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Roles of natural killer cells in immunity to cancer, and applications to immunotherapy

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Abstract | Great strides have been made in recent years towards understanding the roles of natural killer (NK) cells in immunity to tumours and viruses. NK cells are cytotoxic innate lymphoid cells that produce inflammatory cytokines and chemokines. By lysing transformed or infected cells, they limit tumour growth and viral infections. Whereas T cells recognize peptides presented by MHC molecules, NK cells display receptors that recognize stress-induced autologous proteins on cancer cells. At the same time, their functional activity is inhibited by MHC molecules displayed on such cells. The enormous potential of NK cells for immunotherapy for cancer is illustrated by their broad recognition of stressed cells regardless of neoantigen presentation, and enhanced activity against tumours that have lost expression of MHC class I owing to acquired resistance mechanisms. As a result, many efforts are under way to mobilize endogenous NK cells with therapeutics, or to provide populations of ex vivo-expanded NK cells as a cellular therapy, in some cases by equipping the NK cells with chimeric antigen receptors. Here we consider the key features that underlie why NK cells are emerging as important new additions to the cancer therapeutic arsenal.

Natural killer (NK) cells are cytotoxic innate-like lymphocytes that are hardwired to recognize stressed cells, such as tumour cells and certain infected cells, and to mediate the spontaneous killing of tumour cells. A key feature of NK cell recognition is that these cells are inhibited by receptors that bind MHC class I (MHC I) molecules, and hence NK cells preferentially kill MHC I-deficient tumour cells. Loss of MHC I expression has emerged as an important mechanism by which tumour cells acquire resistance to antitumour $CD8^+$ T cell responses, especially in the context of checkpoint blockade immunotherapy. This suggests that the therapeutic use of NK cells may complement therapies that activate antitumour T cells. Moreover, MHC I-expressing tumour cells are often also killed by NK cells, probably because high expression of NK cell-activating ligands by tumour cells can override the inhibition mediated by MHC I recognition.

NK cell targeting of tumour cells can be augmented in numerous ways. For example, NK cells can be activated by cytokines or stimulants of innate immunity, NK cell populations can be activated and expanded ex vivo and equipped with chimeric antigen receptors, or biologic therapeutics can be used that bridge NK receptors to proteins or other ligands expressed on tumour cell membranes. In this Review, we first discuss the roles of NK cells in the endogenous host immune response to tumour cells. We then examine the recent strides made in the therapeutic application of NK cells in preclinical models and in the clinic, focusing on therapeutic strategies such as NK cell activators, checkpoint blockade, cellular therapies, and bispecific and trispecific NK cell activating antibodies.

NK cell biology and regulation

Development and homeostasis of NK cells. NK cells were initially discovered as lymphocytes that can kill tumour cells in the absence of purposeful immunization[1]. They are now recognized as a subset of group 1 innate lymphoid cells (ILCs), a category that also includes ILC1s[2]. Their cytotoxicity and requirement for expression of the transcription factor eomesodermin (EOMES) distinguishes them from ILC1s, although some ILC1s express EOMES[2]. NK cells and other ILCs are derived from lymphoid precursor populations that also give rise to T cells and B cells. The NK cell fate is determined in part by early expression of ID2, while late expression of T-bet and EOMES enforces late-stage maturation and cytotoxic function[3,4]. Sufficient EOMES expression for the development of mature conventional NK cells depends on the transcription factor NFIL3 (REF.[5]). By contrast, ILC1s, unlike NK cells, require the transcription factor PLZF for development[6].

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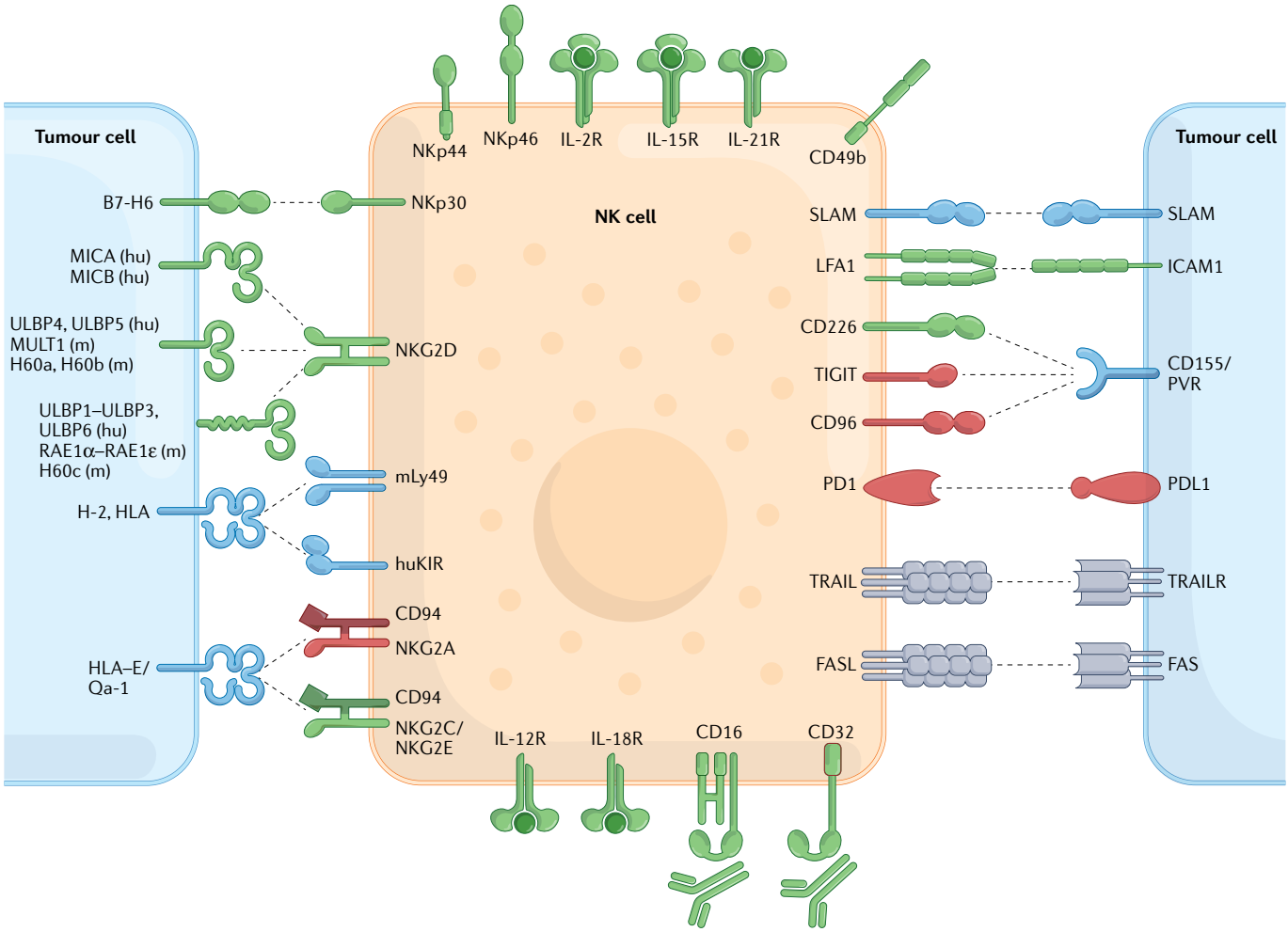


Fig. 1 | **NK cell function is regulated by cell surface receptors and cytokines.** Receptor–ligand interactions that activate (depicted in green) or inhibit (depicted in red) natural killer (NK) cell responses against tumour cells and other cells or that can either activate or inhibit NK cells depending on the specific family member or the context (blue receptors) are shown. Some ligands bind to both inhibitory and activating receptors on NK cells, as indicated. SLAM receptors can impart activating or inhibitory signals depending on expression of adapter proteins by NK cells. Key cytokine receptors are also depicted. The receptors for different IL-2 family cytokines consist of multiple chains, of which one (the common γ-chain) is a shared signalling component. Note that ULBP1–ULBP3, ULBP6, RAE1α–RAE1ε and H60c are all glycosylphosphatidylinositol-linked proteins, whereas the other NKG2D ligands are transmembrane proteins. 'Hu' indicates human only and 'm' indicates mouse only. KIR, killer immunoglobulin-like receptor; TIGIT, T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains.

Once produced, NK cells were originally reported to turn over with a half-life of a single day under homeostatic conditions[7], which raised some concerns regarding their prospects for providing sustained therapeutic effects. Subsequent studies demonstrated a much longer half-life of about 2 weeks — similar to that of effector memory T cells[8–10] — which can be extended greatly by the action of cytokines[11–13].

Target cell recognition by NK cells. NK cells recognize their target cells via a system of germline-encoded activating and inhibitory receptors that recognize membrane-bound ligands on potential target cells[14,15] (FIG. 1). Inhibition and activation are integrated, or balanced against each other, such that inhibition may be overcome by strong activation.

Among the activating receptors expressed by NK cells are the high-affinity Fc receptors CD16 and CD32, which efficiently trigger cytotoxicity and cytokine release and enable NK cells to kill antibody-coated cells via antibody-dependent cellular cytotoxicity (ADCC)[16]. ADCC is a key activity of NK cells that is relevant for antibody-mediated cancer therapeutics.

Other activating receptors expressed by NK cells belong to various broad families, including C-type lectins (such as NKG2D and NK1.1), natural cytotoxicity receptors (such as NKp46 in mice and humans, and NKp44 and NKp30 in humans) and a few of the killer immunoglobulin-like receptors (KIRs) and Ly49 receptors (although most of those receptors are inhibitory). Activating NK receptors generally signal through associated signalling adapter proteins: for instance, NKG2D uses the adaptor DAP10 in humans and both DAP10 and DAP12 in mice; stimulatory KIRs signal with DAP12; natural cytotoxicity receptors signal with CD3ζ or FcRγ; CD16 signals with CD3ζ or FcRγ; Ly49D and Ly49H signal with DAP12 (REF.[17]). Most of the adapters contain immunoreceptor tyrosine-based activation

motifs in their cytoplasmic domain, whereas DAP10 contains a distinct motif that recruits PI3K.

With respect to tumour cell recognition, most of the relevant activating NK cell receptors recognize host gene-encoded ligands that are induced by cell stress pathways. Thus, NK cells are dedicated, in part, to eliminating stressed cells, such as cancer cells. Recognition of this type has been called 'induced-self recognition', and is best studied in the case of the receptor NKG2D. NKG2D recognizes numerous ligands, all of which are distant relatives of MHC I proteins. The human NKG2D ligands include MICA, MICB and ULBP1–ULBP6. In mice the ligands include the RAE1 family members (RAE1α–RAE1ε), MULT1 and three H60 isoforms[18]. NKG2D ligands are upregulated in response to heat shock-induced stress[19,20], the DNA damage response pathway[21], the integrated stress response[22,23], cellular hyperproliferation[24] and activated p53 (REF.[25]). With respect to the other NK cell activating receptors, NKp30 binds to B7-H6, which is also induced by the integrated stress response[26]. Definition of ligands for the other activating natural cytotoxicity receptors on tumour cells has remained mostly elusive, although various immune molecules and glycans that bind these receptors have been reported[17,27–29].

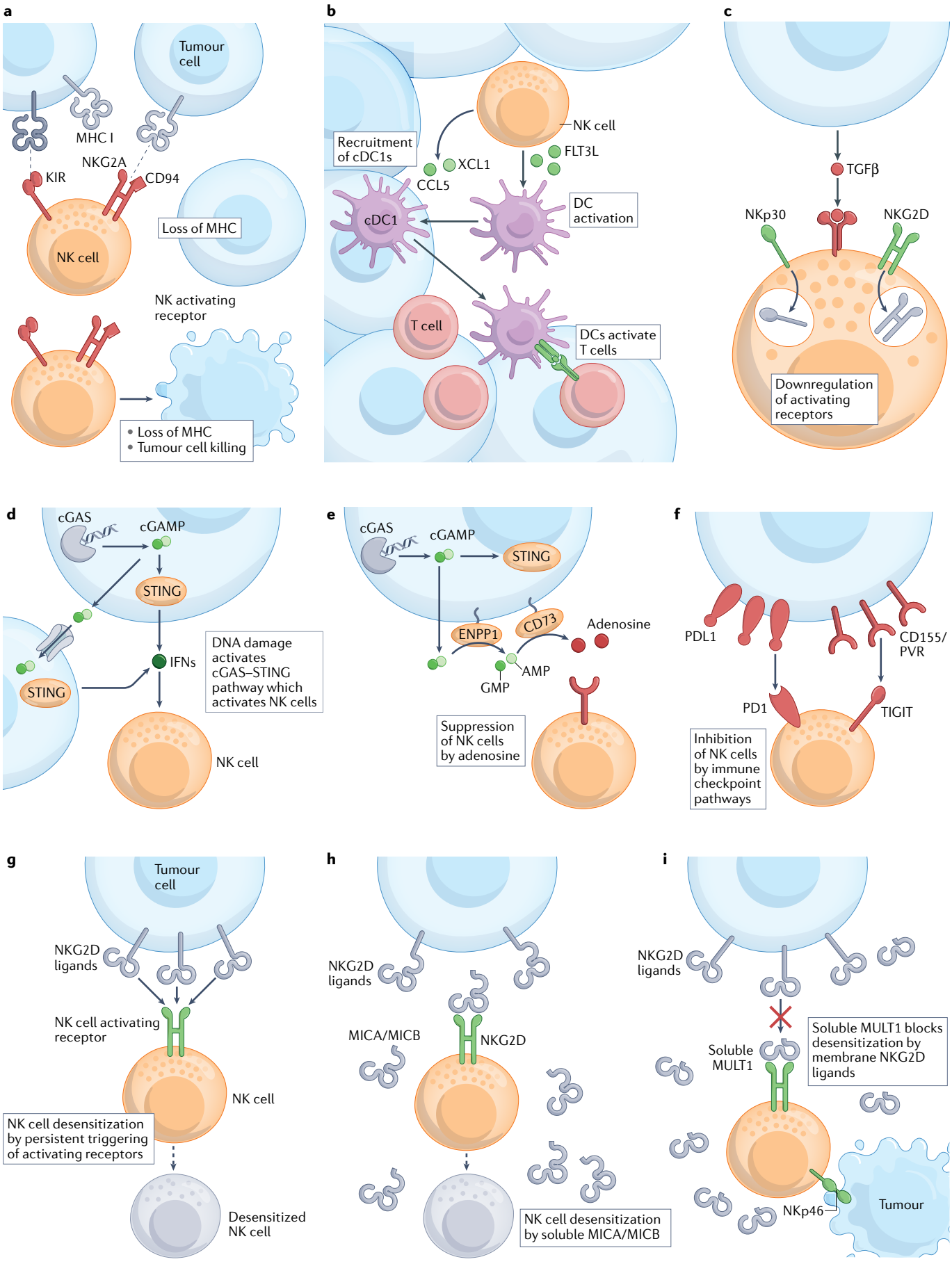
Most inhibitory receptors expressed by NK cells recognize MHC I molecules on target cells (FIG. 1). Hence, target cells that express high levels of MHC I are better protected from NK cell killing, and MHC I loss results in increased NK cell-mediated killing (FIG. 2a). NK cell-mediated killing resulting from the loss of MHC I by a target cell is called 'missing-self recognition'[30,31]. Owing to this property, NK cells are especially promising candidates for mediating therapy for MHC I-deficient tumours, which are resistant to recognition by $CD8^+$ T cells. Inhibitory receptors specific for MHC I signal through immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domains. In humans, the KIR family encodes approximately seven (depending on the genotype) inhibitory receptors, which discriminate different groups of human leukocyte antigen (HLA) class I molecules[32]. Mice lack KIR genes, but instead harbour a family of lectin-like Ly49 receptors, approximately seven of which are inhibitory and discriminate different H-2 class I molecules[33,34]. In addition to these receptors, both humans and mice express a CD94–NKG2A heterodimeric receptor, which recognizes peptides derived from classical MHC I molecules, presented by the non-classical HLA-E molecule (in humans) or Qa-1 molecule (in mice)[14,35]. All of these receptors dampen or prevent killing of cells, including tumour cells, that express high levels of MHC I molecules. Furthermore, the different inhibitory receptors discriminate MHC I allelic products, and are distributed to different subsets of NK cells in an overlapping fashion. A consequence of this is that some NK cells in an individual may be uninhibited if they engage a tumour cell that selectively loses even one of its normal complement of MHC I molecules[36,37], resulting in tumour cell killing. Greater killing is observed in the case of tumour cells that lose most or all MHC I expression, for example because of loss of expression of the MHC I light chain β_2-microglobulin[31]. Although MHC I-deficient cells are most efficiently killed by NK cells, some tumour cells with normal expression of MHC I are sensitive to NK cell killing, presumably because they express sufficiently high levels of activating ligands to overcome inhibitory signalling[38,39]. Therefore, the potential of NK cells in cancer therapy is not restricted to tumours with low MHC I expression.

NK cell cytotoxicity and activation. NK cell cytotoxicity involves the secretion of cytotoxic granules containing the pore-forming protein perforin and procaspase-cleaving granzyme B, which triggers apoptosis in target cells[40]. NK cells may also induce target cell apoptosis via the death receptors TRAIL and FASL[41].

Although apoptosis has been considered the canonical mechanism of NK cell-mediated killing, recent work demonstrates that NK cells can also induce pyroptotic death in target cells, by multiple mechanisms. Once granzyme B from NK cells (or T cells) is delivered into target cells via cytotoxic granules, it can cleave not only caspase 3 to induce apoptosis but also gasdermin E, if the cells express it. Cleaved gasdermin E can insert itself into membranes, resulting in pyroptosis[42]. Caspase 3 can also cleave and activate gasdermin E. In addition to granzyme B, cytotoxic granules in both NK cells and T cells contain granzyme A, which can induce pyroptotic death in cells expressing gasdermin B[43]. Gasdermin B is highly expressed in the epithelia of the digestive tract and tumours derived therefrom. If the activation of pyroptosis results in the release of inflammatory cytokines or other pro-inflammatory mediators, it can drive a feedforward process that amplifies both natural and therapy-induced antitumour immune responses[44].

NK cells also exert antitumour effects through secretion of chemokines and cytokines, which can directly and indirectly recruit other immune cells and induce cytotoxic and cytostatic effects on target cells, in addition to further stimulating broader cellular immune responses. Interferon-γ (IFNγ) and tumour necrosis factor (TNF) produced by stimulated NK cells can mediate tumour cell death[45]. In addition, IFNγ stimulates upregulation of MHC II molecules on antigen-presenting cells, macrophage activation and augmented priming of T cell responses[46]. NK cells also produce chemokines that recruit and activate key antigen-presenting cells at the tissue site of insult[47,48], thereby amplifying T cell immunity. These findings provide further impetus for cancer therapies focused on amplifying NK cell responses in tumours.

The degree to which NK cells are active against tumour cells is highly dependent on the activation state of the NK cells, which is regulated in large part by cytokines derived from other innate immune cells, including dendritic cells (DCs) and monocytes[49,50]. Several cytokines serve overlapping and distinct roles in NK cell activation, including cytokines that signal through the common γ-chain (chiefly IL-2 and IL-15), IL-12 and IL-18, and type I interferons[51] (FIG. 1). In addition to supporting NK cell proliferation and survival, IL-15 and IL-2 promote stronger cytotoxic activity by NK cells[50] (including ADCC[52]), induce human NK cell expression of the activating receptors NKp44 and NKp30 (REF.[53]), and augment NK cell expression of the activating


a
Tumour cell
MHC I
NKG2A
KIR
CD94
NK cell
Loss of MHC
NK activating receptor
• Loss of MHC
• Tumour cell killing
b
Recruitment of cDC1s
XCL1
CCL5
NK cell
FLT3L
DC activation
cDC1
T cell
DCs activate T cells
c
TGFβ
NKp30
NKG2D
Downregulation of activating receptors
d
cGAS
cGAMP
STING
IFNs
STING
DNA damage activates cGAS–STING pathway which activates NK cells
NK cell
e
cGAS
cGAMP
STING
ENPP1
CD73
Adenosine
AMP
GMP
Suppression of NK cells by adenosine
f
PDL1
CD155/ PVR
PD1
TIGIT
Inhibition of NK cells by immune checkpoint pathways
g
Tumour cell
NKG2D ligands
NK cell activating receptor
NK cell
NK cell desensitization by persistent triggering of activating receptors
Desensitized NK cell
h
NKG2D ligands
MICA/MICB
NKG2D
NK cell desensitization by soluble MICA/MICB
i
NKG2D ligands
Soluble MULT1
Soluble MULT1 blocks desensitization by membrane NKG2D ligands
NKp46
Tumour

◀ Fig. 2 | **Influences of the tumour microenvironment on NK cell activation, inhibition and inactivation. a** | Natural killer (NK) cell inhibition by the inhibitory receptors (killer immunoglobulin-like receptor (KIR) and NKG2A) can prevent or depress killing of cells that express surface MHC class I (MHC I) molecules. Tumour cells that lose MHC I expression are susceptible to NK cell killing. **b** | NK cells secrete chemokines that recruit type 1 conventional dendritic cells (cDC1s) and FLT3L, which activates these DCs, which in turn induce antitumour cytotoxic T cells. **c** | The tumour microenvironment can suppress NK cell activity via TGFβ, produced by tumour cells or other cells, which leads to downregulation of activating receptors by NK cells. **d** | DNA damage in tumour cells leads to production of cyclic GMP–AMP (cGAMP) by cGAS, which can activate STING in the same cell or can be transported to neighbouring cells, where it can activate STING. This leads to the production of type 1 interferons (IFNs) and other cytokines that activate NK cells and DCs. **e** | Tumours can in turn evade immune activation by extracellular cGAMP through the action of ENPP1 and CD73, which convert cGAMP into immunosuppressive adenosine. **f** | Tumours can evade NK cell responses by direct inhibition through checkpoint receptor engagement, including the PD1–PDL1 and TIGIT–PVR axes. **g** | Desensitization of NK cells can occur through persistent stimulation of activating receptors by ligands on the surface of tumour cells, in the absence of counterbalancing inhibitory receptor engagement (for example, on MHC I-deficient cells), inducing a hyporesponsive state. **h** | It has been reported that shed NKG2D ligands MICA and MICB directly engage NKG2D on NK cells, leading to a state of systemic NK cell paralysis. The main text points out that the affinities of MICA and MICB for NKG2D are so low that such interactions seem unlikely to have the reported effects unless MICA/MICB is in a multivalent state **i** | The shedding of the high-affinity NKG2D ligand MULT1 can lead to enhanced NK activity, by blocking most NKG2D molecules on NK cells, thus preventing NKG2D engagement by membrane-bound ligands (as in part **g**) and preventing NK cell desensitization. The active NK cell kills tumour cells using other activating receptors. CCL5, CC-chemokine ligand 5; TIGIT, T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains; XCL1, XC-chemokine ligand 1.

receptor NKG2D[50]. IL-12 and IL-18 synergistically stimulate the production of IFNγ by NK cells[54,55]. Type I interferons also play a critical role in activating NK cells, acting directly on NK cells to induce effector molecules such as granzyme B[56,57] and protecting the cells from fratricide by other NK cells[58]. Type I interferons also act indirectly by inducing production of IL-15 by DCs and other cell types[59], which then acts on NK cells[57].

Adaptive features of NK cells

Although NK cells are considered part of the innate immune system, some NK cell responses exhibit features traditionally ascribed to adaptive immunity, specifically a degree of clonal specificity and memory. In contrast to T cells and B cells, however, the clonal specificity of NK cells is relatively limited, and NK cell memory may not be as long-lasting as T cell and B cell memory.

Clonal specificity. Clonal specificity of NK cells arises because subsets of NK cells express different receptors, conferring a variegated pattern of expression in which each receptor is expressed by a more or less random subset of the cells. All of the inhibitory receptors and a few of the activating receptors exhibit this type of variegated expression pattern[35]. That is, the KIRs (or in mice Ly49 receptors) and NKG2A receptors are expressed by overlapping subsets of NK cells such that individual NK cells express between zero and four different inhibitory receptors, chosen in a largely random fashion from the available set of genes. Mass cytometry analysis has revealed surprisingly high clonal diversity in NK cells, with up to 30,000 unique NK cell phenotypes detected in individual humans[60], as well as significant phenotypic diversity[61]. Single-cell RNA sequencing analysis of NK cells in different tissues reveals additional clonal and phenotypic diversity[62,63]. Individual KIRs and Ly49 receptors discriminate in binding different MHC I allelic products[32,64], so depending on the receptors expressed, a given NK cell may or may not display an inhibitory receptor that binds any given MHC I allelic product expressed by the host. Most NK cells express receptors that bind to at least one self MHC protein[60,65,66]. Consequently, those NK cells are inhibited when they encounter a host cell expressing MHC I, generally protecting those cells from NK cell attack. Those same NK cells will preferentially kill cells lacking the corresponding MHC I molecules. The mosaic repertoire of NK cells means that some NK cells are inhibited by only one of the host's MHC I molecules, and are thus empowered to recognize and destroy self cells that extinguish that one MHC I molecule but retain others.

Some of the activating KIRs and Ly49 receptors and the activating receptors NKG2C and NKG2E are also expressed by subsets of NK cells in a similarly random fashion. Hence, such NK cells may preferentially kill or respond to target cells that express a ligand that engages a cognate ligand for that receptor. A well-studied example is the Ly49H receptor, expressed by ~40% of mouse NK cells, which binds to the protein m157 encoded by the mouse cytomegalovirus genome and displayed on the surface of infected cells. Ly49H$^+$ NK cells respond preferentially during mouse cytomegalovirus infections[67,68] (see also BOX 1).

Education. Owing to the stochastic nature of inhibitory receptor acquisition, some NK cells arise that lack any inhibitory receptors specific for self MHC I (REF.[65]). As these NK cells are not inhibited by self MHC I, they have the theoretical potential for autoreactivity. Similarly, some animals and humans harbour mutations that confer MHC I deficiency, yet NK cell development still occurs, again creating a situation where NK cells are not inhibited by self MHC I molecules. In both cases, the NK cells exhibit a functional hyporesponsiveness, which contributes to self-tolerance of NK cells, meaning that they do not kill self cells despite their lack of inhibitory receptors for self MHC molecules or lack of MHC I molecules altogether[31,65,69] (FIG. 2g).

Analyses showed that the functional changes in NK cells that depend on engaging self MHC molecules cannot be categorized as binary, as in responsive versus non-responsive. Instead, NK cells exhibit a range of functional activity that increases depending on how many inhibitory receptors for self MHC I they happen to express and the strength of those interactions[70,71]. Other studies showed that NK cells that express activating receptors that bind self molecules also tend to exhibit lower functional sensitivity[72–75]. The processes that impart NK cells with a varying range of sensitivities depending on the activating and inhibitory receptor engagement they experience in steady-state conditions are variously called NK cell 'education', 'tuning' or 'licensing'. Although the underlying mechanisms are still poorly understood, these processes may be better described as a manifestation of a cellular desensitization mechanism, wherein persistent stimulation by

Box 1 | **NK cell memory**

Memory-like NK cells

Studies demonstrated that exposure of natural killer (NK) cells to a cocktail of cytokines — IL-15, IL-12 and IL-18 — converted the cells into long-lived, activated NK cells called 'memory-like' NK cells[12,188]. These cells persist for weeks or months with augmented functional activity, suggesting their possible utility in cancer immunotherapy (see BOX 3).

Specificity

NK cells specific for virus-encoded entities. The potential for NK cell memory was first suggested by studies of immune responses to mouse cytomegalovirus (MCMV) showing the selective expansion of NK cells with a specific activating receptor, Ly49H, that directly recognizes an MCMV-encoded protein, m157 (REFS[67,68]). During MCMV infections, Ly49H$^+$ NK cells selectively proliferated and conferred protection from viral infection, and these NK cells persisted with augmented functional activity for months after viral infection had subsided[13]. Memory NK cells in mice may not last as long as central memory T cells, but they were shown to provide protection for months after initial exposure in mice[13]. Similarly, humans who are seropositive for human cytomegalovirus (HCMV) often harbour expanded populations of CD57$^+$NKG2C^{hi} NK cells that respond to specific HCMV-encoded peptides[189–191]. Hence, HCMV-exposed patients generally have a sizable population of memory-like NK cells at the steady state, which could provide baseline antitumour effects or could be exploited in the context of NK cell therapies.

Other viruses and haptens. Some evidence suggests that specific NK cell memory is not limited to cytomegalovirus antigens. Exposure of mice to sensitizing haptens was shown to induce an NK cell-dependent memory response to later exposure to the haptens[192], implying an unexpected ability of NK cells to recognize, remember and respond to diverse antigenic stimuli. Furthermore, exposure of mice to proteins of influenza virus, vesicular stomatitis virus or HIV reportedly induced specific, protective, memory NK cell populations resident in the liver[193]. All these studies suggest that NK cells can acquire a memory-like status that contributes to enhanced long-term persistence of the cells accompanied by augmented functional activity, with a broad and diverse range of specificity. The molecules responsible for this diversity — presumably some variable cell surface receptors — remain unknown.

MHC-deficient cells results in varying degrees of desensitization of NK cells to subsequent rounds of stimulation (FIG. 2g). For example, studies demonstrate that even fully mature NK cells become desensitized when they are transferred into MHC I-deficient mice[76,77].

Another important adaptive feature of NK cells is the existence of a form of memory. Studies suggest that elevated NK cell activity can persist for months, if not longer, after an infection and that there is specificity to the NK cell memory response[78] (see BOX 1).

NK cells in host immunity to cancer

Spontaneous NK cell responses to cancer. Numerous studies suggest a role for NK cells in tumour immunosurveillance. It is well established that NK cells potently eliminate various tumour cell lines injected at low doses into syngeneic mice[38,39,79,80], but the more revealing studies used spontaneous models of cancer. Long-term depletion of NK cells resulted in higher incidence or severity of cancer in a carcinogen-induced (methylcholanthrene-induced) model of sarcoma and in a spontaneous B lymphoma model in mice[81,82], suggesting that NK cells normally suppress the development of such tumours. Additional evidence supporting a role for NK cells comes from studies showing a higher incidence of spontaneous tumours, or of aggressive tumours, in mouse models of cancer where the mice lack the key activating receptor NKG2D, although those receptors are expressed by some non-NK cells as well[83]. Furthermore, aggressive tumours arising in one such model of cancer were devoid of NKG2D ligands when the host mice were wild type for *Nkg2d* (also known as *Klrk1*), but expressed NKG2D ligands when the host lacked the NKG2D receptor. These data suggested that tumour cells that grew out in the wild-type mice had been selected for loss of NKG2D ligands[83]. Another line of evidence indicated that early infiltration of tumours with IFNγ-producing NK cells was associated with remodelling of the tumour microenvironment (TME) and unleashed cytotoxic T lymphocyte-mediated tumour eradication in mouse cancer models[84].

Consistent with the evidence that NK cells have a role in suppressing malignancies in mice, numerous studies from The Cancer Genome Atlas have associated NK cell infiltration with overall survival outcomes in several types of cancer, including melanoma, breast cancer, glioma and lung adenocarcinoma[47,84–88]. A caveat to these association studies is that infiltration of tumours by T cells and NK cells is generally correlated. This correlation is consistent with evidence that NK cells play a role in promoting the antitumour T cell responses, but can make it difficult to disaggregate the contributions of each cell type in different types of cancer.

Tumour elimination by NK cells in spontaneous or tumour-transfer models of cancer is often dependent on perforin expression, suggesting a key role for direct tumour killing. However, recent studies indicate an important role for NK cells as sentinels that detect nascent tumours and mobilize more potent immune responses by recruiting DCs to the TME. NK cell-derived CC-chemokine ligand 5 (CCL5) and XC-chemokine ligand 1 (XCL1) were shown to play critical roles in recruiting type 1 conventional DCs (cDC1s) to tumours under experimental conditions[47] (FIG. 2b). Furthermore, intratumoural NK cells are a major source of FLT3 ligand within tumours, which stimulates cDC1 differentiation and expansion[48]. Activated cDC1s activate antitumour responses, including T cell responses. Therefore, tumour infiltration by NK cells that mobilize intratumoural cDC1 populations is likely to be a key step in antitumour immunity, at least in some contexts. Furthermore, features of the TME, such as production of prostaglandins, were shown to impair NK cell-mediated recruitment of cDC1s[47,89]. These findings provide further impetus for the use of cancer therapies that stimulate the recruitment of NK cells to tumours.

Initiation of antitumour NK cell responses. Although it is often stated that NK cells do not require prior sensitization to make productive responses, the cytotoxicity and cytokine responses mediated by NK cells extracted from naive laboratory animals are often extremely weak. Accordingly, NK cell responses in the context of viral infections typically require activation of the cells via cytokines, DCs and other mediators, raising the question of whether similar 'priming' is required for antitumour NK cell responses. Recent evidence demonstrates that the spontaneous rejection of implanted syngeneic tumour cell lines by NK cells requires concomitant activation of innate signalling pathways, most notably the cGAS–STING pathway (FIG. 2d). STING-deficient mice failed to generate spontaneous NK cell-mediated antitumour responses in vivo[85], just as they failed to

generate spontaneous antitumour responses mediated by cytotoxic T lymphocytes[90].

cGAS is a cytosolic DNA-sensing enzyme that catalyses the synthesis of the second messenger molecule 2′3′-cyclic GMP–AMP (2′3′-cGAMP) if it encounters DNA in the cytosol[91]. Once generated, 2′3′-cGAMP binds and activates the endoplasmic reticulum-resident protein STING, which in turn activates TBK1 and the downstream transcription factors IRF3 and NF-κB, leading to transcription of type I interferon genes and genes encoding other cytokines and chemokines[92]. These cytokines play key roles in mobilizing immune responses. See BOX 2 for a discussion of the role of the cGAS–STING pathway in the initiation of antitumour NK cell responses and in the evasion of that response by tumour cells.

Tumour evasion of NK cell surveillance. Evasion of immunity is a hallmark of established tumours. Escape mechanisms include adaptations that prevent NK cell activation or recruitment to tumours or that suppress tumour cell killing by NK cells. Some of these adaptations similarly impact T cell responses.

Numerous molecules produced in the TME are broadly immunosuppressive[93]. Extracellular adenosine and prostaglandins are small molecules that are commonly produced in the TME and clearly suppress NK cell activity[89,94]. Suppressive cytokines, such as TGFβ, also impact NK cells, in part by suppressing expression of the activating receptors NKG2D and NKp30 (REF.[95]) (FIG. 2c) and in part by converting NK cells into intermediate type 1 ILC and ILC1 populations in the TME[96]. TGFβ may also act indirectly by supporting the differentiation of regulatory T cells (T_{reg} cells), which can suppress NK cell responses as well as T cell responses[97,98]. Myeloid-derived suppressor cells and tumour-associated macrophage populations also suppress responses of both NK cells and T cells[99].

The antitumour activity of NK cells, like that of T cells, is generally dampened in obesity[100], and this is associated with metabolic reprogramming of NK cells in the TME[101]. The impact of metabolic states on spontaneous and therapy-induced NK cell responses to cancer is a complex topic that we do not cover in detail in this Review. We direct readers to recent reviews on the topic[102–104].

Additional mechanisms of tumour cell escape from NK cell surveillance involve inhibitory receptor engagement. NK cells express inhibitory receptors that engage MHC I molecules, but can also display other inhibitory receptors, including the immune checkpoint receptors T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), CD96, PD1 and SIRPα[105–109]. Tumour cells may upregulate ligands for these receptors and thus inhibit NK cell-mediated antitumour effects (FIG. 2f). Certain inhibitory receptors, such as PD1 and SIRPα, are upregulated on NK cells after activation or specifically in tumours. Some of the inhibitory ligands, such as PDL1, the ligand for PD1, are upregulated owing to the action of immunostimulatory molecules such as type I interferons or IFNγ, whereas others may be adaptations arising from genetic or epigenetic changes in tumours followed by selection of tumours resistant to immunosurveillance.

Another mechanism of escape is that NK cells within tumours often acquire a desensitized hyporesponsive state, similar to the hyporesponsive state of NK cells in mice that fail to express MHC I-specific inhibitory receptors[110,111]. A comparison of infiltrating immune cells in wild-type mice harbouring implanted MHC I-sufficient or MHC I-deficient tumours showed that NK cells within progressing MHC I-deficient tumours acquired a dysfunctional state, whereas those within MHC I-expressing tumours were more functional[111].

NK cell desensitization also occurs when NK cells are persistently exposed to NK cell-activating ligands in vivo (FIG. 2g). This occurs in many or most tumours[112], but also in disease-free states, as endothelial cells and other cells in healthy mice express activating NKG2D ligands[75]. Expression of such ligands was strongly increased on endothelial cells in the tumour vasculature, raising the possibility that desensitization could significantly impair antitumour responses mediated by NK cells[75]. We also observed in mouse models that macrophages in tumours upregulate NKG2D ligands in response to colony-stimulating factor 1 (CSF1; also known as M-CSF) produced by tumour cells[113]. However, definitive evidence is lacking that interactions of NK cells with NKG2D ligands expressed by macrophages causes desensitization of NK cells within tumours.

NKG2D ligands in the TME. Numerous studies in tumour models have focused on the roles of specific ligands that engage stimulatory receptors on NK cells.

Box 2 | **Initiation of NK cell responses against tumours**

In DNA virus infections, accumulation of viral DNA in the cytosol is thought to trigger cGAS to initiate antiviral immune responses. In the case of cancer cells, chromosomal instability — a hallmark of cancer and/or mitochondrial dysfunction — can lead to accumulation of DNA in the cytosol[194–196]. These alternative sources of cytosolic DNA also result in activation of the cGAS–STING pathway, serving as a means to alert the immune system to the presence of cancer (FIG. 2d).

In some tumour cells, STING activation likely occurs in the same cancer cells in which cGAS activation and cyclic GMP–AMP (cGAMP) production occur, and this may be adequate to drive sufficient production of cytokines by the tumour cells to activate natural killer (NK) cell and T cell responses. In other tumours, however, repression of transcription of *STING* (also known as *STING1*), *TBK1* and/or other genes in tumour cells can thwart the production of immuostimulating cytokines and other mediators by cancer cells themselves[197–199]. Accumulating evidence indicates that these defects can be overcome by mechanisms that transport 2′3′-cGAMP produced in cancer cells to nearby cells such as myeloid cells[85] (FIG. 2d). STING activation in those cells supports an antitumour immune response[85,200,201]. Recently identified membrane transporters of 2′3′-cGAMP, including SLC19A1 and LRCC8 and SLC46 proteins[202–206], may mediate the transport of 2′3′-cGAMP from tumour cells to myeloid cells and other cells in the tumour microenvironment, enabling local immune activation and an antitumour cellular immune response.

With respect to initiating NK cell (and T cell) responses, several mechanisms impair activation of the cGAS–STING pathway in the context of cancer. cGAS expression is repressed in some tumours, preventing production of cGAMP, which serves to initiate the immune response[207]. Perhaps more commonly, the capacity of cancer cells to produce immunoactivating cytokines following cGAS activation is stymied owing to downregulation of STING, TBK1 and other intermediates in the pathway[197–199,207]. In this case, however, transfer of cGAMP from tumour cells to neighbouring cells can bypass STING deficiency[85]. Cancer cells can counter this bypass mechanism by producing excess ENPP1, an extracellular enzyme that degrades cGAMP and has the additional effect of generating in the process AMP, which is converted into immunosuppressive adenosine[201,208] (FIG. 2e). Hence, ENPP1 expression in cancer correlates with reduced infiltration of immune cells and increased metastasis, and agents that inhibit ENPP1 are being tested for their capacity to promote antitumour immune responses.

Ligands for NKG2D are often upregulated by tumour cells[114,115] and stimulate NK cells to kill the tumour cells. However, in both mice and humans, many different NKG2D ligands can be shed by tumour cells, either as a result of proteolytic cleavage by matrix metalloproteinases or in some cases by alternative mRNA splicing that generates secreted versions of the ligands[116–120]. Shedding of membrane-bound NKG2D ligands leads to immune escape by tumour cells, at least in part by reducing the density of ligands anchored in the tumour cell membrane that engage the activating receptors, preventing NK cells from killing the tumour cells. Accordingly, blocking tumour shedding of some NKG2D ligands could improve antitumour responses. Indeed, two sets of studies have characterized antibodies that reduce tumour shedding of MICA and MICB and result in improved antitumour responses in mouse models[121,122].

In some instances, the shed ligands may also impact systemic antitumour responses. Surface MICA and MICB proteins are actively shed by many human tumours, leading to accumulation of soluble MICA and MICB in the plasma[116–118]. Some reports have asserted that soluble MICA and MICB systemically suppress responses of NK cells (and T cells) that express NKG2D, by engaging and downregulating cell surface NKG2D as well as by imparting a state of paralysis on the NK cells[116,123] (FIG. 2h). However, these finding were not corroborated by other studies[118,124,125]. Moreover, the concentrations of tumour-derived soluble MICA and MICB in the plasma in those studies were reported to be less than 1 nM (REFS[116–118]), orders of magnitude below the K_d for binding to NKG2D of approximately 1 μM. The low affinity and low concentration of these soluble ligands means that only a tiny fraction of NKG2D molecules on a cell would be bound by soluble MICA or MICB, suggesting that such binding is unlikely to have a major impact on NKG2D expression, NK cell functional activity or the ability of NK cells to engage other NKG2D ligands or ligand-expressing cells. Furthermore, binding of monomeric soluble ligands does not generally activate stimulatory receptors expressed by immune cells. If the ligands were in an aggregated or multimerized form, on the other hand, binding avidity would increase, and receptor crosslinking and downmodulation from the cell surface would be more likely to occur. These considerations suggest that if cleaved low-affinity NKG2D ligands such as MICA or MICB modulate the activity of NK cells directly, they may do so because they are aggregated or multimerized.

A soluble NKG2D ligand was recently shown to have activating as opposed to inhibitory effects on NK cells, albeit via an indirect mechanism (FIG. 2i). The mouse NKG2D ligand MULT1 is expressed by certain tumours and can be cleaved from the membrane, resulting in relatively high concentrations of soluble MULT1 in the plasma of tumour-bearing mice[119]. MULT1 binds NKG2D with approximately 150 times the affinity of MICA or MICB (in humans) or RAE1δ (in mice)[126], allowing monomeric MULT1 to bind a much larger proportion of NKG2D molecules at physiologically relevant concentrations[119]. Such high-occupancy binding of NKG2D by MULT1 prevents binding by lower-affinity NKG2D ligands[119]. In vivo, soluble MULT1 enhanced NK cell activity systemically[119] apparently by preventing NKG2D binding to lower-affinity NKG2D ligands anchored in the membrane of host cells, presumably endothelial cells[75,119]. As noted earlier herein, binding of NKG2D on NK cells to ligands on endothelial cells in healthy animals was associated with partial desensitization of NK cells[75], and hence blockade by soluble MULT1 has the potential to prevent such desensitization. Note that because the high-affinity ligand blocks NKG2D, the increased antitumour activity cannot be mediated by NKG2D interactions and instead was mediated by other NK activating receptors. Some members of the human ULBP family of NKG2D ligands also bind NKG2D with high affinity and can be shed from cells[118,127], suggesting that similar mechanisms may be operative in humans.

Antitumour NK cell therapies

We have discussed how NK cells are able to detect and respond to tumour cells. In the following sections, we consider how our understanding of these mechanisms is being harnessed to target NK cells therapeutically. Two general approaches are being evaluated for NK cell-mediated cancer immunotherapy: first, therapeutics that activate and mobilize endogenous NK cells (which we detail below) and, second, in vitro expansion and engineering of NK cell populations or NK cell lines for infusion as cellular therapeutics (see BOX 3).

Mobilization of endogenous NK cells by cytokines and superkines. IL-2, originally described as a T cell growth factor, also stimulates NK cell activation and proliferation. Culturing of peripheral blood lymphocytes with a high dose of IL-2 leads to the outgrowth of lymphokine activated killer cells that lyse freshly isolated tumour cells, and many of these lymphokine activated killer cells are NK cells[128]. Adoptive transfer of lymphokine activated killer cells in combination with low-dose IL-2 injections has been shown to inhibit metastasis formation in vivo in a melanoma model in mice. High-dose IL-2 immunotherapy resulted in long-term survival of a small percentage of patients with metastatic renal carcinoma and metastatic melanoma[129], which led to FDA approval of IL-2 for these indications. The degree to which NK cells, as opposed to T cells, mediate these effects is not known. Moreover, the low efficacy and the high toxicity of high-dose IL-2 therapy — with adverse events including vascular leak syndrome, heart failure and liver damage[130] — have limited the utility of this approach in the clinic. Another problematic aspect of this approach is that IL-2 administration selectively expands T_{reg} cell populations, which may counteract the beneficial effects of the therapy.

To optimize the efficacy of IL-2 as an immunotherapeutic agent, groups have modified or engineered IL-2. One approach has been to extend the in vivo half-life of IL-2, which can be accomplished by fusing cytokines to albumin[131] or Fc domains of antibodies, or by conjugation of polyethylene glycol to the molecule[132].

Another powerful approach has been to engineer the molecule to alter its binding to IL-2 receptor components. Native IL-2 binds with high affinity to IL-2Rα (also known as CD25, it is highly expressed by T_{reg} cells)

Box 3 | **Cellular therapies with NK cells**

Numerous studies are under way to evaluate natural killer (NK) cell-based cellular therapy, including infusion of NK cell lines, expanded memory-like NK cells and NK cells endowed with chimeric antigen receptors (CARs) specific for tumour cells. The potential advantages of these approaches compared with T cell therapies include the failure of NK cells to mediate graft-versus-host disease and the findings that they can be used in allogeneic recipients, where they exhibit, if anything, greater antitumour activity than syngeneic NK cells[177,178]. The latter feature, which obviates the need for HLA matching, suggests the potential for generating 'off-the-shelf' NK cell products suitable for many patients.

Early studies demonstrated that transferred partially allogeneic NK cells expanded from peripheral blood with IL-2 persisted with low toxicity in patients receiving certain conditioning regimens, and that some antitumour effects were observed[209]. Another approach takes advantage of the greater in vivo longevity of 'memory-like' NK cells[188], which correspond to NK cells that have been preactivated with a cocktail of cytokines (IL-12, IL-15 and IL-18) for 16 h and subsequently rested for 1 week or more in a low dose of IL-15. Infusion of such NK cells in a mouse syngeneic allograft lymphoma model[160] or xenograft models of acute myeloid leukaemia [11] or melanoma[210] led to heightened antitumour activity compared with infusions of control NK cells. A phase I clinical trial showed expansion of infused allogeneic, HLA-haploidentical, memory-like NK cells in patients with acute myeloid leukaemia[11]. Clinical responses, including complete remissions, were documented in about half of the patients.

A related approach is the infusion of an immortal NK cell line, NK-92. These cells express most activating NK cell receptors and lack most inhibitory killer immunoglobulin-like receptors, and can be dramatically expanded in vitro in serum-free conditions[211]. After irradiation, they have been infused into mice with xenografted tumours or into patients with cancer. Toxic effects were mild in phase I clinical trials[212].

Cultured NK cells, including NK-92 cells, can be genetically engineered in cell cultures, including by disrupting potential inhibitory molecules and/or transduction of CARs with specificity for various tumour antigens, to generate 'CAR NK cells'. Infusion of NK-92 cells expressing CARs into mice with various human tumour xenografts significantly extended their survival[213]. CAR NK cells were also generated from IL-2-expanded NK cells derived from umbilical cord blood or induced pluripotent stem cells[214,215]. Peripheral blood-derived NK cells, engineered with a CD19-specific CAR, an IL-15 expression vector and an inducible suicide gene to ensure safety, were infused into patients with chronic lymphocytic leukaemia[215]. Little toxicity was observed, and the majority of the 11 patients treated showed complete remissions. Numerous studies are currently under way employing CAR NK cells in several types of cancer[216]. CAR NK cells may have several advantages over CAR T cells, such as lower toxicity and the potential to administer a 'one–two' punch, mediating toxicity on the basis of enhanced targeting of cells that express the tumour antigen, exhibit MHC class I deficiency or both.

and with low affinity to IL-2Rβ (also known as CD122), meaning that very high concentrations of IL-2 are required to stimulate cells that lack IL-2Rα, which is the case for most NK cells and resting T cells. A mutant form of IL-2 called super-2, or H9, was selected with five amino acid substitutions that together increase the binding affinity for IL-2Rβ by 200-fold[133] (FIG. 3a). Super-2 showed very potent activity in stimulating NK cells. Furthermore, compared with IL-2, super-2 administration resulted in accumulation of fewer T_{reg} cells and lower toxicity, even though super-2 retains its capacity to bind IL-2Rα. Importantly, in syngeneic mouse tumour models, super-2 showed greater single-agent efficacy against melanoma, colon carcinoma and lung adenocarcinoma tumours than IL-2 (REF.[133]).

An important feature of super-2 is its potential ability to reverse or delay the previously discussed desensitization of NK cells that occurs in tumours[111]. Administration of super-2 in mice with established MHC I-deficient tumours sustained tumour control in an NK-dependent fashion. Future clinical studies will address whether super-2, or similar superkines that do not bind at all to IL-2Rα[134–136], provide benefit to patients with cancer.

Another variant of IL-2, known as bempegaldesleukin (formerly NKTR-214), is a pegylated version of the molecule. In addition to extending the in vivo half-life of the protein, pegylation selectively masks the region of IL-2 that binds to IL-2Rα, making it an IL-2Rβ/IL-2Rγ-biased cytokine[132]. Compared with IL-2 administration, bempegaldesleukin administration resulted in the selective expansion of $CD8^+$ T cells and NK cells and a higher $CD8^+$ T cell to T_{reg} cell ratio. Bempegaldesleukin therapy showed greater single-agent efficacy than IL-2 against transplanted melanoma tumours in mice. It provided synergistic antitumour activity in combination with anti-CTLA4 in mouse tumour models, and was well tolerated in non-human primates, with no evidence of vascular leak syndrome, a common side effect of IL-2 therapy. Bempegaldesleukin is well tolerated in patients[137], and is currently being tested in combination with nivolumab (a PD1 inhibitor) in numerous phase II and phase III clinical trials.

IL-15 therapy has emerged as a promising alternative to IL-2 therapy. IL-15, when bound to the IL-15Rα chain, binds to IL-2Rβ with high affinity, in a similar manner to super-2. Administration of recombinant IL-15, which acts primarily after first binding to available membrane IL-15Rα molecules, resulted in expansion of NK cells and $CD8^+$ T cells and tumour regression in various preclinical cancer models, including melanoma, colon carcinoma and lung adenocarcinoma[138,139]. Clinical trials with recombinant human IL-15 were not promising, which led researchers to modify IL-15 for clinical use. One variant is N-803 (formerly ATL-803), which consists of mutated IL-15 chains with increased affinity for IL-2Rβ–IL-2Rγ complexes, bound to a bivalent IL-15Rα–IgG1-Fc fusion protein[140], which extends the in vivo half-life of the complex. Compared with IL-15, N-803 was superior at treating transplanted melanoma and colon carcinoma tumours in preclinical studies. In phase I clinical studies of N-803-treated patients with advanced solid tumours, NK cell expansion was observed with minimal toxic effects[16]. Another promising IL-15 variant is NKTR-255, a pegylated version of IL-15 (REF.[141]). Numerous phase I and phase II clinical trials are currently being pursued with these IL-15 variants, including some in combination with checkpoint blockade[142].

The cytokines IL-12 and IL-18 are known to potently enhance NK cell (and T cell) functional activities. Each cytokine alone exhibits antitumour effects in mouse tumour models[143–145], but only limited efficacy in patients with cancer[146,147]. The combination of IL-12 and IL-18 is more potent in stimulating IFNγ production by NK cells in vitro, and when injected in vivo, it sustained the survival of mice with certain tumours[148,149] (FIG. 3b). Furthermore, combined IL-12 and IL-18 treatment increased the long-term survival of mice with MHC I^{low} tumours and reversed NK cell desensitization[111]. While potent, the IL-12 and IL-18 combination is associated with increased toxicity, sometimes fatal, owing to elevated serum levels of inflammatory cytokines such as IFNγ[150]. Both cytokines are being engineered to increase their potential efficacy and to reduce toxicity.

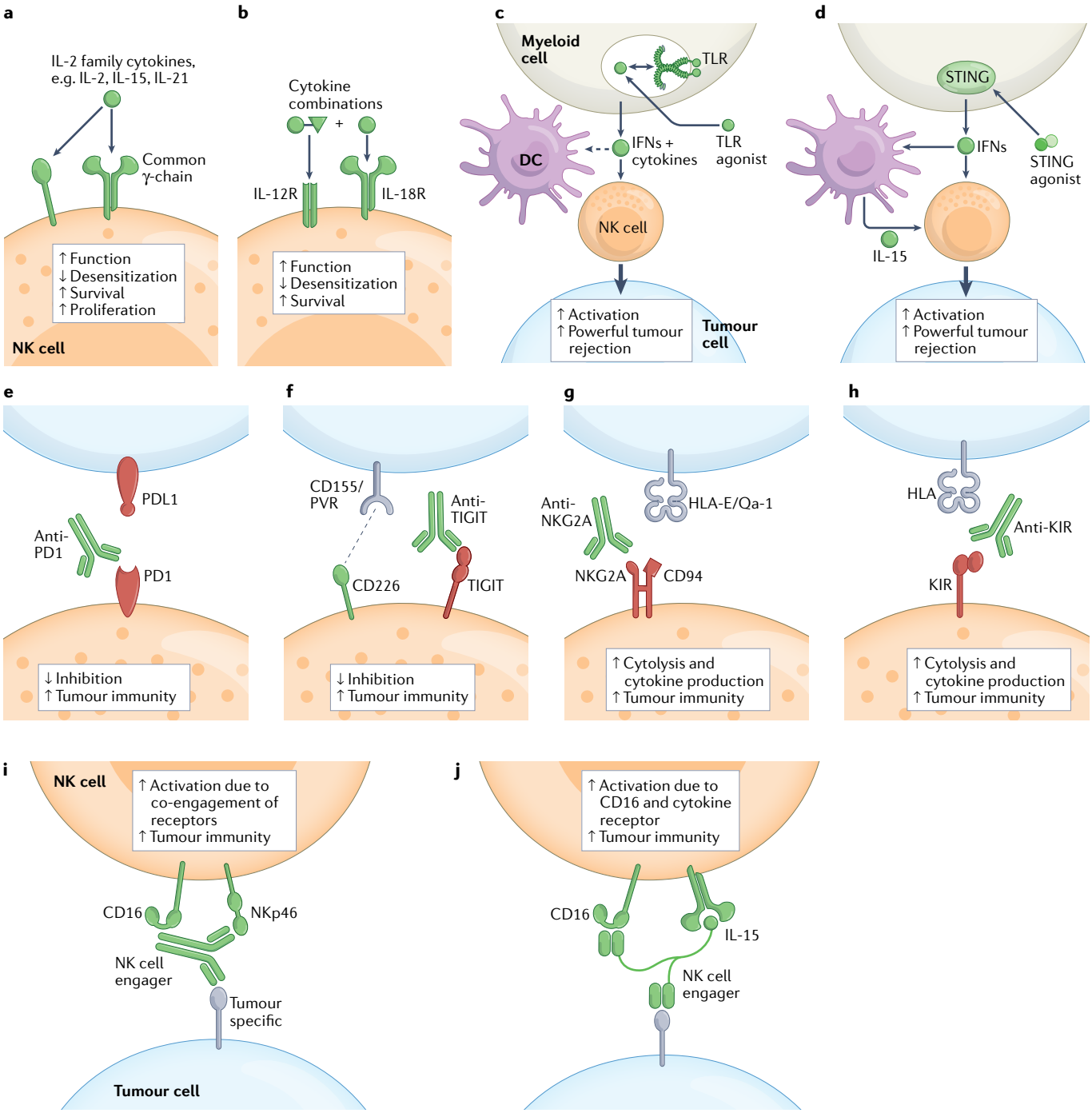


Fig. 3 | **Immunotherapeutic approaches to mobilize antitumour responses by NK cells. a** | Variants of IL-2 family cytokines are optimized to signal through the common γ-chain with less dependence on α-chain expression, leading to increased natural killer (NK) cell functionality and survival. **b** | IL-12 and IL-18 synergistically activate NK cell functional activities. **c** | Delivery of Toll-like receptor (TLR) agonists induces the production of type I interferons (IFNs) and cytokines, leading to direct activation of NK cells, and may also activate dendritic cells (DCs). **d** | Delivery of STING agonists induces the production of type I interferons, which can both directly activate NK cells and indirectly activate NK cells through the production of IL-15–IL-15Rα complexes by DCs. **e** | Anti-PD1 antibodies block the PD1–PDL1 interaction, preventing NK cell inhibition. **f** | Anti-T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) antibodies prevent inhibitory signalling by TIGIT and promote activating signalling through CD226 in NK cells. **g** | Anti-NKG2A antibodies block inhibition by HLA-E/Qa-1, promoting antitumour activity. **h** | Anti-killer immunoglobulin-like receptor (KIR) antibodies block the inhibitory KIR–MHC class I interaction. **i** | Bispecific or trispecific NK engagers bridge NK cells to tumour membrane proteins via antibodies comprising two distinct single-chain variable fragments, with one recognizing activating NK cell receptors and the other recognizing tumour-specific antigens. These are linked through an Fc domain, which provides extra activating function by engaging the activating Fc receptor CD16 on NK cells. **j** | Multifunctional engagers bridge NK cells to tumour cells via two distinct single-chain variable fragments recognizing CD16 on NK cells and tumour-specific antigens. The linker connecting these domains contains an additional segment encoding a cytokine, such as a modified IL-15 sequence, that provides additional stimulation to NK cells. HLA, human leukocyte antigen.

For example, the impact of IL-18 is normally limited by the inhibitory IL-18-binding protein, but an engineered variant of IL-18 that fails to bind the inhibitory protein shows greater efficacy in animal models[151].

Promising results have also been presented in mouse tumour models with a variant of IL-12, IL-12-Fc. Fusion to an Fc fragment increases the stability of the cytokine in vivo, allowing less frequent dosing, leading to enhanced NK cell and T cell responses and tumour rejection[152]. Another promising approach is the production of an IL-12 fusion protein that binds to collagen. Collagen binding retains the IL-12 within solid tumours, minimizing systemic toxicity[153].

IL-21 is structurally similar to IL-2 and IL-15, and signals through a receptor complex containing IL-2Rγ (also known as CD132)[154]. IL-21 has potent effects on all classes of lymphocytes and promotes expansion of NK cell populations in vitro, and the differentiation of new NK cells from bone marrow progenitors. IL-21 administration led to rejection of tumours and suppression of metastases in an NKG2D-dependent manner[119]. As a single therapeutic agent, IL-21 showed better antitumour effects than IL-2 in a mouse melanoma model[155].

Agents that stimulate innate immunity. NK cell activity can be strongly amplified by activators of innate immunity, such as pathogen-associated molecular patterns. The ex vivo activity of NK cells isolated from laboratory mice is often quite low or zero, but can be amplified by prior exposure of mice to Toll-like receptor (TLR) agonists, such as the synthetic double-stranded RNA analogue poly(I:C)[156]. TLR agonists are gaining credence as immunotherapeutic agents, as they are known to induce strong innate and adaptive immune responses (FIG. 3c). The TLR7/TLR8 agonist R848 showed marked efficacy in eliciting tumour-free survival in mouse cancer models when implanted in hydrogel after tumour resection[157]. The antitumour effects depended on T cells and NK cells as shown with cellular depletion studies. In another study, a clinically approved TLR7/TLR8 agonist, imiquimod, was applied at a distant site and stimulated potent antitumour responses against MHC I^{low} tumours that were independent of $CD8^+$ T cells and depended on cooperative actions of NK cells and $CD4^+$ T cells[158]. Numerous phase I and phase II clinical trials are currently being pursued with imiquimod and combination therapies.

More recently, evidence has accumulated that STING agonists, such as cyclic dinucleotides, are potent NK cell activators[85,159]. Injection of STING agonists into tumours mobilized powerful NK cell-mediated antitumour responses in six different MHC I-deficient tumour transplant models in mice[57]. One to three injections of the synthetic STING agonist 2′3′-dithio-(Rp,Rp)-cyclic-di-AMP into established subcutaneous tumours resulted in NK cell-dependent tumour rejection and long-term survival of 50% or more of the mice in four of the six models tested, most of which were solid tumour models. $CD8^+$ T cells played no role in these antitumour responses. This study is one of several showing the potential for NK cells in the therapeutic rejection of solid tumours[57,136,160]. Furthermore, the STING agonist induced potent systemic antitumour effects that impacted the growth of tumours distant from the injection site. These findings suggest that activation of STING locally is sufficient to engender powerful NK cell-dependent tumour rejection responses. The results further suggest that the spontaneous activation of the STING pathway by tumour-derived cGAMP[85], mentioned previously in this Review, provides relatively weak activating signals that are insufficient for clearance of established tumours, perhaps because the amount of cGAMP produced by tumours is limiting. Analysis of tumour rejection induced by a STING agonist in one MHC I-deficient model demonstrated that the response was dependent on type I interferons, which acted directly on NK cells but also indirectly on DCs, where it induced IL-15–IL-15Rα complexes on the cell membrane[57] (FIG. 3d).

As an alternative approach to circumvent the need for intratumoural injections and to mimic aspects of clinical practice, hydrogels containing 2′3′-RR-c-di-AMP were implanted at the site of primary tumour resections in MHC I^+ mouse models[157]. In a breast cancer model and a lung adenocarcinoma model, this led to increased tumour-free survival compared with intratumoural injections, via the action of both $CD8^+$ T cells and NK cells. This approach prevented local tumour reoccurrences and induced systemic antitumour immunity.

Clinical trials with locally applied STING agonists showed that patients tolerated the treatments, but little efficacy has been reported so far[161,162]. Improvements are likely to be in store, as groups have developed orally available STING agonists/mimetics that are effective in animal models[163,164], and various combination therapies are being tested in conjunction with these and other STING agonists.

Blockade of inhibitory receptors. The success of PD1 and PDL1 immune checkpoint blockade therapy is interpreted mainly in the context of amplifying T cell responses. However, evidence has accumulated that NK cells also upregulate the inhibitory receptor PD1 in certain cancers and that PD1 engagement can inhibit NK cells in some instances[107,165–168] (FIG. 3e). In some mouse models, PD1 blockade elicits antitumour NK cell responses, which were necessary for the full therapeutic effect of PD1 and/or PDL1 blockade[107]. These findings suggest that NK cells, in addition to T cells, mediate the effects of PD1/PDL1 blockade in some instances.

As cancers frequently evade T cells responses through downregulation of MHC I, the potential for PD1 blockade in mobilizing NK cell-mediated tumour rejection has emerged as an important issue. Interestingly, PD1 blockade is extremely effective in relapsed/refractory classical Hodgkin lymphoma, despite the very high incidence of MHC I downregulation observed in that indication, raising the possibility that NK cells contribute to the efficacy of treatment[169–171].

TIGIT is an inhibitory receptor expressed on both T cells and NK cells that has been shown to inhibit both types of lymphocytes through interactions with its ligand, CD155, on antigen-presenting cells or on tumour cells[105,172] (FIG. 2f). TIGIT was correlated with NK cell exhaustion in both human colon cancer and mouse cancer models[173]. Blockade of TIGIT with antibodies

reversed NK cell exhaustion in multiple tumour models. In some of the models studied, NK cells were indispensable for the therapeutic effects of TIGIT blockade alone or when anti-TIGIT was combined with anti-PD1/anti-PDL1 (REF.[173]), and in other cases $CD8^+$ T cells played a central role[174] (FIG. 3f).

As described earlier in this Review, NK cells express families of activating and inhibitory receptors specific for MHC I. One such receptor, the NKG2A–CD94 heterodimer, binds to the non-classical MHC molecule HLA-E (FIG. 1). Approximately half of naive NK cells express NKG2A, and while resting T cells do not express the receptor, virtually all activated T cells do. Binding of NKG2A–CD94 to HLA-E leads to inhibition of NK cell and T cell effector functions. HLA-E is reportedly overexpressed in several kinds of cancer, and a correlation has been documented between higher HLA-E expression and poorer prognosis[175].

Monalizumab, a humanized anti-NKG2A monoclonal antibody, blocks NKG2A, unleashing NK cell and T cell function when target cells express HLA-E[176] (FIG. 3g). In vivo, monalizumab enhanced tumour immunity by promoting NK cell and $CD8^+$ T cell effector functions[176]. In preclinical testing, monalizumab showed better results when combined with anti-PDL1. Better results were also observed when monalizumab was combined with cetuximab, an antibody specific for epidermal growth factor receptor (EGFR), which may mediate ADCC of tumour cells. Encouraging indications of efficacy were documented in early phases of a clinical trial of monalizumab combined with cetuximab in head and neck cancer[176]. Several clinical trials are under way with monalizumab as a monotherapy or in combination with anti-PDL1 and/or cetuximab.

The major families of inhibitory receptors for MHC I on NK cells are the KIRs in humans and the similarly functioning Ly49 receptors in mice. In some cases of allogeneic haplo-mismatched bone marrow transplants, where NK cells are included in the bone marrow cell preparations, many of the donor NK cells express KIRs that fail to engage the MHC I molecules of the recipient. In those cases, the NK cells can mediate a powerful 'graft-versus-leukaemia' effect, leading to better outcomes for patients[177,178]. These findings supported the notion that antibody-mediated blockade of KIRs on a patient's own NK cells might provide a similar antitumour effect. Mouse modelling supported this contention by demonstrating that blockade of Ly49 receptors could provide antitumour efficacy, especially when combined with IL-2 therapy[179]. Human anti-KIR antibody specific for the inhibitory receptors KIR2DL1, KIR2DL2 and KIR2DL3 enhanced NK cell function against malignant cells in multiple tumour models[180–182] (FIG. 3h). Anti-KIR antibody was tested alone or in combination with checkpoint therapies in phase I and phase II clinical trials for lymphoid malignancies, and while the patients tolerated the treatment, little clinical efficacy has been documented[183,184]. Interestingly, some patients treated with the anti-KIR antibody showed contraction and reduced cytotoxic functions of their circulating $KIR2D^+$ NK cells. Although not definitive, these findings raise the possibility that KIR blockade in some cases induces a state of desensitization[185], possibly by imposing a state of persistent stimulation of the NK cells[76].

NK cell engagers. An alternative approach has been to amplify NK cell function against tumours by generating 'NK cell engagers', consisting of bispecific or trispecific antibodies that bridge NK cell activating receptors and molecules specific to tumour cells. The approach is similar in conception to T cell engagers, which bridge cytotoxic T cells to tumour cells. A possible advantage of NK cell engagers is that NK cell activation may be associated with less toxicity than persistent T cell activation.

One set of NK cell engagers consisted of a single-chain variable fragment (scFv) of an antibody that binds the activating NKp46 receptor on NK cells linked to an Fc domain that binds to the CD16 activating Fc receptor on NK cells (FIG. 3i). The other arm of the engagers consisted of scFv domains that bind various tumour-associated antigens, including CD19, CD20 and EGFR[186]. In mouse models, the NK cell engagers were more potent in vivo than the corresponding antibodies specific for the tumour antigens, which mediate ADCC. The anti-CD20 NK cell engager promoted better clearance in preclinical mouse models of solid and invasive cancer and induced accumulation of NK cells in tumours.

In another approach, a killer engager consisted of an scFv specific for CD16 linked to an scFv specific for CD33, which is expressed by myelodysplastic cells. The linker connecting these scFv domains included a modified IL-15 sequence, meant to provide cytokine stimulation in concert with the bridging effects of the antibody domains[187] (FIG. 3j). In preclinical studies, this NK cell engager enhanced NK cell effector functions, survival and proliferation, and induced robust antitumour effects in mouse xenograft models of human myeloid dysplastic syndrome.

Summary

There has been an explosive growth in the effort to mobilize endogenous or infused NK cells for cancer immunotherapies, based on the logic of their potential for complementing T cell therapies, and the apparent low toxicities associated with NK cell therapy so far. Emerging findings suggest that some aspects of the TME will need to be overridden, including desensitizing stimuli, dysfunction associated with metabolic factors, and inhibitory cytokines and ligands that suppress NK cell activity. The diversity of NK cells in different tissues[61] also raises the question of whether the efficacy of therapeutic approaches may vary for tumours located in different tissues. Nevertheless, early data are suggestive of the promise of mobilizing NK cells against cancer, including preclinical evidence that therapeutic intervention can result in NK cell-mediated rejection of established solid tumours[57,136,160]. These findings provide a powerful impetus for continuing efforts to exploit these cells for cancer therapy, Given the rapid progress in these efforts, one can predict the emergence of NK cells as a complementary therapeutic modality to T cells, and they have the potential to supplant T cell-targeted therapies in some indications.

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Competing interests
D.H.R. cofounded Dragonfly Therapeutics and served or serves on the scientific advisory boards of Dragonfly Therapeutics, Aduro Biotech and Innate Pharma; he has a financial interest in all three companies and could benefit from commercialization of the results of his research in cancer immunology. The other authors declare that they have no competing interests.

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