal cancer patients. *Int. J. Oncol.* **26**, 41–47 (2005).
54. Chen, E.-B. et al. The miR-561-5p/CX_3CL1 signaling axis regulates pulmonary metastasis in hepatocellular carcinoma involving CX_3CR1^{+} natural killer cells infiltration. *Theranostics* **9**, 4779–4794 (2019).
55. Liu, J. et al. Increased CX3CL1 mRNA expression level is a positive prognostic factor in patients with lung adenocarcinoma. *Oncol. Lett.* **17**, 4877–4890 (2019).
56. Castriconi, R. et al. Neuroblastoma-derived TGF-β1 modulates the chemokine receptor repertoire of human resting NK cells. *J. Immunol.* **190**, 5321–5328 (2013).
57. Castriconi, R. et al. Molecular mechanisms directing migration and retention of natural killer cells in human tissues. *Front. Immunol.* **9**, 2324 (2018).
58. Regis, S. et al. TGF-β1 downregulates the expression of CX_3CR1 by inducing miR-27a-5p in primary human NK cells. *Front. Immunol.* **8**, 868 (2017).
59. Carosella, E. D., Rouas-Freiss, N., Tronik-Le Roux, D., Moreau, P. & LeMaoult, J. In *Adv. Immunol.* Vol. 127 (ed. Alt, F. W.) 33–144 (Science Direct, 2015).
60. Lindaman, A., Dowden, A. & Zavazava, N. Soluble HLA-G molecules induce apoptosis in natural killer cells. *Am. J. Reprod. Immunol.* **56**, 68–76 (2006).
61. Lesport, E. et al. Human melanoma cell secreting human leukocyte antigen-G5 inhibit natural killer cell cytotoxicity by impairing lytic granules polarization toward target cell. *Hum. Immunol.* **70**, 1000–1005 (2009).
62. Feng, M. et al. Phagocytosis checkpoints as new targets for cancer immunotherapy. *Nat. Rev. Cancer* **19**, 568–586 (2019).
63. Hagenaars, M. et al. Characteristics of tumor infiltration by adoptively transferred and endogenous natural-killer cells in a syngeneic rat model: implications for the mechanism behind antitumor responses. *Int. J. Cancer* **78**, 783–789 (1998).
64. Morvan, M. G. & Lanier, L. L. NK cells and cancer: you can teach innate cells new tricks. *Nat. Rev. Cancer* **16**, 7–19 (2016).
65. Shifrin, N., Raulet, D. H. & Ardolino, M. NK cell self tolerance, responsiveness and missing self recognition. *Semin. Immunol.* **26**, 138–144 (2014).
66. Raulet, D. H., Vance, R. E. & McMahon, C. W. Regulation of the natural killer cell receptor repertoire. *Annu. Rev. Immunol.* **19**, 291–330 (2001).
67. Vivier, E., Nunès, J. A. & Vély, F. Natural killer cell signaling pathways. *Science* **306**, 1517–1519 (2004).
68. Braud, V. M. et al. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* **391**, 795–799 (1998).
69. Vance, R. E., Kraft, J. R., Altman, J. D., Jensen, P. E. & Raulet, D. H. Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical major histocompatibility complex (MHC) class I molecule Qa-1^{b}. *J. Exp. Med.* **188**, 1841–1848 (1998).
70. Barrow, A. D., Martin, C. J. & Colonna, M. The natural cytotoxicity receptors in health and disease. *Front. Immunol.* **10**, 909 (2019).
71. Brandt, C. S. et al. The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans. *J. Exp. Med.* **206**, 1495–1503 (2009).
72. Pogge von Strandmann, E. et al. Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells. *Immunity* **27**, 965–974 (2007).
73. Welte, S., Kuttruff, S., Waldhauer, I. & Steinle, A. Mutual activation of natural killer cells and monocytes mediated by NKp80-AICL interaction. *Nat. Immunol.* **7**, 1334–1342 (2006).
74. Barrow, A. D. et al. Natural killer cells control tumor growth by sensing a growth factor. *Cell* **172**, 534–548.e19 (2018).
75. Lee, S. C., Srivastava, R. M., López-Albaitero, A., Ferrone, S. & Ferris, R. L. Natural killer (NK): dendritic cell (DC) cross talk induced by therapeutic monoclonal antibody triggers tumor antigen-specific T cell immunity. *Immunol. Res.* **50**, 248–254 (2011).
76. Gaggero, S. et al. Nidogen-1 is a novel extracellular ligand for the NKp44 activating receptor. *Oncoimmunology* **7**, e1470730 (2018).
77. Diefenbach, A. et al. Selective associations with signaling proteins determine stimulatory versus costimulatory activity of NKG2D. *Nat. Immunol.* **3**, 1142–1149 (2002).
78. Gilfillan, S., Ho, E. L., Cella, M., Yokoyama, W. M. & Colonna, M. NKG2D recruits two distinct adapters to trigger NK cell activation and costimulation. *Nat. Immunol.* **3**, 1150–1155 (2002).
79. Cerwenka, A. et al. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* **12**, 721–727 (2000).
80. Diefenbach, A., Jensen, E. R., Jamieson, A. M. & Raulet, D. H. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* **413**, 165–171 (2001).
81. Urlaub, D., Höfer, K., Müller, M.-L. & Watzl, C. LFA-1 activation in NK cells and their subsets: influence of receptors, maturation, and cytokine stimulation. *J. Immunol.* **198**, 1944–1951 (2017).
82. Martinet, L. et al. DNAM-1 expression marks an alternative program of NK cell maturation. *Cell Rep.* **11**, 85–97 (2015).

83. Chan, C. J. et al. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. *Nat. Immunol.* **15**, 431–438 (2014).
84. Baginska, J. et al. The critical role of the tumor microenvironment in shaping natural killer cell-mediated antitumor immunity. *Front. Immunol.* **4**, 490 (2013).
85. Stiff, A. et al. Nitric oxide production by myeloid-derived suppressor cells plays a role in impairing Fc receptor–mediated natural killer cell function. *Clin. Cancer Res.* **24**, 1891–1904 (2018).
86. Viel, S. et al. TGF-β inhibits the activation and functions of NK cells by repressing the mTOR pathway. *Sci. Signal* **9**, ra19 (2016).
87. Gao, Y. et al. Tumor immunoevasion by the conversion of effector NK cells into type 1 innate lymphoid cells. *Nat. Immunol.* **18**, 1004–1015 (2017).
88. Krzywinska, E. et al. Loss of HIF-1α in natural killer cells inhibits tumour growth by stimulating non-productive angiogenesis. *Nat. Commun.* **8**, 1597 (2017).
89. Michelet, X. et al. Metabolic reprogramming of natural killer cells in obesity limits antitumor responses. *Nat. Immunol.* **19**, 1330–1340 (2018).
90. O'Brien, K. L. & Finlay, D. K. Immunometabolism and natural killer cell responses. *Nat. Rev. Immunol.* **19**, 282–290 (2019).
91. Skak, K., Frederiksen, K. S. & Lundsgaard, D. Interleukin-21 activates human natural killer cells and modulates their surface receptor expression. *Immunology* **123**, 575–583 (2008).
92. Swann, J. B. et al. Type I IFN contributes to NK cell homeostasis, activation, and antitumor function. *J. Immunol.* **178**, 7540–7549 (2007).
93. Souza-Fonseca-Guimaraes, F. et al. NK cells require IL-28R for optimal in vivo activity. *Proc. Natl Acad. Sci. USA* **112**, E2376–E2384 (2015).
94. Rajagopalan, S. HLA-G-mediated NK cell senescence promotes vascular remodeling: implications for reproduction. *Cell Mol. Immunol.* **11**, 460–466 (2014).
95. Molfetta, R. et al. c-Cbl regulates MICA- but not ULBP2-induced NKG2D down-modulation in human NK cells. *Eur. J. Immunol.* **44**, 2761–2770 (2014).
96. Deng, W. et al. A shed NKG2D ligand that promotes natural killer cell activation and tumor rejection. *Science* **348**, 136–139 (2015).
97. Imai, K., Matsuyama, S., Miyake, S., Suga, K. & Nakachi, K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet* **356**, 1795–1799 (2000).
98. Melaiu, O., Lucarini, V., Cifaldi, L. & Fruci, D. Influence of the tumor microenvironment on NK cell function in solid tumors. *Front. Immunol.* **10**, 3038 (2019).
99. Peritt, D. et al. Differentiation of human NK cells into NK1 and NK2 subsets. *J. Immunol.* **161**, 5821–5824 (1998).
100. Alspach, E., Lussier, D. M. & Schreiber, R. D. Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. *Cold Spring Harb. Perspect. Biol.* **11**, a028480 (2019).
101. Burke, J. D. & Young, H. A. IFN-γ: a cytokine at the right time, is in the right place. *Semin. Immunol.* **43**, 101280 (2019).
102. Schreiber, R. D., Hicks, L. J., Celada, A., Buchmeier, N. A. & Gray, P. W. Monoclonal antibodies to murine gamma-interferon which differentially modulate macrophage activation and antiviral activity. *J. Immunol.* **134**, 1609–1618 (1985).
103. Pan, J. et al. Interferon-γ is an autocrine mediator for dendritic cell maturation. *Immunol. Lett.* **94**, 141–151 (2004).
104. Kelly, J. M. et al. Induction of tumor-specific T cell memory by NK cell-mediated tumor rejection. *Nat. Immunol.* **3**, 83–90 (2002).
105. Cerwenka, A., Baron, J. L. & Lanier, L. L. 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. USA* **98**, 11521–11526 (2001).
106. Mocikat, R. et al. Natural killer cells activated by MHC class I^{low} targets prime dendritic cells to induce protective CD8 T cell responses. *Immunity* **19**, 561–569 (2003).
107. Morandi, B. et al. NK cells provide helper signal for $CD8^+$ T cells by inducing the expression of membrane-bound IL-15 on DCs. *Int. Immunol.* **21**, 599–606 (2009).
108. Walzer, T., Dalod, M., Robbins, S. H., Zitvogel, L. & Vivier, E. Natural-killer cells and dendritic cells: "l'union fait la force". *Blood* **106**, 2252–2258 (2005).
109. Shankaran, V. et al. IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* **410**, 1107–1111 (2001).
110. Kaplan, D. H. et al. Demonstration of an interferon γ-dependent tumor surveillance system in immunocompetent mice. *Proc. Natl Acad. Sci. USA* **95**, 7556–7561 (1998).
111. Bander, N. H. et al. MHC class I and II expression in prostate carcinoma and modulation by interferon-alpha and -gamma. *Prostate* **33**, 233–239 (1997).
112. Seliger, B. et al. IFN-γ-mediated coordinated transcriptional regulation of the human *TAP-1* and *LMP-2* genes in human renal cell carcinoma. *Clin. Cancer Res.* **3**, 573–578 (1997).
113. Hisamatsu, H. et al. Newly identified pair of proteasomal subunits regulated reciprocally by interferon γ. *J. Exp. Med.* **183**, 1807–1816 (1996).
114. Castro, F., Cardoso, A. P., Gonçalves, R. M., Serre, K. & Oliveira, M. J. Interferon-gamma at the crossroads of tumor immune surveillance or evasion. *Front. Immunol.* **9**, 847 (2018).
115. Reschner, A., Hubert, P., Delvenne, P., Boniver, J. & Jacobs, N. Innate lymphocyte and dendritic cell cross-talk: a key factor in the regulation of the immune response. *Clin. Exp. Immunol.* **152**, 219–226 (2008).
116. Wallace, M. E. & Smyth, M. J. The role of natural killer cells in tumor control—effectors and regulators of adaptive immunity. *Springe. Semin. Immunopathol.* **27**, 49–64 (2005).
117. Wculek, S. K. et al. Dendritic cells in cancer immunology and immunotherapy. *Nat. Rev. Immunol.* **20**, 7–24 (2020).
118. Carbone, E. et al. Recognition of autologous dendritic cells by human NK cells. *Eur. J. Immunol.* **29**, 4022–4029 (1999).
119. Wilson, J. L. et al. Targeting of human dendritic cells by autologous NK cells. *J. Immunol.* **163**, 6365–6370 (1999).
120. Fernandez, N. C. et al. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate antitumor immune responses in vivo. *Nat. Med.* **5**, 405–411 (1999).
121. Della Chiesa, M. et al. The natural killer cell-mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors. *Eur. J. Immunol.* **33**, 1657–1666 (2003).
122. Ferlazzo, G. et al. The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells. *Eur. J. Immunol.* **33**, 306–313 (2003).
123. Piccioli, D., Sbrana, S., Melandri, E. & Valiante, N. M. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J. Exp. Med.* **195**, 335–341 (2002).
124. Adam, C. et al. DC-NK cell cross talk as a novel $CD4^+$ T-cell-independent pathway for antitumor CTL induction. *Blood* **106**, 338–344 (2005).
125. Broz, M. L. et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell* **26**, 638–652 (2014).
126. Liu, K. & Nussenzweig, M. C. Origin and development of dendritic cells. *Immunol. Rev.* **234**, 45–54 (2010).
127. Pulendran, B. et al. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc. Natl Acad. Sci. USA* **96**, 1036–1041 (1999).
128. Jahchan, N. S. et al. Tuning the tumor myeloid microenvironment to fight cancer. *Front. Immunol.* **10**, 1611 (2019).
129. Böttcher, J. P. & Reis e Sousa, C. The role of type 1 conventional dendritic cells in cancer immunity. *Trends Cancer* **4**, 784–792 (2018).
130. Broz, M. L. & Krummel, M. F. The emerging understanding of myeloid cells as partners and targets in tumor rejection. *Cancer Immunol. Res.* **3**, 313–319 (2015).
131. Shimasaki, N., Jain, A. & Campana, D. NK cells for cancer immunotherapy. *Nat. Rev. Drug Discov.* **19**, 200–218 (2020).
132. Bachanova, V. et al. Haploidentical natural killer cells induce remissions in non-Hodgkin lymphoma patients with low levels of immune-suppressor cells. *Cancer Immunol. Immunother.* **67**, 483–494 (2018).
133. Björklund, A. T. et al. Complete remission with reduction of high-risk clones following haploidentical NK-cell therapy against MDS and AML. *Clin. Cancer Res.* **24**, 1834–1844 (2018).
134. Parihar, R. et al. NK cells expressing a chimeric activating receptor eliminate MDSCs and rescue impaired CAR-T cell activity against solid tumors. *Cancer Immunol. Res.* **7**, 363–375 (2019).
135. Chang, Y.-H. et al. A chimeric receptor with NKG2D specificity enhances natural killer cell activation and killing of tumor cells. *Cancer Res.* **73**, 1777–1786 (2013).
136. Kremer, V. et al. Genetic engineering of human NK cells to express CXCR2 improves migration to renal cell carcinoma. *J. Immunother. Cancer* **5**, 73 (2017).
137. Imamura, M. et al. Autonomous growth and increased cytotoxicity of natural killer cells expressing membrane-bound interleukin-15. *Blood* **124**, 1081–1088 (2014).
138. Kamiya, T., Seow, S. V., Wong, D., Robinson, M. & Campana, D. Blocking expression of inhibitory receptor NKG2A overcomes tumor resistance to NK cells. *J. Clin. Invest.* **129**, 2094–2106 (2019).
139. Afolabi, L. O., Adeshakin, A. O., Sani, M. M., Bi, J. & Wan, X. Genetic reprogramming for NK cell cancer immunotherapy with CRISPR/Cas9. *Immunology* **158**, 63–69 (2019).
140. Imai, C., Iwamoto, S. & Campana, D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. *Blood* **106**, 376–383 (2005).
141. Margolin, K. et al. Phase I trial of ALT-803, a novel recombinant IL15 complex, in patients with advanced solid tumors. *Clin. Cancer Res.* **24**, 5552–5561 (2018).
142. Liu, E. et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N. Engl. J. Med.* **382**, 545–553 (2020).

143. O'Donnell, J. S., Teng, M. W. L. & Smyth, M. J. Cancer immunoediting and resistance to T cell-based immunotherapy. *Nat. Rev. Clin. Oncol.* **16**, 151–167 (2019).
144. Bi, J. & Tian, Z. NK cell dysfunction and checkpoint immunotherapy. *Front. Immunol.* **10**, 1999 (2019).
145. Minetto, P. et al. Harnessing NK cells for cancer treatment. *Front. Immunol.* **10**, 2836 (2019).
146. Beldi-Ferchiou, A. & Caillat-Zucman, S. Control of NK cell activation by immune checkpoint molecules. *Int. J. Mol. Sci.* **18**, 2129 (2017).
147. Romagné, F. et al. Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer–mediated killing of tumor cells. *Blood* **114**, 2667–2677 (2009).
148. Benson, D. M. Jr. et al. A phase I trial of the anti-KIR antibody IPH2101 and lenalidomide in patients with relapsed/refractory multiple myeloma. *Clin. Cancer Res.* **21**, 4055–4061 (2015).
149. Benson, D. M. Jr. et al. A phase 1 trial of the anti-KIR antibody IPH2101 in patients with relapsed/refractory multiple myeloma. *Blood* **120**, 4324–4333 (2012).
150. Guillerey, C., Huntington, N. D. & Smyth, M. J. Targeting natural killer cells in cancer immunotherapy. *Nat. Immunol.* **17**, 1025–1036 (2016).
151. Korde, N. et al. A phase II trial of pan-KIR2D blockade with IPH2101 in smoldering multiple myeloma. *Haematologica* **99**, e81–e83 (2014).
152. Carlsten, M. et al. Checkpoint inhibition of KIR2D with the monoclonal antibody IPH2101 induces contraction and hyporesponsiveness of NK cells in patients with myeloma. *Clin. Cancer Res.* **22**, 5211–5222 (2016).
153. Felices, M. & Miller, J. S. Targeting KIR blockade in multiple myeloma: trouble in checkpoint paradise? *Clin. Cancer Res.* **22**, 5161–5163 (2016).
154. van Hall, T. et al. Monalizumab: inhibiting the novel immune checkpoint NKG2A. *J. Immunother. Cancer* **7**, 263 (2019).
155. Haanen, J. B. & Cerundolo, V. NKG2A, a new kid on the immune checkpoint block. *Cell* **175**, 1720–1722 (2018).
156. van Montfoort, N. et al. NKG2A blockade potentiates CD8 T cell immunity induced by cancer vaccines. *Cell* **175**, 1744–1755.e15 (2018).
157. André, P. et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes antitumor immunity by unleashing both T and NK cells. *Cell* **175**, 1731–1743.e13 (2018).
158. Chen, Z., Yang, Y., Liu, L. L. & Lundqvist, A. Strategies to augment natural killer (NK) cell activity against solid tumors. *Cancers (Basel)* **11**, 1040 (2019).
159. Doberstein, S. K. Bempegaldesleukin (NKTR-214): a CD-122-biased IL-2 receptor agonist for cancer immunotherapy. *Expert Opin. Biol. Ther.* **19**, 1223–1228 (2019).
160. Bentebibel, S.-E. et al. A first-in-human study and biomarker analysis of NKTR-214, a novel IL2Rβγ-biased cytokine, in patients with advanced or metastatic solid tumors. *Cancer Discov.* **9**, 711–721 (2019).
161. Donskov, F., Jensen, N. V., Smidt-Hansen, T., Brøndum, L. & Geertsen, P. A randomized phase II trial of interleukin-2 and interferon-α plus bevacizumab versus interleukin-2 and interferon-α in metastatic renal-cell carcinoma (mRCC): results from the Danish Renal Cancer Group (DaRenCa) study-1. *Acta Oncol.* **57**, 589–594 (2018).
162. Conlon, K. C. et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. *J. Clin. Oncol.* **33**, 74–82 (2015).
163. Miller, J. S. 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.* **24**, 1525–1535 (2018).
164. Cooley, S. et al. First-in-human trial of rhIL-15 and haploidentical natural killer cell therapy for advanced acute myeloid leukemia. *Blood Adv.* **3**, 1970–1980 (2019).
165. Guo, Y., Luan, L., Patil, N. K. & Sherwood, E. R. Immunobiology of the IL-15/IL-15Rα complex as an antitumor and antiviral agent. *Cytokine Growth Factor Rev.* **38**, 10–21 (2017).
166. Romee, R. et al. First-in-human phase 1 clinical study of the IL-15 superagonist complex ALT-803 to treat relapse after transplantation. *Blood* **131**, 2515–2527 (2018).
167. Wrangle, J. M. 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.* **19**, 694–704 (2018).
168. Smits, E. L., Anguille, S. & Berneman, Z. N. Interferon α may be back on track to treat acute myeloid leukemia. *Oncoimmunology* **2**, e23619 (2013).
169. Mo, X.-D. et al. IFN-α is effective for treatment of minimal residual disease in patients with acute leukemia after allogeneic hematopoietic stem cell transplantation: results of a registry study. *Biol. Blood Marrow Transplant.* **23**, 1303–1310 (2017).
170. Mo, X. et al. Minimal residual disease-directed immunotherapy for high-risk myelodysplastic syndrome after allogeneic hematopoietic stem cell transplantation. *Front. Med.* **13**, 354–364 (2019).
171. Felices, M., Lenvik, T. R., Davis, Z. B., Miller, J. S. & Vallera, D. A. In *Natural Killer Cells Methods and Protocols* Vol. 1441 (ed. Somanchi, S. S.) 333–346 (Humana Press, 2016).
172. Runcie, K., Budman, D. R., John, V. & Seetharamu, N. Bi-specific and tri-specific antibodies- the next big thing in solid tumor therapeutics. *Mol. Med.* **24**, 50 (2018).
173. Labrijn, A. F., Janmaat, M. L., Reichert, J. M. & Parren, P. W. H. I. Bispecific antibodies: a mechanistic review of the pipeline. *Nat. Rev. Drug Discov.* **18**, 585–608 (2019).
174. Ferrari de Andrade, L. et al. Antibody-mediated inhibition of MICA and MICB shedding promotes NK cell–driven tumor immunity. *Science* **359**, 1537–1542 (2018).
175. Ravi, R. et al. Bifunctional immune checkpoint-targeted antibody-ligand traps that simultaneously disable TGFβ enhance the efficacy of cancer immunotherapy. *Nat. Commun.* **9**, 741 (2018).
176. Nayyar, G., Chu, Y. & Cairo, M. S. Overcoming resistance to natural killer cell based immunotherapies for solid tumors. *Front. Oncol.* **9**, 51 (2019).
177. Schreiber, R. D., Old, L. J. & Smyth, M. J. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* **331**, 1565–1570 (2011).
178. Vesely, M. D., Kershaw, M. H., Schreiber, R. D. & Smyth, M. J. Natural innate and adaptive immunity to cancer. *Annu. Rev. Immunol.* **29**, 235–271 (2011).
179. Rosenberg, J. & Huang, J. CD8$^+$ T cells and NK cells: parallel and complementary soldiers of immunotherapy. *Curr. Opin. Chem. Eng.* **19**, 9–20 (2018).
180. Ingegnere, T. et al. Human CAR NK cells: a new non-viral method allowing high efficient transfection and strong tumor cell killing. *Front. Immunol.* **10**, 957 (2019).
181. Deguine, J., Breart, B., Lemaître, F., Di Santo, J. P. & Bousso, P. Intravital imaging reveals distinct dynamics for natural killer and CD8$^+$ T cells during tumor regression. *Immunity* **33**, 632–644 (2010).
182. Halle, S., Halle, O. & Förster, R. Mechanisms and dynamics of T cell-mediated cytotoxicity in vivo. *Trends Immunol.* **38**, 432–443 (2017).
183. Chiang, S. C. C. et al. Comparison of primary human cytotoxic T-cell and natural killer cell responses reveal similar molecular requirements for lytic granule exocytosis but differences in cytokine production. *Blood* **121**, 1345–1356 (2013).
184. Vanherberghen, B. et al. Classification of human natural killer cells based on migration behavior and cytotoxic response. *Blood* **121**, 1326–1334 (2013).
185. Choi, P. J. & Mitchison, T. J. Imaging burst kinetics and spatial coordination during serial killing by single natural killer cells. *Proc. Natl Acad. Sci. USA* **110**, 6488–6493 (2013).
186. Vasconcelos, Z. et al. Individual human cytotoxic T lymphocytes exhibit intraclonal heterogeneity during sustained killing. *Cell Rep.* **11**, 1474–1485 (2015).
187. Sun, J. C. & Lanier, L. L. NK cell development, homeostasis and function: parallels with CD8$^+$ T cells. *Nat. Rev. Immunol.* **11**, 645–657 (2011).
188. Sun, J. C., Beilke, J. N. & Lanier, L. L. Adaptive immune features of natural killer cells. *Nature* **457**, 557–561 (2009).
189. Dokun, A. O. et al. Specific and nonspecific NK cell activation during virus infection. *Nat. Immunol.* **2**, 951–956 (2001).
190. Bonifant, C. L., Jackson, H. J., Brentjens, R. J. & Curran, K. J. Toxicity and management in CAR T-cell therapy. *Mol. Ther. Oncolytics* **3**, 16011 (2016).
191. Yu, S., Yi, M., Qin, S. & Wu, K. Next generation chimeric antigen receptor T cells: safety strategies to overcome toxicity. *Mol. Cancer* **18**, 125 (2019).
192. Shimabukuro-Vornhagen, A. et al. Cytokine release syndrome. *J. Immunother. Cancer* **6**, 56 (2018).
193. Santomasso, B. D. et al. Clinical and biological correlates of neurotoxicity associated with CAR T-cell therapy in patients with B-cell acute lymphoblastic leukemia. *Cancer Discov.* **8**, 958–971 (2018).
194. Rezvani, K., Rouce, R., Liu, E. & Shpall, E. Engineering natural killer cells for cancer immunotherapy. *Mol. Ther.* **25**, 1769–1781 (2017).
195. Lupo, K. B. & Matosevic, S. Natural killer cells as allogeneic effectors in adoptive cancer immunotherapy. *Cancers (Basel)* **11**, 769 (2019).
196. Miller, J. S. et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* **105**, 3051–3057 (2005).
197. Rubnitz, J. E. 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.* **28**, 955–959 (2010).
198. Barkholt, L. et al. Safety analysis of ex vivo-expanded NK and NK-like T cells administered to cancer patients: a phase I clinical study. *Immunotherapy* **1**, 753–764 (2009).
199. Vacca, P. et al. Exploiting human NK cells in tumor therapy. *Front. Immunol.* **10**, 3013 (2019).
200. Woan, K. V. & Miller, J. S. Harnessing natural killer cell antitumor immunity: from the bench to bedside. *Cancer Immunol. Res.* **7**, 1742–1747 (2019).
201. Sparrow, E. & Bodman-Smith, M. D. Granulysin: the attractive side of a natural born killer. *Immunol. Lett.* **217**, 126–132 (2020).

202. Kumar, S. Natural killer cell cytotoxicity and its regulation by inhibitory receptors. *Immunology* **154**, 383–393 (2018).
203. Orange, J. S. Formation and function of the lytic NK-cell immunological synapse. *Nat. Rev. Immunol.* **8**, 713–725 (2008).
204. Bengsch, B. et al. Deep immune profiling by mass cytometry links human T and NK cell differentiation and cytotoxic molecule expression patterns. *J. Immunol. Methods* **453**, 3–10 (2018).
205. Smyth, M. J., Street, S. E. A. & Trapani, J. A. Cutting edge: granzymes A and B are not essential for perforin-mediated tumor rejection. *J. Immunol.* **171**, 515–518 (2003).
206. Takeda, K. et al. TRAIL identifies immature natural killer cells in newborn mice and adult mouse liver. *Blood* **105**, 2082–2089 (2005).
207. Smyth, M. J. et al. Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) contributes to interferon γ–dependent natural killer cell protection from tumor metastasis. *J. Exp. Med.* **193**, 661–670 (2001).
208. Hayakawa, Y. et al. NK cell TRAIL eliminates immature dendritic cells in vivo and limits dendritic cell vaccination efficacy. *J. Immunol.* **172**, 123–129 (2004).
209. Josephs, S. F. et al. Unleashing endogenous TNF-alpha as a cancer immunotherapeutic. *J. Transl. Med.* **16**, 242 (2018).
210. Balkwill, F. Tumour necrosis factor and cancer. *Nat. Rev. Cancer* **9**, 361–371 (2009).
211. Rafiq, S., Hackett, C. S. & Brentjens, R. J. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat. Rev. Clin. Oncol.* **17**, 147–167 (2020).
212. Long, E. O., Kim, H. S., Liu, D., Peterson, M. E. & Rajagopalan, S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Annu. Rev. Immunol.* **31**, 227–258 (2013).
213. Li, F. et al. Blocking the natural killer cell inhibitory receptor NKG2A increases activity of human natural killer cells and clears hepatitis B virus infection in mice. *Gastroenterology* **144**, 392–401 (2013).
214. Sun, C. et al. High NKG2A expression contributes to NK cell exhaustion and predicts a poor prognosis of patients with liver cancer. *Oncoimmunology* **6**, e1264562 (2017).

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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 Squib, 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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