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NK cells and cancer: you can teach innate cells new tricks

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Abstract | Natural killer (NK) cells are the prototype innate lymphoid cells endowed with potent cytolytic function that provide host defence against microbial infection and tumours. Here, we review evidence for the role of NK cells in immune surveillance against cancer and highlight new therapeutic approaches for targeting NK cells in the treatment of cancer.

Innate lymphoid cell
(ILC). A lymphocyte that participates in the innate immune responses. ILCs are present in RAG-deficient (*Rag1*$^{-/-}$ and *Rag2*$^{-/-}$) mice, so do not require gene rearrangement for their development or recognition.

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Although they were discovered more than 40 years ago, natural killer (NK) cells have recently been attracting attention for their potential in immune-based therapies. Initially believed to be just an annoying background activity in cytotoxicity assays or an artefact, NK cells are now considered to be an important part of the immune system by controlling microbial infections and tumour progression. Recently, it has been appreciated that NK cells are the founding member of the innate lymphoid cell (ILC) family[1]. Although the ILC family subtypes are characterized primarily by their signature cytokine secretion profiles — ILC1 produces interferon-γ (IFNγ), ILC2 produces interleukin-5 (IL-5) and IL-13, and ILC3 produces IL-17 and IL-22 — the NK cells are the predominant population with specialized cytolytic function.

In patients and animal models, impaired NK cells or NK cell deficiency have been associated not only with recurring virus infections, but also with an increased incidence of various types of cancer[2]. NK cell functions are tightly regulated by a balance between activating and inhibitory signals (FIG. 1a) delivered by a multitude of receptors expressed at the cell surface[3] (TABLE 1). Using these immune receptors, NK cells are able to recognize and then spontaneously kill 'stressed' cells, such as infected or tumour cells, without prior sensitization. Indeed, these abnormal cells can initiate NK cell effector functions, including cytotoxicity, cytokine production and proliferation, either through the loss of self identifying molecules such as major histocompatibility complex (MHC) class I that bind to inhibitory receptors on the NK cells (detection of 'missing self', FIG. 1b) or by upregulating the expression of ligands for activating receptors on the NK cells that can overcome the inhibitory signals (FIG. 1c). Furthermore, as opposed to other immune cells that require a considerable length of time to acquire cytolytic activity, NK cells are 'ready to kill', which provides a powerful tool for use in immunotherapeutic approaches. In this Review, we describe the established role of NK cells in tumour surveillance, as well as that of other immune cells expressing NK cell receptors that may contribute to their antitumour immune responses. Given the successful anticancer therapeutic approaches involving blockade of T cell-directed immune checkpoints, we discuss how these and new combination strategies could also affect the antitumour functions of NK cells. To conclude, we focus on recent progress in genetic engineering of immune cells, and how this field could benefit from targeting NK cells and their receptors.

NK cell receptors and their ligands

NK cells are characterized phenotypically as CD3$^-$CD56$^+$ lymphocytes in humans and CD3$^-$NK1.1$^+$ lymphocytes in mice, or as CD3$^-$NKp46$^+$ lymphocytes in both species. On the basis of their level of CD56 expression, human NK cells can be further divided into two distinct subsets identified as CD16$^+$CD56^{dim}, which are the most numerous in blood, and CD16$^-$CD56^{bright}, which characterizes a less mature population. NK cells express receptors that bind to MHC class I molecules, including the killer cell immunoglobulin (Ig)-like receptors (KIRs) in humans[4] and the Ly49 (also known as KLRA) receptors in mice[5]. The KIR and Ly49 receptors are generated by a multigenic family of polymorphic genes that encode both inhibitory and activating receptors[4,5]. Another receptor family conserved in both mice and humans is the CD94 (also known as KLRD1)–NKG2 receptor system. In this family, the CD94 receptor forms a disulfide-linked heterodimer with the inhibitory NKG2A receptor or with the activating NKG2C receptor[6]. Unlike KIRs and Ly49, the CD94–NKG2 receptors are invariant and recognize a relatively non-polymorphic MHC class I ligand, human leukocyte antigen-E (HLA-E) in humans[7–9] and Qa1 in mice[10]. The inhibitory KIR, Ly49 and CD94–NKG2A receptors function to detect self-MHC class I ligands on healthy cells and prevent or dampen NK cell activation (FIG. 1a). Activating receptors in these families may recognize pathogen-encoded ligands or detect alterations in the peptide repertoire in MHC class I molecules, which results in NK cell activation[11–13].

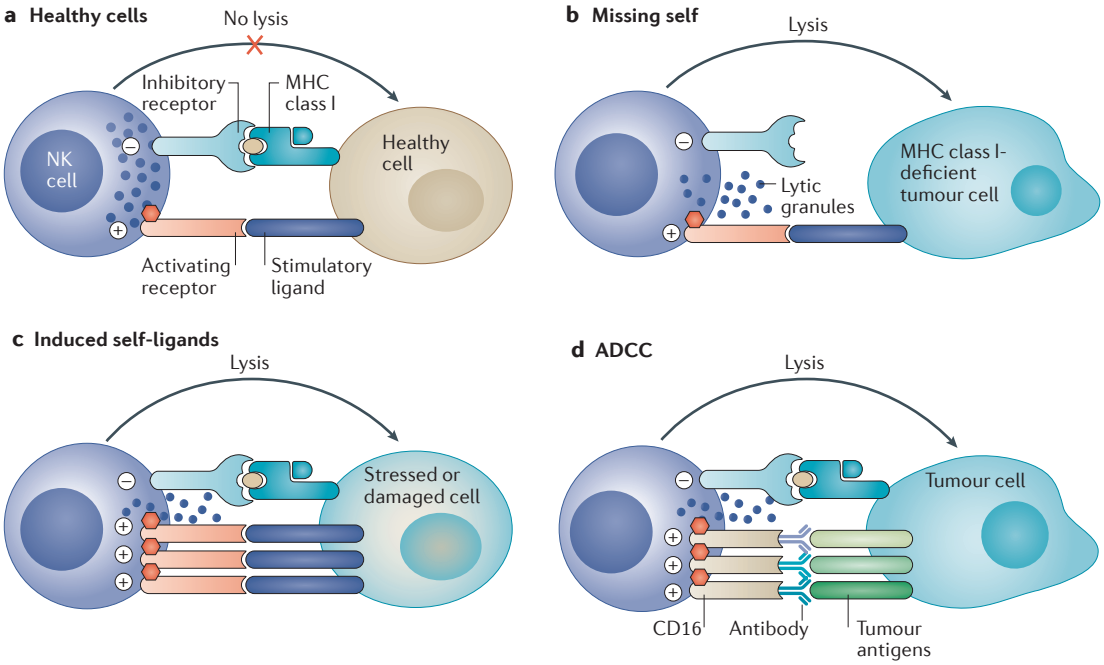

Figure 1 | **Schematic representation of physiological NK cell functions. a** | A balance of signals delivered by activating and inhibitory receptors regulates the recognition of healthy cells by natural killer (NK) cells. **b** | Tumour cells that downregulate major histocompatibility complex (MHC) class I molecules are detected as 'missing self' and are lysed by NK cells. **c** | Tumour cells can overexpress induced stress ligands recognized by activating NK cell receptors, which override the inhibitory signals and elicit target cell lysis. **d** | Tumour antigen-specific antibodies bind to CD16 and elicit antibody-dependent NK cell-mediated cytotoxicity.

NK cells use inhibitory receptors to prevent the killing of healthy cells, whereas they need strong activating signals to initiate an immune response against infected cells or tumour cells (FIG. 1b,c). NK cells possess numerous receptors that can trigger their effector functions when they are engaged alone or in combination[14]. NKG2D (also known as CD314 and KLRK1), the natural cytotoxicity receptors (NCRs), DNAM1 (also known as CD226) and CD16 are the best-characterized activating NK cell receptors implicated in immune responses against cancer. NKG2D is a particularly important and well-studied receptor, which is expressed by all NK cells, as well as by $CD8^+$ T cells and γδ T cells[15]. This type II transmembrane-anchored protein has unique properties, which include binding to an extensive array of ligands. In humans, NKG2D ligands (NKG2DLs) are MHC class I polypeptide-related sequence A (MICA), MICB, retinoic acid early transcript 1E protein (RAET1E), RAET1G, RAET1H, RAET1I, RAET1L and RAET1N (also known as ULBP1–ULBP6), whereas in mice its ligands are the RAE1 family (α, β, γ, δ and ε), the histocompatibility antigen 60 (H60) family (H60a, H60b and H60c) and mouse UL16-binding protein-like transcript 1 (MULT1)[16,17]. These ligands are not present on the cell surface of most healthy tissues, but can be expressed on rapidly proliferating cells, infected cells, transformed cells and tumour cells[17]. Regulation of the NKG2DLs is a very complex process that can involve transcriptional, translational and post-translational control, and there are no general rules that apply for the induction and expression of the different NKG2DLs in different cell types or tumours[16,17]. Some viruses and tumour cells have developed escape mechanisms to circumvent NKG2D-mediated immunosurveillance, for example, by ligand shedding, intracellular retention of ligands and induction of ligands on neighbouring bystander cells, which demonstrates the importance of this interaction during immune responses[16,17].

NCRs are another family of activating receptors that were discovered on NK cells in the 1990s, and include three receptors: NKp30 (also known as NCR3 and CD337), NKp44 (also known as NCR2 and CD336) and NKp46 (also known as NCR1 and CD335)[18]. NKp30 (REF. 19) and NKp46 (REF. 20) transmit their activating signal by associating with the immunoreceptor tyrosine-based activation motif (ITAM)-bearing adaptor protein CD3ζ, and the high-affinity IgE receptor-γ (FcεRIγ), whereas NKp44 associates with DAP12 (also known as TYROBP)[21]. NKp46 and NKp30 are expressed constitutively on NK cells, but NKp44 is expressed only after activation of NK cells. NKp46 is highly conserved among species including in mice[22], whereas NKp30 and NKp44 are not present in inbred mice[23], which limits the ability to probe their *in vivo* function. A wide variety of NCR ligands have been reported, including ligands encoded by genes from viruses, bacteria and parasites; however, most of these are not well defined[18] (TABLE I). Although as yet not characterized, NCR ligands have been detected on many tumour cells and some healthy tissues[18].

DNAM1 is an activating receptor expressed by NK cells, subsets of T cells and myeloid cells[24]. Its ligands are CD112 (also known as PVRL2 and nectin 2) and

Natural cytotoxicity receptors
(NCRs). A family of activating receptors expressed by NK cells and some innate lymphoid cells that recognize various, mostly ill-defined, ligands. The three members of this family in humans are NKp46 (encoded by *NCR1*), NKp44 (encoded by *NCR2*) and NKp30 (encoded by *NCR3*). Mice possess only a functional *Ncr1* gene and not orthologues of NKp30 or NKp44.

γδ T cells
A subset of T cells that express a distinct T cell receptor (TCR) composed of one γ-chain and one δ-chain, instead of the α- and β-chains that comprise the TCR on the majority of T cells. Like natural killer cells, they are considered to be a bridge between innate and adaptive immunity.

Table 1 | **Natural killer cell receptors and their ligands**

Receptor	CD number	Inhibitory	Activating	Expressed on other cells	Ligands
KIR (humans)	CD158	X	X	Effector and/or memory T cells	MHC class I molecules
Ly49 (mice)	n/a	X	X	Effector and/or memory T cells	MHC class I molecules
NKG2A	CD159a	X		Effector and/or memory T cells	HLA-E (humans) and Qa1 (mice)
NKG2C	CD159c		X	Effector and/or memory T cells	HLA-E (humans) and Qa1 (mice)
NKG2D	CD314		X	T cells and activated macrophages	MICA, MICB, ULBP1–6 (humans) and RAE1α–ε, H60a–c, MULT1 (mice)
DNAM1	CD226		X	T cells and monocytes	PVRL2 (CD112) and PVR (CD155)
TIGIT	n/a	X		T cells	PVRL2 (CD112) and PVR (CD155)
Tactile	CD96	X		T cells	PVR (CD155)
FcγRIIIA	CD16a		X	Activated monocytes and macrophages	Fc domain of IgG antibodies
NKp46	CD335		X	αβ and γδ T cell subsets and ILC3	HSs*, HNs*, HAs*, vimentin*, PfEMP1*, unknown bacterial components and unknown extracellular ligands
NKp44	CD336		X	αβ and γδ T cell subsets, ILC3 and plasmacytoid dendritic cells	HSs*, PCNA*, NKp44L, HNs*, HAs*, viral envelope glycoproteins* and unknown bacterial components
NKp30	CD337		X	αβ and γδ T cell subsets and epithelial cells	B7-H6, HSs*, BAT3*, HA*, HCMV pp65* and PfEMP1*
PD1	CD279	X		Activated T and B cells and myeloid cells	PDL1 and PDL2
4-1BB	CD137		X	T cells, dendritic cells and endothelial cells	4-1BBL

HA, haemagglutinin; HCMV, human cytomegalovirus; HLA-E, human leukocyte antigen-E; HN, haemagglutinin neuramidase; HS, heparan sulfate; IgG, immunoglobulin G; ILC3, innate lymphoid cell 3; KIR, killer cell immunoglobulin-like receptor; MIC, major histocompatibility complex (MHC) class I polypeptide-related sequence; MULT1, mouse UL16-binding protein-like transcript 1; n/a, not applicable; PCNA, proliferating cell nuclear antigen; PD1, programmed cell death protein 1; PDL, PD1 ligand; PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1; PVR, poliovirus receptor; RAE1, retinoic acid early inducible 1; Tactile, T cell-activated increased late expression; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; ULBP, UL16-binding protein. *These natural cytotoxicity receptor ligands have not been confirmed by independent research groups or by co-crystal structures.

CD155 (also known as PVR), which are members of the Nectin family of molecules and are broadly distributed on haematopoietic and non-haematopoietic cells, including on many tumour cells. DNAM1 is involved in NK cell-mediated killing of certain tumours, including melanomas[25], and *Cd226*$^{-/-}$ mice are more susceptible to primary and transplantable tumours[26,27]. Two other inhibitory receptors, T cell immunoreceptor with Ig and ITIM domains (TIGIT) and CD96, also bind to DNAM1 ligands and oppose DNAM1 function[24,28]. TIGIT can disrupt DNAM1 signalling by interacting with it in *cis* to form heterodimers, and blockade of TIGIT with monoclonal antibodies augments the anti-tumour and antiviral activity of NK cells and T cells *in vivo* in mouse models of cancer and viral infection[29].

A potent activator of NK cells is CD16 (also known as FcγRIIIA), which is expressed predominantly on the CD56^{dim} subset of NK cells in humans and on most mouse NK cells[30]. This receptor recognizes the constant region (Fc) of IgG antibodies, predominantly IgG1 in humans, and is responsible for antibody-dependent cell-mediated cytotoxicity (ADCC) (BOX 1; FIG. 1d). Although the role of CD16 in NK cell-mediated immunosurveillance in mouse models has not been addressed, allelic polymorphisms of *FCGR3A* (which encodes CD16) in humans that affect the affinity of the receptor have been correlated with the efficacy of rituximab (anti-CD20) antibody therapy[31]. CD16 is also expressed on activated macrophages, thus both NK cells and macrophages might contribute to the therapeutic benefit of ADCC in antibody-mediated cancer therapies.

NK cell functions

Mature NK cells are morphologically characterized as large granular lymphocytes. These granules contain both perforin (a membrane-disrupting protein) and granzymes (a family of proteolytic enzymes), which are responsible for NK cell-mediated killing[32]. When NK cells interact with target cells, an immunological synapse forms, by which these granules are exocytosed[33], leading to the specific lysis of the target cell. Additionally, NK cells are able to kill tumour cells by using molecules in the tumour necrosis factor (TNF) family. NK cells express both soluble and membrane-bound TNF, and activation of NK cells induces death-inducing ligands, such as FAS ligand (FASLG; also known as TNFSF6) and TNF-related apoptosis-inducing ligand (TRAIL; encoded by *TNFSF10*) at the NK cell surface[34]. When bound to these ligands, death receptors on target cells (for example, FAS) can activate the caspase enzymatic cascade that

Box 1 | **Antibody-dependent cell-mediated cytotoxicity**

Natural killer (NK) cells express CD16 (or FcγRIIIA), a cell surface receptor for the highly conserved Fc portion of immunoglobulin G (IgG) antibodies. When human IgG1 antibodies bind to specific antigens displayed by infected or transformed cells, these antibodies are recognized by CD16 and trigger NK cell activation and killing of the antibody-coated target cells. This mechanism is referred to as antibody-dependent cell-mediated cytotoxicity (ADCC). In humans, circulating mature NK cells express CD16 and exhibit potent ADCC effector function. A polymorphism at residue 158 in human CD16 that encodes either valine (higher affinity) or phenylalanine (lower affinity) affects the affinity of binding of CD16 to IgG1 and influences the magnitude of the ADCC response initiated by therapeutic antibodies such as rituximab. Engineering the Fc region of these antibodies in order to increase their affinity for both CD16 variants is a promising avenue for improving ADCC responses against tumours in all patients.

causes apoptosis[35]. *Tnfsf10*$^{-/-}$ mice are more susceptible to spontaneous and transplantable tumours in an NK cell-dependent manner[36].

When activated by target cell interactions or by cytokines in the microenvironment, NK cells secrete cytokines and chemokines that shape the innate and adaptive immune responses[14]. The best-characterized cytokine produced by NK cells is IFNγ, but they can also secrete numerous interleukins (for example, IL-10), TNF, growth factors (for example, granulocyte–macrophage colony-stimulating factor (GM-CSF))[37] and chemokines (for example, chemokine (C-C motif) ligand 3 (CCL3), CCL4 and CCL5)[38]. Secretion of these cytokines recruits other immune cells to the inflammation site and induces activation and proliferation of these cells. NK cells are an early and potent producer of IFNγ, which has many effects on the immune response, including induction of MHC class II molecules on antigen-presenting cells, activation of myeloid cells and induction of T helper 1 (T_H1) cells, as well as effects on angiogenesis. Macrophage activation by NK cell-derived IFNγ has been shown to be essential for resistance to chemical carcinogenesis in a mouse model of primary tumorigenesis[39].

Tumour surveillance by NK cells

NK cells were first implicated in tumour immunosurveillance in the 1980s, when several studies reported a higher incidence of cancers in individuals with defective NK cell function caused by genetic disorders such as Chēdiak–Higashi syndrome and X-linked lymphoproliferative syndrome[40,41]. During the same period, increased tumour growth and metastasis were described in mutant mice with impaired NK cell activity[42] and in mice treated with an NK cell-depleting antibody[43]. Numerous studies then found decreased NK cell function in cancer patients[44–46] or their families[47,48], including in a landmark long-term epidemiological study reporting that subjects with low NK cell activity had a higher risk of developing various types of cancer[49]. NK cell deficiencies[2], characterized by the absence of NK cells or NK cell function, and caused by genetic mutations in genes such as *GATA2* (REF. 50) or *MCM4* (REF. 51), lead to higher rates of malignancy. These primary immunodeficiencies affecting NK cells indicate how important these cells are in tumour immune surveillance. However, these immunodeficiencies affect other immune cells in addition to NK cells, and the relationship between increased cancer risk and low NK cell activity (frequently measured by *in vitro* cytotoxicity assays using the K562 leukaemia cell line as the target) is only correlative. NK cells have been shown to control the growth and metastasis of transplantable tumours in numerous mouse models by antibody depletion of NK cells[52]. However, a significant limitation to these studies has been the ability to selectively ablate NK cells for the long periods of time that are often necessary to establish primary tumours. There are no truly NK cell-specific molecules that can serve as targets for genetic or antibody-mediated ablation of NK cells. A recent comprehensive transcriptional profiling study of mouse leukocyte populations by the Immunological Genome Consortium revealed that essentially all genes expressed by NK cells are also expressed by other immune cell types, usually by subsets of T cells or other ILCs[53]. In many models, mouse NK cells are depleted using an antibody against NK1.1 (also known as KLRB1C); however, NK1.1 is expressed on invariant natural killer T cells (iNKT cells) and some activated $CD8^+$ T cells, as well as some ILCs. Perhaps the most NK cell-restricted marker is NKp46, but this receptor is also expressed by a subset of γδ T cells and other ILC populations[54]. Although RAG-deficient (for example, *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$) mice can be used to exclude B and T cell contributions to antitumour effects, the shared expression of many molecules by NK cells and other ILC subsets[55] must be considered.

Although NK cells have been documented to infiltrate primary tumours in mice and humans, they typically comprise only a minor population, raising questions about their importance[56]. Given the paucity of NK cells usually detected in solid tumours, and their relative abundance in the circulation, it has been speculated that the predominant role of NK cells might be to prevent metastasis of tumours[43]. However, even if NK cells fail to accumulate in solid tumours, this does not exclude that they significantly contribute to antitumour immune responses by their cytolytic functions and by secreting chemokines or cytokines that attract or activate tumour-infiltrating T cells or myeloid cells. Many new reagents and imaging technologies are being developed that provide more sensitive and dynamic methods of probing the leukocyte populations that infiltrate tumours, which should provide important insights into the recruitment and function of NK cells within tumour sites.

What renders tumours susceptible to NK cell-mediated attack? As noted above, it is the net balance of activating and inhibitory signals that regulates whether an NK cell attacks and eliminates a potential target cell. As demonstrated by the ability of NK cells in healthy mice to reject transferred splenocytes from β2-microglobulin-deficient (*B2m*$^{-/-}$) mice[57], normal cells express relevant ligands for activating receptors on NK cells to initiate killing of these cells if unopposed by signals from the NK cell inhibitory receptors for MHC class I. Therefore, NK cells can eliminate tumours that downregulate expression of MHC class I (FIG. 1b),

Chēdiak–Higashi syndrome and X-linked lymphoproliferative syndrome
Autosomal recessive genetic disorders that impair natural killer cell and T cell effector functions.

Invariant natural killer T cells
(iNKT cells). A subset of T cells that express certain natural killer cell markers as well as an invariant T cell receptor α-chain. Their recognition is restricted to glycolipids presented by the CD1d antigen-presenting molecule.

possibly in response to selective pressure exerted by $CD8^+$ T cells, although still displaying one or several ligands for an activating NK cell receptor. Although most tumours have retained expression of MHC class I, it is impossible to know the frequency with which nascent tumours losing MHC class I are eliminated by NK cells before becoming established. Alternatively, NK cells can kill tumour cells that retain full expression of MHC class I if they have upregulated ligands that engage activating NK cell receptors, thus overriding the inhibitory signals (FIG. 1c).

Perhaps the best-characterized ligands for an activating NK receptor that are abundantly expressed on transformed cells from various origins[58] are the NKG2D ligands[17]. NK cell activation induced by NKG2D ligand expression on tumour cells is sufficient to overcome inhibitory signals delivered by MHC class I receptors, thereby enabling NK cells to eliminate tumours expressing normal levels of MHC class I[59,60]. Furthermore, mice deficient for NKG2D (*Klrk1*$^{-/-}$), or treated with an NKG2D-neutralizing antibody, are more susceptible to primary tumorigenesis[61,62], confirming the crucial role of NKG2D in tumour immunosurveillance, although it has not yet been established whether NKG2D-mediated protection is conferred by NK cells, T cells or both. One of the mechanisms used by tumour cells to evade this surveillance is the shedding of NKG2DLs through cleavage by metalloproteinases, which decreases the amount of ligand at the tumour cell surface[63–68] (FIG. 2). Soluble NKG2DLs have been found in the sera of patients with many different types of cancer[69] and might be used as a diagnostic marker[70]. Another novel escape mechanism involves the induction of NKG2DLs on healthy host myeloid cells by soluble factors (such as lactate dehydrogenase) secreted by tumour cells[71]. Chronic stimulation of NKG2D by ligand-bearing cells, whether on tumours or on healthy host cells, results in downregulation of NKG2D and desensitization of the NKG2D pathway, thereby impairing NKG2D-dependent tumour immunosurveillance[72,73]. Therapeutic strategies to preserve or induce NKG2DL expression on tumours and prevent desensitization of the NKG2D receptor on NK cells and T cells provide an attractive target for immunotherapy. Recent work in a mouse model suggests that a soluble high-affinity NKG2DL, MULT1, can stabilize expression of NKG2D on the surface of NK cells and augment NKG2D-dependent NK cell antitumour activity[74].

Several other mechanisms that enable tumours to evade NK cell-mediated surveillance have been documented. For example, tumours that activate platelets can avoid NK cell recognition, either directly by disrupting activating ligand expression[75] and displaying ligands for NK cell inhibitory receptors[76,77], or indirectly by releasing immunoregulatory factors, such as transforming growth factor-β (TGFβ)[78] (FIG. 2). Additionally, tumours themselves secrete immunomodulatory molecules that compromise the activity of immune cells in general, and NK cells in particular. Factors that can suppress NK cell effector functions include TGFβ[79], prostaglandin E_2 (REFS 80,81), indoleamine 2,3-dioxygenase[81] and adenosine[82] (FIG. 2). This may not only impair NK cell function, but also block their maturation process, for example, by downregulating expression of the IL-15 receptor[83]. Tumours can also produce IL-10, a cytokine known mainly for its anti-inflammatory and immunosuppressive properties; however, IL-10 has also been shown to display immunostimulatory activity, especially on NK cells, in some cancer settings[84,85].

The NCRs have been implicated in antitumour immune responses on the basis of the ability of monoclonal antibodies against these receptors to block human NK cell killing of various tumour cell lines using *in vitro* cytotoxicity assays[86]. In many cases, combining the antibodies against NKp30, NKp44 and

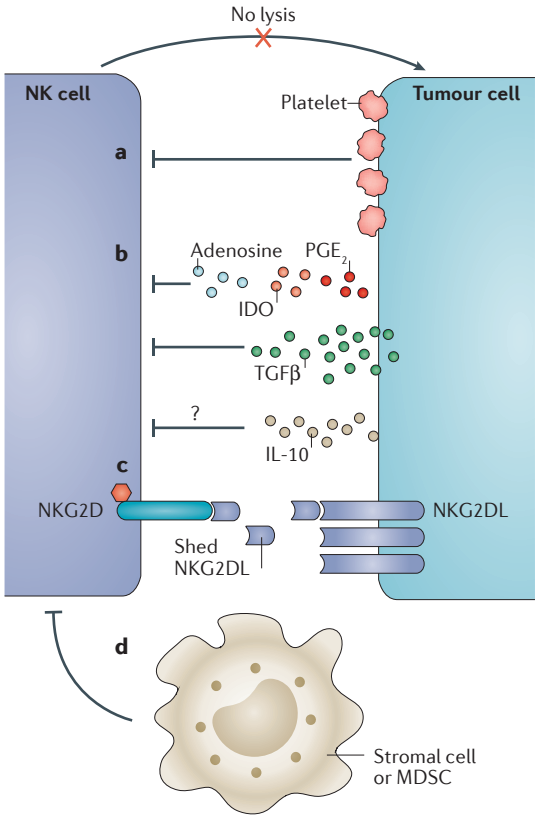

Figure 2 | **Schematic representations of immune escape mechanisms used by tumour cells. a** | Platelets coating tumour cells can secrete immunosuppressive factors, such as transforming growth factor-β (TGFβ), and can display ligands for inhibitory receptors (such as glucocorticoid-induced TNFR-related protein (GITR)) in order to dampen natural killer (NK) cell activation or down-modulate activating receptors (such as NKG2D) on NK cells. **b** | Tumour cells can secrete immunomodulatory molecules such as prostaglandin E_2 (PGE_2), indoleamine 2,3-dioxygenase (IDO), adenosine, TGFβ and interleukin-10 (IL-10) (although in some cases IL-10 has also been shown to activate NK cells). **c** | Tumour cells can shed NKG2D ligands (NKG2DLs), and these soluble ligands mask or down-modulate NKG2D on NK cells. **d** | Stromal cells or myeloid-derived suppressor cells (MDSCs) in the tumour microenvironment can secrete immunosuppressive molecules or can express NKG2DLs, which can cause a chronic interaction with NKG2D on NK cells that leads to down-modulation of the receptor.

NKp46 demonstrated more efficient blocking of lysis of tumours than the same antibodies individually, implying the existence of different ligands on the target cells. Fc-fusion proteins containing the extracellular domains of the NCRs bind to a broad array of tumour cells, as well as healthy cells, suggesting that the ligands of these receptors are widely expressed[87,88]. Several putative ligands expressed by tumours have been proposed for the NCRs (TABLE 1), including heparan sulfates[18]; however, the only well-validated cellular ligand of an NCR is B7-H6 (also known as NCR3LG1)[89], which has recently been co-crystalized with NKp30 (REF. 90). B7-H6 is expressed preferentially on tumours, including leukaemias, lymphomas, melanomas and carcinomas[89], but is induced on healthy Toll-like receptor (TLR)-activated myeloid cells[91]. NKp46-deficient (*Ncr1*$^{-/-}$) mice have been reported to have an impaired ability to control growth of the PD1.6 lymphoma cell line[92] and metastasis of the B16 melanoma and Lewis lung carcinoma cell lines injected in the footpad or in the tail vein[93], although NKp46 ligands on these tumours have not been identified. NKp46 has also been implicated in the rejection of glioblastomas induced by infection with Herpes simplex virus in a mouse model[94]. Collectively, the NCRs are an important part of the NK cell repertoire of receptors that provide defence against tumours, and a better understanding of their recognition may suggest new approaches to cancer therapy.

Schreiber and colleagues[95] have demonstrated that, as they evolve in the host, tumours are shaped or edited by encounters with the immune system. Although the adaptive immune system has a major role in editing nascent tumours, there is evidence for immune-based editing by NK cells. Bui and colleagues[39] observed a higher incidence of methylcholanthrene-induced sarcomas in RAG-deficient (*Rag2*$^{-/-}$) mice lacking the IL-2-receptor-γ chain (*Il2rg*$^{-/-}$), which lack NK cells, compared with *Rag2*$^{-/-}$ mice, which possess NK cells, and found that tumours arising in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were more immunogenic when transplanted into wild-type immunocompetent mice. Further, they demonstrated that this impairment in tumour immune surveillance was dependent on IFNγ production by NK cells and the activation of macrophages within the tumour microenvironment.

There is also evidence for immune-based editing mediated by the DNAM1, NKG2D and NKp46 receptors. DNAM1-deficient (*Cd226*$^{-/-}$) mice developed significantly more DNAM1 ligand-expressing fibrosarcoma and papilloma cells when induced by methylcholanthrene and DMBA, respectively, than did wild-type mice[26]. Similarly, Smyth and colleagues[96] reported the preferential growth of methylcholanthrene-induced sarcomas expressing high levels of NKG2DLs in perforin-deficient (*Prf*$^{-/-}$) mice compared with wild-type mice, and Guerra *et al.*[61] have also observed evidence for NKG2D-mediated editing of primary *Myc*-driven B cell tumours arising in NKG2D-deficient (*Klrk1*$^{-/-}$) mice compared with wild-type mice. Although methylcholanthrene-induced sarcomas were induced at the same frequency in NKp46-deficient (*Ncr1*$^{-/-}$) and wild-type mice, analysis of cell lines derived from these tumours revealed higher levels of expression of NKp46 ligands (detected by staining with a NKp46–Ig fusion protein) on sarcomas isolated from NKp46-deficient (*Ncr1*$^{-/-}$) mice[97]. Together, these studies suggest a role for NK cells in the immunoediting of primary tumours; however, because DNAM1, NKG2D and NKp46 are also expressed on T cells and other ILCs, further studies are needed to discriminate the contribution of NK cells and other lymphocytes bearing these receptors.

Although NK cells and NK receptors have been shown to provide host protection, the challenge now is to devise strategies to augment their activity against tumours that have evolved mechanisms to escape detection and elimination by NK cells.

Checkpoint blockade of NK cells

Given their very strong antitumour potential, current immunotherapy approaches could also take advantage of NK cell activity. Recent reports have shown that under some circumstances activated NK cells can express programmed cell death protein 1 (PD1; also known as PDCD1)[98,99] and cytotoxic T lymphocyte-associated antigen 4 (CTLA4)[100], which are the target molecules of several US Food and Drug Administration (FDA)-approved cancer immunotherapy drugs (ipilimumab against CTLA4 and nivolumab and pembrolizumab against PD1) that restore T cell activation. A promising study showed that, in patients with multiple myeloma, NK cells express PD1 and that a PD1 antibody (CT-011) could restore NK cell-mediated antitumour activity[101]. Additionally, NK cells are able to potently kill tumour cells treated with a human IgG1 PD1 ligand 1 (PDL1) monoclonal antibody owing to the synergistic effect of the inhibitory PD1–PDL1 interaction being blocked and ADCC induced through the activating CD16 Fc receptor on NK cells[102]. Although the role of CTLA4 in NK cells is unclear[103,104], NK cells might have a functional impact in patients treated with CTLA4 antibodies. For example, cytokines produced by activated T cells by CTLA4 blockade therapy might activate NK cells and initiate their antitumour effector functions. CTLA4 can also be expressed on some tumour cells[105] and thus induce ADCC after treatment with CTLA4 antibodies[106]. Moreover, tumour cells infected with oncolytic viruses, such as Newcastle Disease virus[107] and H-1PV rat parvovirus[108], have been shown to stimulate NK cells directly by inducing activating ligands on the virus-infected cells. A recent study that tested the combination of CTLA4 blockade and oncolytic virus infection demonstrated that the strong antitumour effect obtained was, in part, dependent on NK cells[109]. Because several antibodies targeting the PD1 and CTLA4 pathways have been approved or are in clinical trials, studying their impact on NK cell activity in more depth is now crucial. Furthermore, given the successes of these immune checkpoint inhibitors, novel approaches are being developed that could use a combination of current and new targets, some of them being expressed by NK cells.

Because their interaction with self-MHC class I molecules, which are expressed on every nucleated cell in the host including tumour cells, strongly inhibits NK cell-mediated cytotoxicity, inhibitory KIRs have become a target of choice for direct immune checkpoint blockade of NK cells (FIG. 3a). IPH2102, a monoclonal antibody that blocks KIR2DL1, KIR2DL2 and KIR2DL3 — three inhibitory KIRs that are specific for all HLA-C molecules — has been shown to increase NK cell activity against tumour cells *in vitro*[110], while maintaining tolerance to healthy cells[111]. Because anti-KIR enhanced NK cell-mediated, rituximab-dependent cytotoxicity against lymphomas *in vitro* and *in vivo* in KIR transgenic and syngeneic mouse lymphoma models[112], KIR blockade provides an attractive target for cancer immunotherapy. Further, it should be appreciated that inhibitory KIRs are expressed on a subset of effector and/or memory $CD4^+$ and $CD8^+$ T cells in addition to NK cells, so KIR blockade might augment the antitumour activity of T cells in addition to NK cells. Phase I and Phase II trials in patients with acute myeloid leukaemia (AML) or multiple myeloma have demonstrated that treatment with anti-KIR is safe and has minimal side effects[113–115]. Although these early trials using anti-KIR as a monotherapy have not demonstrated significant efficacy, one might envisage synergy when it is combined with CTLA4 or PD1 blockade, which are known to induce systemic induction of pro-inflammatory cytokines that may further boost NK cell function.

NKG2A is another inhibitory receptor on NK cells and some T cells that recognizes an MHC class I ligand, HLA-E, and is planned for testing in human cancer patients[116]. Finally, NK cells express T cell Ig mucin receptor 3 (TIM3; also known as HAVCR2)[117] and lymphocyte activation gene 3 (LAG3)[118], two inhibitory immune checkpoint receptors currently under development as potent targets when combined with PD1 blockade[119–121], promising that NK cell antitumour activity might also be unleashed by these new immunotherapy combinations.

An alternative strategy to blocking inhibitory receptors with monoclonal antibodies for cancer therapy is to use agonist antibodies to augment the effector functions of T cells or NK cells. CD137 (also known as 4-1BB and TNFRSF9) is expressed on both cytotoxic T cells and activated NK cells, and agonist monoclonal antibodies augment antitumour activity in mouse models[122], although there are conflicting data on the role of CD137 in NK cell activation[123]. Agonist CD137 antibodies enhance the human NK cell-mediated killing of breast tumours[124] and B cell lymphomas[125] induced by trastuzumab and rituximab, respectively. Although agonist CD137 antibodies showed significant toxicities when used at high doses in the early clinical trials[126], lower doses might serve to increase antitumour efficacy when combined with other checkpoint blockade therapeutics or with tumour-specific antibodies that induce ADCC. However, care must be taken when combining agonist antibodies against co-stimulatory receptors, as this might increase off-target toxicities.

Other co-stimulatory molecules that have shown efficacy in mouse tumour models that are being considered as targets for agonist antibodies in cancer therapy include glucocorticoid-induced TNFR-related protein (GITR; also known as CD357 and TNFRSF18) and CD27 (REF. 127), both of which are expressed on NK cells. Combination of these agonists of NK cell co-stimulatory receptors with blockade of inhibitory receptors expressed by T cells and/or NK cells is a promising approach in future cancer therapies.

Using NK cells for cancer therapy

The first clinical use of NK cells involved infusing IL-2-activated (lymphokine-activated killer cells (LAK cells)) into cancer patients in the 1980s[128]. *In vitro* studies had documented the ability of activated NK cells to kill autologous tumours in patients, but the clinical efficacy of LAK therapy was limited by the toxicity of IL-2 and possibly by the IL-2-driven expansion of T regulatory (T_{Reg}) cells, which were unknown at that time. Further, the inhibitory receptors on NK cells, such as KIR and NKG2A, may limit their antitumour potential. The first evidence for the clinical benefit of NK cells was reported in 2002 by Velardi and colleagues[129] who observed that patients with AML who received allogeneic bone marrow transplants that were mismatched for KIR and HLA-C experienced a significantly lower rate of relapse, suggesting that the donor-derived NK cells were mediating an alloantigen-specific response against the AML without causing graft-versus-host disease (GVHD) (FIG. 3b). This strategy was extended by the adoptive cell transfer of *in vitro* activated allogeneic KIR-mismatched NK cells into patients with AML[130] and autologous NK cells into patients with malignant gliomas[131], which demonstrated partial, but modest, success. Results have seemed most promising when there is evidence of persistence and expansion of the NK cells after infusion[130]. A recent study has reported severe GVHD in some cancer patients given T cell-depleted allogeneic haematopoietic stem cell transplants infused with allogeneic NK cells pre-activated *in vitro* with IL-15 and TNFSF9 (also known as 4-1BBL)[132]. Although it is not known whether the GVHD was caused directly by the NK cells, or the NK cells induced a T cell-mediated GVHD response, this outcome was surprising given that NK cells have not been shown to cause GVHD in previous human clinical trials or in preclinical mouse models.

Although the therapeutic efficiency of adoptively transferred IL-2-activated NK cells seems to be limited, recent studies have reported that mouse NK cells activated *in vitro* with a combination of IL-12, IL-15 and IL-18 survived longer after adoptive cell transfer than naive cells and produced more IFNγ after stimulation[133]. These pre-activated NK cells had better effector functions *in vitro*, as well as enhanced proliferation, tumour infiltration and tumour control *in vivo* when tested in mouse models of lymphoma and melanoma[134,135]. Furthermore, recent work by Ardolino *et al.*[136,137] showed that treatment with these cytokines *in vivo* reversed the tumour-induced anergic state of NK

Lymphokine-activated killer cells
(LAK cells). Cytolytic lymphocytes, predominantly natural killer cells, that have been generated after stimulation with interleukin-2 and can spontaneously lyse cancer cells.

Alloantigen-specific response
An immune response directed towards polymorphic antigens expressed by cells of the same species, such as those from transplanted cells or tissues.

Graft-versus-host disease
(GVHD). A complication of allogeneic haematopoietic stem cell transplantation that occurs when contaminating T cells present in the graft recognize the recipient cells as foreign and mount an immune response against them.

Adoptive cell transfer
Refers to the administration of autologous or allogeneic cells to a recipient.

Anergic state
Characterized by the unresponsiveness of immune cells as a result of chronic stimulation or other immunosuppressive mechanisms.

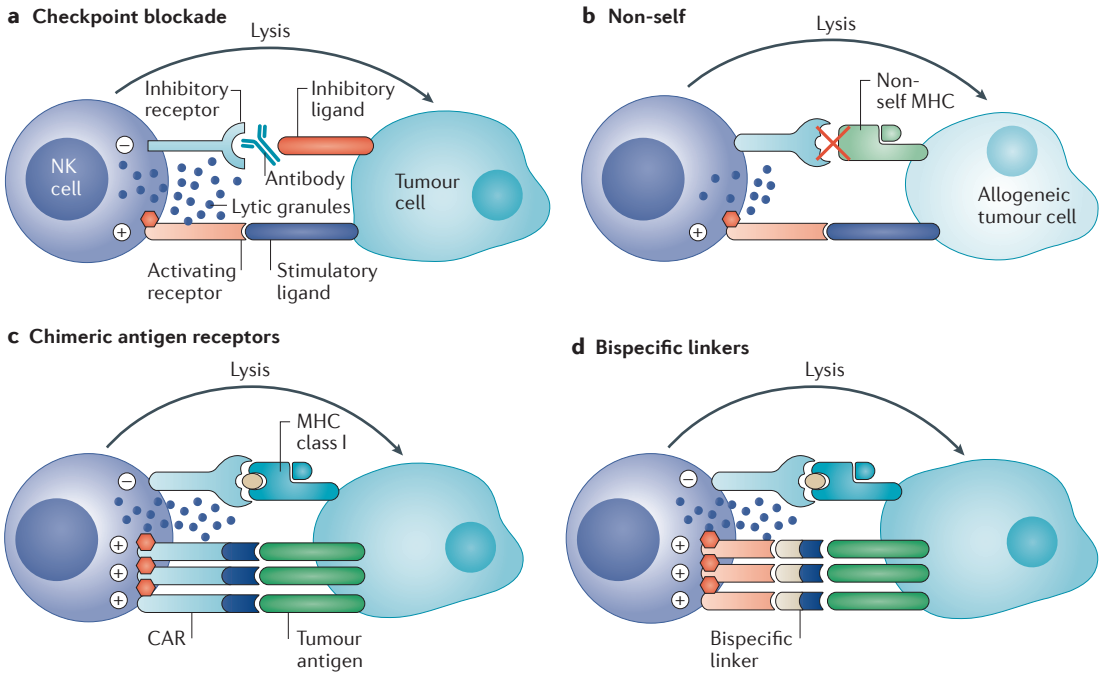

Figure 3 | **Schematic representation of therapeutic approaches that take advantage of NK cell potential.** **a** | Using checkpoint blockade that prevents the interaction of natural killer (NK) cell inhibitory receptors with their ligands, the signals dampening NK cell activation are suppressed. **b** | In the case of killer cell immunoglobulin-like receptors (KIRs) and major histocompatibility complex (MHC) class I mismatch between donor and recipient, inhibitory KIRs on adoptively transferred NK cells do not recognize MHC class I molecules expressed by allogeneic tumour cells, which are subsequently eliminated. **c** | NK cells can be transduced with activating chimeric antigen receptors (CARs) that specifically bind to antigens overexpressed by tumour cells. **d** | Bispecific molecules have been designed to recognize activating NK cell receptors on one arm and tumour antigens on the other, in order to redirect NK cell lysis towards tumours overexpressing these antigens.

cells in a mouse leukaemia model. Although cytokine therapy can augment NK cell antitumour activity, this approach may be limited by the systemic toxicity of cytokines such as IL-2, IL-12 and IL-15 (REFS 138,139).

Several challenges must be overcome to optimize the use of NK cells in cancer therapy, including blocking the inhibitory receptors that dampen NK cell activation (FIG. 3a), eliminating T_{Reg} cells that suppress their function, enabling NK cells to traffic into solid tumours, neutralizing immunosuppressive cytokines such as TGFβ secreted by the tumour (FIG. 2), eliminating myeloid-derived suppressor cells (MDSCs), and providing essential growth factors and cytokines that are required for NK cell activation, proliferation and persistence. Strategies to address these issues are rapidly being developed and may improve NK cell-based therapies in the near future.

Chimeric antigen receptors and NK cells. Whereas genetic engineering of immune cells has focused mainly on T cells, NK cells are potent effectors of antitumour immune responses and genetically modified NK cells provide another approach to improve the outcome of cancer immunotherapy[140] (FIG. 3c). Several recent studies have documented success using NK cells that were engineered to express activating chimeric antigen receptors (CARs) that are specific for tumour antigens[141]. In some studies, the NK-92 cell line (an NK cell leukaemia cell line) were transduced to express CARs specific for CD19 (REF. 142) and CD20 (REF. 143) expressed on B cell malignancies; disialoganglioside G_{D2}, a glycolipid expressed on neuroblastoma[144] and various other cancer types[145]; HER2 (also known as ERBB2), an antigen expressed by tumours of epithelial origin[146–148]; epithelial cell adhesion molecule (EPCAM), a molecule overexpressed by carcinomas and cancer stem cells[149]; HLA-A2 loaded with the melanoma antigen gp100 (also known as PMEL)[150]; prostate stem cell antigen (PSCA)[151]; and CD138 (also known as SYND1), which is expressed by multiple myeloma cells[152]. Because NK-92 cells are a tumour cell line infected with Epstein–Barr virus (EBV), they must be irradiated to prevent their proliferation before being adoptively transferred. For this reason, and also because NK-92 cells are allogeneic and have lost some important NK cell-activating receptors, including NCRs and CD16, other studies have used primary NK cells isolated from peripheral blood or derived from pluripotent stem cells[153,154]. In these studies, primary human NK cells that were transduced to express CARs specific for CS1 (also known as SLAMF7; which is expressed on multiple myeloma cells)[155], mesothelin (overexpressed by ovarian cancer)[156], CD19 (REFS 157–159), HER2 (REF. 160) and G_{D2} (REF. 161) showed increased responses to tumour cells *in vitro* and suppressed tumour growth when tested *in vivo* in xenograft models[162,163]. Although initially considered as receptors providing 'built-in' ADCC-like

activity against specific tumour antigens, CARs actually elicit a significantly stronger NK cell cytotoxic response than ADCC mediated by antibodies against the same targets[164]. This may be because the ligand-binding portion of the CAR has higher affinity than the Fc region of the IgG antibody for the CD16 Fc receptor on NK cells and because the cytoplasmic domain of the CAR has been engineered to maximize intracellular signalling. Because of their shorter lifespan, CAR-expressing NK cells might be superior to T cells, as they would not need 'suicide genes' to limit their expansion, in that NK cells, unlike T cells, are not able to produce autocrine growth factors such as IL-2, and this may also limit off-target side effects by CAR-bearing NK cells[165]. Additionally, even if the targeted antigens were rapidly lost on tumours, CAR-expressing NK cells would still be able to be stimulated by their endogenous activating receptors such as NKG2D, DNAM1 and NCRs. We look forward to the results of the Phase I clinical trials using primary NK cells expressing CARs for the treatment of leukaemia, as these will help to answer questions about how these modified NK cells behave in patients and compare with CAR-bearing T cells with respect to efficacy, longevity and side effects.

Conversely, because of their potent recognition of 'induced-self' ligands expressed by some tumour cells, some NK cell-specific receptors are potential candidates for CAR-engineered T cells. Studies by Sentman and colleagues[166] have shown the *in vitro* efficacy of T cells expressing CARs based on NKG2D. These T cells are cytotoxic against tumour cells, but also against immunosuppressive myeloid cells and T_{Reg} cells that transiently express NKG2DLs[167–169]. These 'improved' NKG2D CARs present advantages over the endogenous NKG2D receptors, because some of these CAR variants that do not associate with DAP10 (the signalling adaptor required for endogenous NKG2D receptor expression) are not down-modulated after exposure to the ligands[170]. Other NKG2D CAR variants enhance NK cell proliferation and cytokine production owing to their engineered signalling domains. However, a very serious concern with enhanced NKG2D CAR T cells is that NKG2DLs can be expressed on non-tumour tissues, which might cause autoimmune reactions. Indeed, a recent study reported that NKG2D CARs transduced into mouse T cells induced rapid lethal toxicity when adoptively transferred into an autologous mouse model[171]. As yet, there is limited experience *in vivo* with CAR-engineered NK cells to know the efficacy versus toxicity outcomes of this strategy.

Nonetheless, human T cells expressing a CD16-based CAR showed considerable antitumour activity when administered in combination with therapeutic antibodies in xenograft models[172]. Promising studies using T cells expressing CARs based on other NK cell receptors such as NKp46 (REF. 173) or NKp30 (REF. 174) showed good efficacy against various tumours expressing their respective ligands, although again expression of undefined ligands on healthy cells in the host might result in undesired toxicities. In summary, NK cells are very promising candidates for CAR-based therapies owing to their vast array of activating receptors that trigger spontaneous lysis of tumours via natural cytotoxicity or ADCC, in addition to CAR-mediated activation. Further, these NK receptors themselves represent considerable opportunities for the design of various new CAR strategies in T cells.

NK cell-targeted bispecific antibodies. In addition to conventional antibodies that are specific for tumour antigens that elicit ADCC, some bispecific proteins have been designed to recognize tumour antigens on one arm and bind to activating NK cell receptors on the other arm (FIG. 3d). The resulting crosslinking promotes the interaction between NK cells and tumour cells, while stimulating NK cells and triggering target cell lysis. Given the potency of ADCC, many of these bispecific proteins were designed in order to provide stronger and more stable binding to CD16 than the Fc region of conventional antibodies, given their relatively low affinity for CD16. Various approaches, such as using CD16-targeting Fv domains with higher affinity[175] and tetravalent proteins with two binding sites for CD16 for added stability[176], have proved successful in redirecting NK cell lysis towards tumour cells. Different tumour types have been targeted by linking these anti-CD16 Fv domains to Fv domains against CD19 (REF. 177) and HLA class II for B cell malignancies[178], CD30 for Hodgkin's lymphoma[176,179], epidermal growth factor receptor (EGFR), which is overexpressed in several epithelial

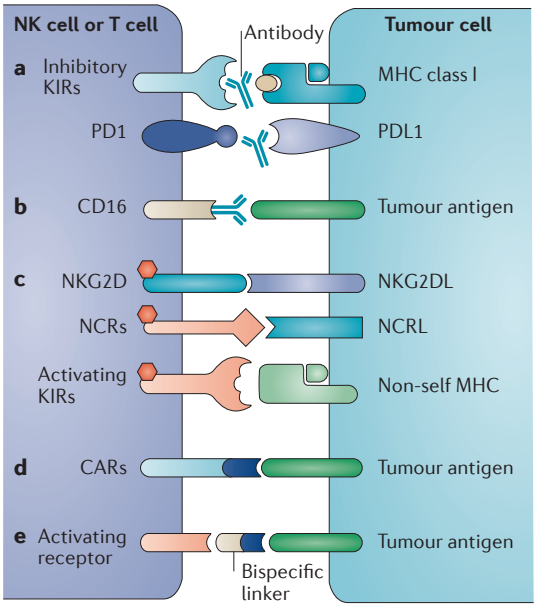

Figure 4 | **Schematic representation summarizing the different NK cell-based therapeutic approaches.** These strategies could be combined for optimal antitumour immune responses. Many natural killer (NK) cell receptors are also expressed on T cells, and T cells can also be transduced with chimeric antigen receptors (CARs) based on NK cell receptors; therefore, these immunotherapeutic combinations might involve both NK cells and T cells. KIR, killer cell immunoglobulin-like receptor; MHC, major histocompatibility complex; NCR, natural cytotoxicity receptor; NCRL, NCR ligand; NKG2DL, NKG2D ligand; PD1, programmed cell death protein 1; PDL1, PD1 ligand 1.

cancer types[175], HER2 (REFS 180,181) for breast cancer, CD33 for AML[182,183] and EPCAM[184] for carcinomas. The *in vitro* and *in vivo* efficacy of these optimized proteins in eliciting specific antitumour activity is encouraging and should be further developed in clinical settings.

Another NK cell receptor that has been targeted in bispecific protein design is NKG2D. One of these approaches was to create a bispecific protein that recognizes NKG2DLs expressed by tumour cells and CD3 in order to redirect T cell lysis[169]. Other NKG2D-based bispecific reagents have attempted to trigger NKG2D-dependent cytotoxicity using an NKG2DL on one arm and a tumour antigen-specific Fv domain on the other arm[185]. The first of this kind was a RAET1H–anti-CD138 fusion protein that bound to CD138, which is overexpressed in multiple myeloma[186], and crosslinked NKG2D on NK cells. Similarly, a RAET1H–anti-carcinoembryonic antigen (CEA) bispecific protein showed significant *in vitro* and *in vivo* efficacy against colon carcinoma[187]. Another study used recombinant MICA conjugated to monoclonal antibodies specific for CEA, HER2 or CD20, and their use resulted in NKG2D-dependent tumour cell lysis by NK cells[188]. Because T cells also express NKG2D, all bispecific proteins targeting this receptor have the advantage of co-stimulating both NK and T cells, as demonstrated in a study that used a fusion protein containing RAET1H and Fv domains specific for CD33 to redirect NK and T cell lysis towards AML cells[189]. For this reason and because they use a pathway that tumours try to evade by various mechanisms, this versatile NKG2D-based bispecific approach is very promising.

Conclusions

NK cells have a crucial role in immunosurveillance against tumour formation. However, when both the innate and adaptive immune systems fail and tumours develop, NK cells and their receptors can still be targeted in many therapeutic approaches (FIG. 4). From the adoptive transfer of genetically modified NK cells to optimization of the existing checkpoint blockade therapeutics and tumour antigen-specific antibodies and newly engineering bispecific molecules to engage an NK cell response, there are many opportunities to exploit NK cells in immunotherapeutic strategies to eliminate cancer. Many challenges remain, in particular that of overcoming the tolerance mechanisms used by NK cells to 'do no harm to self'. As such, it is important to continue the molecular and functional characterization of NK cells and their receptors, both at the bench and in clinical trials, so that future immunotherapies can be innovated and improved accordingly.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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