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Harnessing natural killer cells for cancer immunotherapy: dispatching the first responders

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Abstract

Natural killer (NK) cells have critical roles in the innate immunosurveillance of cancer and viral infections. They are ‘first responders’ that can spontaneously recognize abnormal cells in the body, rapidly eliminate them through focused cytotoxicity mechanisms, and potently produce proinflammatory cytokines and chemokines that recruit and activate other immune cells to initiate an adaptive response. From the initial discovery of the diverse cell surface receptors on NK cells to the characterization of regulatory events controlling their development and function, our understanding of the basic biology of NK cells has improved dramatically in the past three decades. This advanced knowledge has revealed increased mechanistic complexity, which has opened the doors to the development of a plethora of exciting new therapeutics that can effectively manipulate and target NK cell functional responses, particularly in cancer patients. This translational progression of the field is well underway, as modern medicine is learning to effectively exploit the beneficial innate effector responses of NK cells to effectively combat cancer. Here, we summarize the basic mechanisms regulating NK cell biology, review a wide variety of drugs, cytokines, and antibodies currently being developed and employed to stimulate NK cell responses, and briefly outline newly evolving NK cell adoptive transfer approaches to treat cancer.

Natural killer (NK) cells are innate lymphocytes with unique capacity to rapidly kill infected, transformed, allogeneic, or stressed cells without prior encounter. T and B cells are essential mediators of durable immune responses to infections and cancer and for decades have shared center stage in the debate of immune relevance. Interest in NK cells has continued to blossom, however, since it has become clear that they perform as both direct and supporting actors in inflammatory responses. Whereas T and B cells generate adaptive immunity toward ‘non-self’ foreign antigens through the expression of antigen receptors, NK cells can identify and rapidly attack stressed cells, notably tumour cells, that have downregulated class I major histocompatibility complex (MHC-I) expression, which was termed ‘missing self’ recognition[1]. Importantly, NK cells can also swiftly generate potent cytokine production, particularly the secretion of interferon (IFN)-γ, tumour necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokines that recruit other immune cells and promote T and B cells to generate a robust secondary adaptive response[2,3]. In addition, NK cells can be triggered by cells targeted by

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immunoglobulin g (IgG) opsonization to mediate antibody-dependent cellular cytotoxicity (ADCC)[2]. Although originally named for their capacity to spontaneously eradicate aberrant cells, NK cells have safety features, as they rarely elicit autoimmunity and can actually promote immune homeostasis to counteract autoimmune disease[3]. In addition, a subset of long-lived NK cells can exhibit memory-like recall responses, which is characteristic of adaptive immunity[4]. Thus, NK cells have numerous beneficial attributes, making them attractive targets for immunotherapeutics that can harness their potent antitumour effector mechanisms.

Substantial evidence supports a crucial role for NK cells in routine surveillance against cancer. For example, an eleven-year study found higher incidence of cancer development in individuals with low peripheral NK cell cytotoxicity responses[5]. Furthermore, patients with congenital NK cell deficiencies experience increased incidence of malignancies, although most of these are driven by a concomitant increase in tumour-driving Epstein Barr virus or human papillomavirus infections[6]. Given their prevalence in peripheral blood, NK cells are most effective in directly counteracting haematopoietic cancers, including acute myeloid leukemia (AML), multiple myeloma (MM), non-Hodgkin lymphoma (NHL), and chronic lymphocytic leukemia (CLL)[7,8]. They also have an important role in eradicating metastasizing tumour cells, as illustrated in recent noteworthy studies that demonstrated selective elimination of single breast tumour cells in circulation by NK cells as opposed to circulating clusters of cancer cells that are more resistant, and preferential NK cell-mediated attack of *SOX2*^{high} regenerative-lineage metastatic lung cancer cells, while *SOX9*^{high} cells escape[9,10]. Furthermore, preneoplastic senescent cells are targeted by NK cells, thereby potentially removing these cells that might otherwise progress to carcinomas[11]. In addition to direct tumour cell cytolysis, potent production of IFN-γ by NK cells can induce expression of a wide array of interferon response genes to elicit inflammatory antitumour effects[12].

An exciting era of translational research has led to the rapid development of numerous immunotherapeutic strategies to boost antitumour responses by NK cells. In this article, we review this trajectory by first summarizing the basic foundations of NK cell biology and how NK cells can become functionally exhausted within the immunosuppressive tumour microenvironment (TME). Next, we outline therapeutic agents that augment NK cell-mediated antitumour responses, ranging from current drugs and other innate immune stimulants under exploration to cytokines as well as antibodies that activate NK cells through blocking inhibitory checkpoint receptors or targeting ADCC responses. Finally, we briefly describe current strategies of adoptive NK cell therapy, including arming adoptively transferred NK cells with chimeric antigen receptors (CARs) to enhance antitumour specificity.

The biology of human NK cells

What defines a NK cell?

To understand how NK cells can be manipulated therapeutically, we must first comprehend how they are hard wired. NK cells are the predominant member of group 1 innate lymphoid cells (ILC1), and they are characterized by the production of the type 1 cytokines, IFN-γ

and TNF. The ILC1 family also includes a minor population of tissue resident intraepithelial cells that produce type 1 cytokines too, but also express both natural cytotoxicity triggering receptor 2 (NCR2, also known as NKp44) and integrin alpha-E (also known as CD103), unlike conventional NK cells[13]. NK cells constitute 5–15% of peripheral blood lymphocytes and are readily found in bone marrow, spleen, liver, lung, and other tissues, but are rare in lymph nodes[2,4,14]. The classic surface marker profile defining human NK cells in peripheral blood is $CD3^{-}CD56^{+}$, although expression of NKp80 (encoded by *KLRF1*) or CD7 can differentiate them from $CD56^{+}$ myeloid cells[4,15]. Two major subsets of circulating human NK cells are distinguished by expression levels of CD56, namely $CD56^{bright}$ NK cells, which are immature and constitute 5–10% of peripheral blood NK cells, and $CD56^{dim}$ NK cells, which are more mature and mediate robust serial cytotoxicity of aberrant cells[4,14]. $CD56^{bright}$ cells can extravasate from the circulation into tissues and lymph nodes through expression of L-selectin (also known as CD62L) and C-C chemokine receptor type 7 (CCR7) and potently produce cytokines, but exhibit limited cytotoxicity responses[2,4,14]. $CD56^{dim}$ cells can also produce cytokines, but are highly cytolytic toward susceptible target cells and can mediate ADCC, owing to expression of IgG Fc region receptor III-A (FcγRIIIa, also known as CD16)[2,4]. Importantly, the majority of tissue-resident NK cells are $CD56^{bright}$ and often express CD69 and CXCR6 in lymph node, spleen, bone marrow, tonsil, and liver[16,17]. Furthermore, upon stimulation with IL-2 or IL-15, both $CD56^{bright}$ and $CD56^{dim}$ NK cells express high levels of CD56 and become highly cytolytic[18]. Recent high resolution molecular studies have defined more intricate subsets of NK cells in a variety of tissues beyond the simple $CD56^{dim}$ versus $CD56^{bright}$ delineation, which was established based on studies in peripheral blood[13,16,19–21].

NK cells develop from bone marrow-derived progenitor cells in a thymus-independent manner[22]. In contrast to T and B cells, which express diverse antigen receptors generated by gene rearrangements mediated by recombination activating genes (*RAG1/2*), NK cells express a broad array of germline-encoded receptors[2,4]. IL-15 strongly stimulates NK cell proliferation, activation, and cytotoxicity and is essential for normal NK cell development and survival[23,24]. IL-2 can substitute for IL-15 to support NK cell proliferation *ex vivo* (as receptors for IL-2 and IL-15 (IL-2R and IL-15R) share common β (CD122) and γ chains (CD132) but is dispensable *in vivo*, since NK cells develop and function normally in IL-2-deficient mice[14,25].

How do receptors regulate NK function?

NK cells are regulated by a dynamic balance between signals transduced by a diverse set of activating and inhibitory receptors. NK cells explore their environment through physical interactions with adjacent cells, whereby NK receptors engage with ligands on other cells at contact interfaces called immune synapses. Most healthy cells display ubiquitous MHC-I expression, also known as class I human leukocyte antigens (HLA), which thereby elicits dominant inhibitory signaling, disengagement, and tolerance by NK cells[26]. Virus-infected and tumour cells often downregulate MHC-I[2,27], however, thereby escaping recognition by $CD8^{+}$ cytolytic T lymphocytes (CTL), since CTL rely upon an antigen receptor that detects antigenic peptides presented by MHC-I. By contrast, loss of this MHC-I inhibitory receptor ligand and/or upregulation of activating receptor ligands tips the balance toward

activation in the NK cell to trigger spontaneous attack[2]. An activating immune synapse initiates strong adhesion and focused exocytosis of cytolytic granules to release perforins that form membrane pores, and granzymes that induce apoptosis of the target cell[26]. Focused degranulation prevents damage to healthy adjacent bystander cells[26]. NK cells can also mediate delayed killing by expressing TNF ligand superfamily member 6 (also known as Fas ligand, FASL, or CD95L) or TNFSF10 (also known as TRAIL or CD253), to induce target cell apoptosis[2,3].

Fundamental signaling in NK cells involves activating receptor-induced stimulation of protein tyrosine kinases (especially Syk and ZAP-70) and inhibitory receptors that counteract tyrosine phosphorylation events by recruiting and activating protein tyrosine phosphatases (especially SHP-1 (also known as PTPN6) and SHP-2 (also known as PTPN11)[26]. The major activating and inhibitory receptors on human NK cells and their ligands are summarized in Table 1. The primary inhibitory receptors that recognize MHC-I are killer cell Ig-like receptors (KIRs, which detect the classical MHC-I molecules: HLA-A, HLA -B, and HLA-C), NKG2A (which heterodimerizes with CD94 and binds the non-classical MHC-I: HLA-E), and leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB-1, also known as ILT-2 or CD85j, which has broad specificity to classical and non-classical MHC-I)[2,28,29]. Activating receptors include the NCRs, (namely NKp30, NKp44, and NKp46, which recognize various ligands on tumour and stressed cells), CD16, NKG2D (which binds MICA/MICB and ULBP family stress molecules), DNAM-1 (also known as CD226, and which engages with Poliovirus receptor PVR and nectin-2, also known as CD155 and CD112, respectively), and signaling lymphocytic activation molecule (SLAM) family receptors (especially 2B4, which recognizes CD48, and SLAMF7, which is a self-ligand receptor)[2,4,30]. Some of these receptors are linked to dimers of transmembrane adaptors (TYRO protein tyrosine kinase-binding protein, also known as DAP-12, TCR-ζ, or FcR-γ, which contain immunoreceptor tyrosine-based activating motifs (ITAMs) for robust activation or DAP-10, which is co-stimulatory), whereas others contain cytoplasmic tyrosine motifs for intracellular signaling[26]. Several adhesion molecules on NK cells are also critical for assembling activating immune synapses with target cells, especially integrin alpha-L (also known as LFA-1)[26]. Importantly, subsets of T cells readily express many of these NK cell receptors, so therapeutic targeting of them will also impact some T cells.

NK cells also express structurally-related families of receptors that contain both activating and inhibitory members, some of which interact with the same ligands. For example, KIRs have distinct members (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5, and KIR3DL1, KIR3DL2 and KIR3DL3) with long cytoplasmic domains (L) that contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) to mediate inhibitory function. Alternatively, other KIRs (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5 and KIR3DS1) have short (S) cytoplasmic domains lacking the ITIM, but associate with DAP12 to transduce activation signals[28]. Similarly, whereas NKG2A is an inhibitory receptor containing ITIMs, NKG2C is an activating receptor lacking ITIMs and associating with DAP12[26]. Additionally, NCR family members have differentially spliced gene products, some that encode classic activating receptors, and others that encode suppressive forms[30]. The complexities and expression conditions for differentially spliced NCRs are still under investigation. Another mixed function NK receptor family consists of the co-stimulatory DNAM-1 and suppressive

CD96 (also known as TACTILE), TIGIT, and PVRIG (also known as D112R)[31,32], all of which share ligands upregulated on various tumours (Table 1).

KIRs diversify, tolerize, and mature

KIRs are highly polymorphic and provide the human NK cell repertoire with extensive diversity, through three strategic attributes.[28] First, each person is capable of inheriting a different haplotype of the 14 available KIR family member genes (Figure 1A). This varied gene content can include almost entirely inhibitory KIRs (haplotype A), or a more diverse repertoire enriched with more activating KIRs (haplotype B). Second, individual KIRs recognize distinct subsets of the classical MHC-I ligands (Figure 1B). The inhibitory KIRs have co-evolved with MHC-I in primates to detect HLA-C via KIR2DL1, KIR2DL2, or KIR2DL3, subsets of HLA-B and HLA-A (those containing the Bw4 epitope) by KIR3DL1, and certain HLA-A (particularly HLA-A3 and -A11) by KIR3DL2. Importantly, since KIR and HLA gene loci are located on separate chromosomes, they are differentially inherited, and some KIR are inherited and expressed, yet lack an available cognate ‘self’ MHC-I ligand. Third, the available KIR genes are randomly expressed on individual NK cells to generate a diverse repertoire. Owing to this variegated KIR expression, the loss of one allele of MHC-I could make a tumour cell susceptible to a subset of NK cells that expressed only the KIR that has lost its cognate ligand (Figure 1C).

In view of the variegated expression of KIRs and other receptors, mass cytometry analysis of receptors on peripheral blood NK cells in adult healthy donors revealed a repertoire of between 6,000 and 30,000 distinct NK cell ‘clones’ within each donor, as defined by distinct combinations of expressed receptors[33,34]. Owing to this diversity, a subset of NK cell clones would potentially have an optimal receptor expression phenotype to respond to the unique ligand expression characteristics of a particular tumour cell. Once an NK cell clone has been activated toward a compromised target cell, it can proliferate and terminally differentiate to generate long-lived ‘memory’ NK cells capable of mounting a more rapid secondary response, thereby providing an adaptive immune response[35]. A prominent example is the expansion of $NKG2C^{+}$ $CD57^{+}$ NK cells in response to human cytomegalovirus infection, which are also referred to as ‘adaptive-like’ NK cells that are long-lived and exhibit strong antitumour responsiveness[21,36,37].

The inheritance of certain haplotypes of KIR and MHC-I ligands has been associated with susceptibility to diseases, including psoriatic arthritis, viral infections, cancer, and pre-eclampsia in pregnant women[38]. Furthermore, inhibitory KIR/MHC-I mismatch has been shown to be beneficial under defined conditions of haematopoietic stem cell transplantation and adoptive NK cell therapies[39].

Although inhibitory KIRs and NKG2A have key roles in enabling NK cells to tolerize healthy MHC-I-expressing cells, they also have a second critical role in instructing NK cells to be functionally competent during their developmental maturation. During normal development, early stage NK cell precursors stochastically express individual KIR genes in ‘sequential acquisition’ until one inhibitory receptor recognizes a ‘self’ MHC-I molecule to effectively transduce a negative signal and restrict expression of additional inhibitory receptor genes[40]. This process generates a repertoire of NK cells, each of

which permanently expresses a defined combination of inhibitory receptors. Successful engagement of an inhibitory KIR or NKG2A with MHC-I ligand promotes maturation to a functionally responsive NK cell through a process that has been called ‘education’ or ‘licensing’ (Figure 1D). Educated mature NK cells are subsequently capable of being activated upon encounter of an MHC-I-deficient target cell[41,42]. By contrast, a small subset of NK cells never successfully expresses an inhibitory receptor that can recognize endogenous MHC-I, and these remain hyporesponsive. NK cells in patients who are deficient for antigen peptide transporter 1 (TAP1), who lack normal MHC-I expression, are similarly hyporesponsive[43]. Unlicensed or uneducated NK cells can, however, be ‘primed’ to achieve a functional state if cultured with IL-2 or IL-15, which suggests they may become activated under certain conditions *in vivo* and may have a lower threshold of activation toward MHC-I-expressing tumours[44]. Education is also evident in patients after receiving MHC-I-mismatched cord blood transplants, as the most responsive NK cells that develop in these patients express a KIR for which both donor and recipient express the cognate ligand[45]. If, however, the patient lacks the cognate MHC-I ligand for a particular inhibitory KIR, the NK cells expressing that KIR lose some responsiveness[45].

As an overview, maturation, survival and functionality of NK cells strongly depends on suppression of activation signaling mediated by tonic inhibitory receptors, which starkly contrasts with the primary need in T and B cells for antigen receptor-mediated activation signaling to support the same processes. In a striking example of this fundamental signaling dichotomy, mice deficient in the activation signaling protein B-cell adaptor for phosphoinositide 3-kinase (BCAP, encoded by *PIK3AP1*) have B cells that are less mature, less functional, and more susceptible to apoptosis, whereas NK cells that develop in the same mouse have the exact opposite phenotype[46].

NK Cells in the TME

While haematopoietic tumours in peripheral blood are readily accessible to NK cells, solid tumours are more challenging to reach and infiltrate. Nonetheless, increased influx and activation of NK cells in solid tumours has been associated with longer overall survival in a variety of cancers[47]. To reach solid tumours, NK cells must extravasate from the blood and traverse the extracellular matrix and tumour stroma. The expression of L-selectin allows immature $CD56^{bright}$ NK cells to contact high endothelial venules and leave the vasculature for secondary lymphoid organs, and CCR7 expression also draws them toward C-C motif chemokine 21 (CCL21) expressed on these lymphatic endothelial cells and toward CCL19 and CCL21 in T cell zones of lymph nodes[48,49]. NK cells can degrade and thereby traverse the extracellular matrix through expression of matrix metalloproteinases, urokinase plasminogen activator, and serine dipeptidylpeptidase IV[50]. In contrast to those found in blood, NK cells in most normal peripheral tissues are mainly $CD56^{bright}$, and this subset is even more enriched in tumours[51]. Once in a tumour, however, NK cell responsiveness is often hindered by the immunosuppressive TME.

The immunosuppressive TME can drive NK cells into an exhausted state. Tumour growth factor beta (TGF-β) is readily expressed in most TMEs and is an important suppressor of NK cells through a variety of mechanisms[52,53]. TGF-β drives differentiation of NK

cells into intraepithelial ILC1, which are less capable of mediating antitumour cytolytic responses, are retained in tissue upon upregulation of integrin alpha-1 (also known as CD49a) and CD103, and lose expression of eomesodermin homolog (EOMES), the loss of which is a sign of NK cell exhaustion[52,54,55]. The TME is also hypoxic, which can downregulate expression of NCRs and NKG2D and reduce natural cytotoxicity by NK cells[56]. Hypoxia also induces hypoxia-inducible factor 1α (HIF-1α) expression in tumour-infiltrating NK cells, which suppresses their antitumour and IFN-γ production responses[57], and deletion or pharmacological inhibition of HIF-1α substantially potentiates NK cell antitumour responses[57]. Dysfunctional NK cells in liver cancer exhibit mitochondrial fragmentation, which is associated with lower oxidative phosphorylation, increased reactive oxygen species (ROS), and a hypoxic gene expression signature that contributes to poor antitumour response and increased apoptosis[58]. Also, NK cell dysfunction in the TME is driven by oxidative stress-induced suppression of glucose metabolism[29]. Additional suppressive mediators in the TME that can inhibit NK cell function include prostaglandin E2[59], 5'-deoxy-5'-methylthioadenosine[60], indoleamine 2,3-dioxygenase (IDO)-mediated catabolism of tryptophan to L-kynurenine[61], lactate-mediated acidification causing mitochondrial distress[62], and adenosine produced from ATP by the ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, also known as CD39) and 5'-nucleotidase (also known as CD73) ectoenzymes[63], as well as limited glucose availability to support glycolysis by NK cells[64]. Furthermore, expression of members of the a disintegrin and metalloproteinase (ADAM) family within the TME can cleave and release soluble forms of ligands for NKG2D and NKp30, thereby limiting NK cell recognition and activation[65]. Given the harsh environment in the TME, successful pharmacological potentiation of NK cell responses in tumours will likely require multiple approaches to overcome these many immunosuppressive barriers.

Agents enhancing NK cell function

Immunomodulatory imide drugs

Immunomodulatory imide drugs (IMiDs) are a class of drugs containing an imide group that enhance the function of lymphocytes. The IMiDs include thalidomide and the related compounds, lenalidomide and pomalidomide, which are used to treat MM, myelodysplastic syndrome (MDS) and some lymphomas[66]. Pomalidomide has higher potency and shows efficacy in patients who are resistant to lenalidomide and thalidomide[67]. At least part of the immunotherapeutic benefit of IMiDs is believed to result from enhanced NK cell activity. One of the primary mechanisms by which IMiDs activate NK cells is through inducing IL-2 production by T cells. Lenalidomide, however, can also directly enhance NK cell responses by lowering their activation threshold for CD16- and NKG2D-mediated stimulation of IFN-γ production[68]. In addition, IMiDs can make malignant plasma cells more susceptible to NK cells by increasing their expression of the NKG2D and DNAM-1 receptor ligands, MICA and PVR, respectively[69].

A primary molecular mechanism of IMiD action involves interaction with cereblon (CRBN), which forms a complex with cullin-4A (CUL4A), DNA damage binding protein-1 (DDB1), and E3 ubiquitin-protein ligase RBX1 (also known as ROC1) to mediate E3 ubiquitin ligase

activity[70–72]. Thalidomide, lenalidomide and pomalidomide have similar binding modes and affinities for CRBN, and this binding promotes CRBN to more efficiently interact with the transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) and induce their targeted ubiquitination and proteolytic degradation[71–73]. IKZF1 and IKZF3 normally suppress *IL2* mRNA expression in T cells, and their degradation is responsible for the increased secretion of IL-2 in lenalidomide-treated T cells[71].

Pomalidomide can also directly bind and activate tyrosine-protein kinase ZAP-70 in T cells and NK cells, and kinase activation was independent of CRBN[74]. In addition, pomalidomide can induce granzyme-B expression in NK cells, and this increase depends on ZAP-70 activation[74]. Therefore, IMiDs can enhance NK and T cell function by ZAP-70-mediated and CRBN-mediated mechanisms and offer potential to restore the activity of NK cells in the immunosuppressive TME. Additional next-generation CRBN-binding drugs are also under development with improved capacity to degrade IKZF1 and IKZF2 ubiquitylation targets[73].

Proteasome inhibitors

In vitro treatment of tumour cell lines with the proteasome inhibitor bortezomib upregulates expression of ligands for NKG2D and DNAM-1 substantially, increasing their susceptibility to NK cell-mediated cytotoxicity[75–77]. MICA upregulation by bortezomib is dependent on the ATM/CHK2 DNA damage response pathway[78]. To further increase susceptibility to NK cells, bortezomib also downregulates MHC-I expression on MM cells treated overnight *in vitro* or when assayed in blood from patients 48 hours after a single dose[79].

STING agonists

The cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes protein (STING) pathway has a key role in innate immune sensing of aberrant cytosolic DNA from viral or bacterial pathogens. Activation of this pathway generates type I interferons (IFN-α and IFN-β) and proinflammatory cytokines and chemokines[80,81]. cGAS converts double-stranded viral DNA into 2′,3′-cyclic guanosine monophosphate–adenosine monophosphate (cGAMP)[80], which binds to STING in the endoplasmic reticulum membrane[80]. The cGAMP-STING complex recruits protein kinase TBK1 and activates interferon regulatory factor 3 (IRF3) and nuclear factor kappa B (NFκB) transcription factors[80]. In mice, tumour cells were found to produce cGAMP, which activated STING in neighboring myeloid cells to produce IFN-β that subsequently activated NK cells to mediate an antitumour immune response[82].

STING agonists, such as cyclic dinucleotides (CDN), can stimulate $CD8^+$ T cells and NK cells[83]. CDN treatment slowed tumour growth in a wide variety of mouse cancer models and this effect was independent of $CD8^+$ T cells[84]. NK-mediated antitumour effects were dependent on type I IFNs produced by dendritic cells (DCs), macrophages, monocytes, and endothelial cells[84]. These cells can have a pivotal role in the antitumour effect of intratumoral CDN injection[83,85,86]. The type I IFNs produced in response to CDN treatment induced IL-15Rα and IL-15 production by DCs, which resulted in systemic activation of NK

cells[84]. Several clinical trials investigating different STING agonists to treat lymphomas are currently ongoing (e.g. NCT04144140 and NCT04609579).

Cytokines to stimulate NK cells

A number of cytokines have an effect on NK cell function and are being investigated for stimulating NK cells in patients using various forms of the activating cytokines or antagonists of an inhibitory cytokine. The general impacts and active clinical trials of these are listed in Table 2.

IL-2 and IL-15

IL-2 has long been known to directly stimulate the proliferation and activation of T and NK cells[87,88]. Originally, stimulation of human peripheral blood mononuclear cells with IL-2 was found to expand cytotoxic cells, which were named lymphocyte-activated killer (LAK) cells[87–89]. Subsequent work found that tumour destruction by LAK cells was predominantly mediated by NK cells[90]. IL-2 is still commonly used for *ex vivo* NK cell stimulation, but therapeutic use is limited by common toxicity, which ranges from flu-like symptoms to severe capillary leak syndrome with high doses[91]. Moreover, IL-2 induces the proliferation and activation of highly suppressive regulatory T (Treg) cells expressing inducible T-cell costimulator (ICOS)[92]. Various groups are developing modernized variations of IL-2 with improved toxicity profiles, such as ALKS 4230 and F42K-modified IL-2, which induce greater activation and expansion of NK cells with reduced expansion of Treg cells[92,93].

The most promising therapeutic substitute for IL-2 is IL-15. Importantly, IL-15 stimulates NK and $CD8^+$ T cells, but not Treg cells[94]. As mentioned before, both IL-2 and IL-15 receptors share common receptor elements, namely β-chain and common γ-chain heterodimers, that are expressed on NK cells. IL-2R also utilizes the IL-2Rα chain (CD25) for high affinity binding, whereas IL-15R uses the IL-15Rα chain (CD215) for high affinity IL-15 binding. The primary method of IL-15 signaling in NK cells involves ‘transpresentation’ of the cytokine in a bioactive complex with IL-15Rα expressed on the surface of another cell, often DCs and monocytes, as well as in a soluble paracrine complex in serum[95,96].

In vitro studies have shown that IL-15 can potentiate the function of NK cells exhausted by the TME. Treatment of dysfunctional tumour-derived NK cells with IL-15 under hypoxic conditions restored mitochondrial integrity, increased expression of granzyme B, reduced apoptosis, and enhanced cytotoxicity and IFN-γ production[58]. Treatment with IL-15 also restored the function of NK cells exposed to H_2O_2-mediated oxidative stress by upregulating expression and activity of the anti-oxidant, thioredoxin-1, to increase cell surface thiol levels and reduce accumulation of ROS[97].

Owing to strong impact on NK and cytotoxic T cells, several recombinant forms of IL-15 have been engineered for clinical use. Subcutaneous dosing of recombinant IL-15 stimulated activation and robust expansion of NK and $CD8^+$ T cells, and was well tolerated in patients, although serious cytokine release syndrome was observed with high dosage in patients that had received lymphodepletion and adoptive transfer therapies[98,99]. More recent

innovations involve engineered fusion complexes of recombinant IL-15 and IL-15Rα. One heterodimeric fusion (hetIL-15; NIZ985) packaged in extracellular vesicles is currently in phase 1/1b clinical trials[100]. In preclinical studies, hetIL-15 slowed tumour growth and increased tumour infiltration with NK cells and $CD8^+$ T cells with increased expression of IFN-γ, cytotoxic granule components, and anti-apoptotic BCL-2[101]. Another promising IL-15 'superagonist' is N-803 (formerly called ALT-803), which consists of recombinant IL-15 containing an N72D mutation that increases affinity to a dimeric IL-15Rα sushi domain-IgG1 fusion protein to facilitate transpresentation and prolong half-life in blood[102]. In preclinical studies, N-803 induced tumour regression in mouse models and prolonged survival compare to unconjugated IL-15[103,104]. Subcutaneous administration of N-803 in patients with leukaemia or lymphoma was safe, stimulated expansion of NK cells and $CD8^+$ T cells, but not of Treg cells, induced expression of NK cell receptors, and showed some indications of clinical efficacy[105,106]. Early injections induced transient increase in serum IL-6 and IFN-γ associated with minor flu-like symptoms[105,106]. N-803 is currently being tested in numerous clinical trials (Table 2). Caution must be stressed in treating patients with IL-15, however, since long term exposure has been shown to cause NK cell dysfunction through metabolic and epigenetic reprogramming mechanisms[107,108].

IL-12

IL-12 is a heterodimeric pro-inflammatory cytokine primarily produced by antigen-presenting cells, especially DCs and macrophages that stimulates the recruitment, effector functions, and IFN-γ production of $CD8^+$ T cells and NK cells[109]. In addition to direct activation of NK cells, IL-12 stimulates T helper 1 (T_H1) cells to produce IFN-γ and IL-2, which subsequently can stimulate NK cells to produce more IFN-γ, perforin, and granzyme B[110].

Despite these positive effects on NK cell function, systemic administration of IL-12 causes numerous adverse side effects, including flu-like symptoms (fever, fatigue, headache, chills, and arthromyalgia) toxicity to liver (increased transaminases, hyperbilirubinemia, and hypoalbuminemia) and bone marrow (neutropenia and thrombocytopenia)[111]. Owing to these adverse effects, maximum tolerated doses have shown limited anti-cancer efficacy, so alternative methods of IL-12 delivery are being developed.

Alternative IL-12 delivery strategies can be divided into three groups: fusion molecules of IL-12 with tumour binding antibodies, introducing genetic material encoding IL-12, and controlled release of recombinant IL-12 from a delivery system, such as nanoparticles[112]. In mouse models immunocytokine constructs fusing IL-12 protein to targeting antibody domains can activate T cells and NK cells more efficiently at tumour sites than soluble IL-12[113,114]. Direct intratumoural delivery of DNA or RNA encoding IL-12 into tumour has been explored. A recent phase II clinical trial testing electroporation of accessible melanomas with the *IL12*-encoding plasmid tavokinogene telseplasmid (tavo) in combination with the programmed cell death protein 1 (PD-1) inhibitor pembrolizumab showed increased NK cell-associated gene expression in tumours and enhanced antitumour immune responses[115]. Engineered viruses expressing IL-12 are also being tested, with several clinical trials ongoing (Table 2). Nanoparticle approaches are also being tested to

deliver IL-12[112]. Another promising strategy is intratumoural injection of IL-12 expressed on the surface of exosomes, which increased tumour infiltrating NK and $CD8^+$ T cells in mouse studies and kept the cytokine localized to the injection site in mice and monkeys to reduce systemic toxicity[116]. Clinical trials delivering IL-12 with many of these technologies are still pending, but promising preclinical studies indicate that localized delivery of the cytokine will reduce adverse effects substantially.

IL-18

IL-18 is a proinflammatory member of the IL-1 cytokine family produced by a wide variety of cells, including myeloid cells, intestinal epithelial cells, keratinocytes, and endothelial cells[117]. It is produced as pro-IL-18 and converted into mature cytokine by caspase 1-mediated cleavage through activation of the inflammasome pathway, whereupon it binds a heterodimeric receptor composed of IL-18Rα and IL-18Rβ.[118] IL-18 (especially in combination with IL-12) induces FASL expression in NK cells and potentiates production of IFN-γ and TNF[118]. Moreover, IL-18 induces proliferation of NK cells and expression of the CCR7 chemokine receptor for CCL21, which promotes migration to secondary lymphoid organs[118]. In mice, the combination of IL-18 antibodies against PD-1 and cytotoxic T-lymphocyte protein 4 (CTLA4) is much more effective than the antibodies alone, and the response was significantly abrogated upon depletion of NK or $CD8^+$ T cells[119].

Owing to these favourable functions, IL-18 is being investigated in the clinic, with initial clinical trials showing tolerable toxicity[120]. Phase I trials testing the combination of IL-18 and anti-CD20 antibodies for treatment of B-cell lymphomas showed increased IFN-γ in serum and objective responses in some patients who were previously refractory to anti-CD20 antibodies alone[121,122]. Nonetheless, despite the relatively low toxicity profile, few clinical trials have studied IL-18 infusions to treat cancer, since efficacy has been limited. Impediments to the positive therapeutic impact of IL-18 on NK cells are the endogenous immune proteins interleukin-1 receptor 8 long isoform (IL-1R8, which suppresses IL-18 receptor function) and IL-18 binding protein (IL-18BP, which inactivates IL-18 in serum)[123,124]. A modified form of IL-18 that is unable to bind to IL-18BP, but still stimulates the IL-18 receptor is a promising alternative currently in clinical trial (NCT04787042)[125].

IL-21

IL-21 is produced by T_H17 cells and DCs, and signals through a heterodimeric receptor composed of IL-12Rα and γc, which is the common γ chain also shared by IL-2R and IL-15R[118]. IL-21 enhances the maturation and proliferation of NK cells and potentiates their cytotoxic activity by increasing expression of perforin and granzyme B[126], as well as upregulation of NKp30 and NK cell receptor 2B4[127]. By contrast, however, culture of human primary NK and $CD8^+$ T cells with IL-21 and IL-2 significantly decreases cell surface expression of NKG2D, compared to IL-2 alone, which parallels reduced DAP10 transcription[127]. The combination of IL-21 with other cytokines such as IL-2, IL-15 and IL-18 increases NK cell proliferation, cytotoxicity and IFN-γ production[128,129]. IL-21 infusions were safe and well tolerated in clinical trials, although clinical efficacy of the cytokine alone was limited, which has prompted testing in combination therapies[130]. It

should also be cautioned that IL-21 is a growth factor for B cells and can thereby promote the proliferation of some B cell neoplasms, such as MM, Burkitt lymphoma, and Hodgkin lymphoma[130].

TGF-β inhibitors

TGF-β strongly inhibits NK cell functions and promotes NK cells to differentiate into intraepithelial ILC1, which are tissue resident and less cytolytic toward tumours[52,54,55]. TGF-β is often expressed within the TME, so a strategy to inhibit TGF-β in patients has potential to restore NK cell function and suppress tumour growth.

Approaches to inhibit TGF-β that have been tested in clinical and pre-clinical studies include neutralizing antibodies, ligand traps, and receptor kinase inhibitors[131]. The neutralizing monoclonal antibody (mAb) anti-TGF-β1/TGF-β2 fresolimumab (GC1008) has been tested in several clinical trials and was well tolerated, but exhibited only modest responses and elicited reversable cutaneous keratoacanthomas, hyperkeratosis, and carcinomas[132]. A clinical trial of fresolimumab with local hypofractionated radiation in patients with metastatic breast cancer found increased circulating NK cells and $CD8^+$ effector and central memory T cells, but surprisingly also increased Treg cells, demonstrating broad disruption of T cell homeostasis[133]. Galunisertib (LY2157299) is a small molecule inhibitor of TGF-β receptor complex TGFβRI kinase that was found to improve survival of neuroblastoma-bearing NSG mice treated with an ADCC-inducing antibody and adoptively transferred NK cells[134]. Treatment of patients with liver and pancreatic cancer with galunisertib was well tolerated and showed improved overall survival, although NK cells were not analyzed in treated patients[135,136]. Although TGF-β targeting agents show promising potential, their effects on NK cell activity and functions have been limited in patients.

Antibodies to boost NK cell function

Checkpoint blocking antibodies

Since NK cell functional responses are determined by the integration of activating and inhibitory receptor signaling, targeting inhibitory receptors with blocking antibodies offers an enticing strategy to potentiate the NK cell antitumour response in cancer patients (Figure 2). Below, we summarize the major checkpoint targeted therapies impacting NK cells that are currently undergoing clinical trials.

PD-1, PD-L1, and CTLA-4 inhibitors—Immune checkpoint blockade targeting PD-1, its ligand PD-L1, or CTLA4 has established a revolutionary breakthrough for overcoming T cell exhaustion to reinvigorate antitumour responsiveness. Characterization of immune changes in response to these treatments indicate that NK cells can play an ancillary part to support antitumour responses during T cell-directed checkpoint blockade[137,138]. Although the direct impact of PD-1/PD-L1 blockade is less obvious for NK cells than T cells, preclinical studies have shown that it can enhance ADCC-induced antitumour function[139,140]. Also, ADCC-induced IFN-γ production by NK cells can promote expression of PD-L1 on the surface of tumour cells, thereby indicating a synergistic value

in adding PD-1 blockade to ADCC immunotherapy[138,141]. A subset of NK cells can express PD-1[142], including low-level expression on resting NK cells and a single chain Fv of PD-1 antibody was recently shown to increase their responsiveness toward PD-L1-expressing target cells[143], which suggests that NK cells can also be a direct target for stimulation by PD-1 blockade. The low levels of PD-1 detected on the surface of NK cells, however, has raised doubts of their inhibitory potential when engaged with PD-L1 on tumours[144]. In fact, it has been suggested that PD-1 expression on NK cells may be passively acquired from other cells through trogocytosis[144], which is a mechanism by which NK cells can acquire a variety of transmembrane proteins[145,146]. In contrast, many NK cells reportedly express PD-1 mRNA transcripts and protein in the cytoplasm, suggesting that certain TMEs may be able to induce upregulation of PD-1 on the NK cell surface[147]. Accordingly, in mouse models of cytomegalovirus infection, the production of glucocorticoids resulted in upregulation of PD-1 surface expression on mouse NK cells[148]. Interestingly, corticosterone upregulated PD-1 on mouse NK cells when cultured with IL-15 and IL-18, but further addition of IL-12 abrogated this effect[148]. Alternatively, an intriguing study found that PD-L1 is upregulated on activated NK cells, and binding of PD-L1 blocking antibodies to these NK cells stimulates their cytolytic and cytokine responses to PD-L1^{-} tumour cells[149,150]. The authors propose that this mechanism could explain the effectiveness of PD-L1 antibody therapies in patients with PD-L1^{-} tumours.

Whereas CTLA-4 blockade has generally been studied within the context of T cells, several studies suggest that NK cells can contribute to positive outcomes in patients with melanoma treated with the anti-CTLA-4 mAb, ipilimumab[151,152]. Sanseviero et al. observed in mouse tumour models treated with CTLA-4 blockade that NK cells assisted in tumour control by clearing anti-CTLA-4 opsonized Treg cells, which has implications for human therapy as well, since ipilumumab is an IgG1 that can mediate ADCC[152]. Also, intracellular CTLA-4 protein was found in NK cells derived from human lung tumour tissue and in human NK cells stimulated with cytokines[153,154]. It is unclear, however, if CTLA-4 blockade can directly impact NK cell-mediated tumour clearance in view of its low surface expression, similar to PD-1[155].

KIRs and NKG2A inhibitors—The first immune checkpoint blocking antibody developed to stimulate NK cell responses was the KIR antibody, lirilumab (BMS-986015). Lirilumab is a fully human IgG4 that targets KIRs with two Ig-like domains, specifically inhibitory KIR2DL1, KIR2DL2, and KIR2DL3, as well as activating KIR2DS1 and KIR2DS2[156]. Therefore, it has broad specificity for the HLA-C-specific family of KIR2D receptors. Promising preclinical evidence supported the potential for use of lirilumab in patients[157]. In the first results of a Phase II clinical trial studying lirilumab as a monotherapy for treating AML, however, there was no measurable benefit compared with placebo (NCT01687387). After this setback, lirilumab trials have focused on combination therapies, most notably with the PD-1 antibody, nivolumab. Currently only one basket clinical trial (NCT02813135) is actively recruiting paediatric patients with refractory or recurrent malignancies to receive lirilumab in combination with nivolumab.

Another NK cell checkpoint blocking antibody is monalizumab (IPH2201), which is a humanized IgG4 blocking NKG2A. NKG2A is prominently found on solid tumour-

infiltrating NK cells[51,158], whereas its ligand HLA-E is also upregulated in many different types of tumours, most notably in ovarian cancer, breast cancers, non-small cell lung cancer (NSCLC), and head and neck cancer[159,160]. Similar to lirilumab, phase II results of monalizumab suggested limited effectiveness as a monotherapy[161]. Several clinical trials of monalizumab in combination with other agents are currently active (NCT04590963, NCT02643550, NCT04307329, NCT02671435). It should be noted that a Phase I/II trial studying the combination of monalizumab and the tyrosine-protein kinase BTK inhibitor ibrutinib to treat CLL was recently terminated owing to serious adverse effects (NCT02557516). However, ibrutinib is known to interact with many off-target kinases and has been shown to disrupt NK cytotoxicity in patients[162], so the adverse results in this combination may not reflect the safety and efficacy of monalizumab treatment in other contexts.

We have previously reported that a cocktail of antibodies blocking NKG2A and all KIRs was necessary to significantly enhance *in vitro* ADCC responses by primary human NK cells[163]. Therefore, combination therapy with both monalizumab and lirilumab may be worth testing in clinical trials to achieve an NK cell activation threshold more effectively than with either antibody alone, particularly in combination with an additional ADCC-inducing antitumour antibody.

Caution should be exercised in KIR and NKG2A antibody blocking therapies, however, owing to the potential risk of disrupting NK cell functional responsiveness. As previously discussed, inhibitory receptors not only tolerize mature NK cells from attacking normal cells but are also critical in educating the developing NK cells to become fully functional. Accordingly, lirilumab blockade of KIR2D receptors in patients suppressed the responsiveness of the KIR2D-expressing NK cells in peripheral blood, and the authors concluded that this was due to disrupting the normal education process, thereby reverting the NK cells to a hyporesponsive state that would serve as poor effectors for tumour clearance[164]. This does not entirely rule out checkpoint blockade as a therapeutic option to enhance antitumour responses by NK cells, but it may not be effective as a long term therapy and may require short-term or pulsed treatment regimens to periodically boost NK cell responses.

TIGIT, TIM3, and LAG3 inhibitors—There is potential for expanding checkpoint blocking therapy in NK cells by exploring checkpoint receptors that have already been established in T cells. Some of these receptors, such as TIGIT, hepatitis A virus cellular receptor 2 (also known as TIM-3), and lymphocyte activation gene 3 protein (LAG-3) are also expressed on peripheral and tumour infiltrating NK cells, and can be further induced with *in vitro* activation stimuli (for example with IL-15 or IL-12)[108,165,166]. How these receptors impact NK cell effector responses is being characterized in preclinical models. TIGIT blockade promotes antitumour responses by NK cells in multiple mouse models and reverses exhaustion in NK cells derived from patients with colon cancer.[167] Blockade of TIM-3 *in vitro* can also reverse exhaustion in NK cells derived from patients with metastatic melanoma and bladder cancer, promoting proliferation, increased IFN-γ production, and increased cytotoxicity[168,169]. Finally, therapies that pair IL-12 and/or IL-15 stimulation of NK cells with TIGIT and/or LAG-3 blockade in mouse tumour models have shown

promise[165,166]. TIGIT, TIM-3, and LAG-3 blocking antibodies are already in clinical trials for T cell targeting cancer therapies[170]. This offers opportunities to examine their impacts on NK cell responses in patients, including whether blocking TIGIT and/or TIM-3 may impact NK cell education[171].

Additional checkpoints on NK Cells—Additional receptor–ligand pairs have been identified as promising checkpoint blockade targets on NK cells, namely NKp44 and proliferating cell nuclear antigen (PCNA), KIR3DL3 and HHLA2, and SIRPα and CD47. Several years ago, NKp44 was shown to mediate inhibitory signaling in NK cells when engaged with PCNA as a ligand on the surface of target cells[172]. This result was puzzling, since NKp44 was originally considered an activating receptor linked to DAP12, and PCNA was considered a nuclear protein[173,174]. However, the cytoplasmic domain of NKp44 contains an ITIM, which was required to transduce the inhibitory signaling. In addition, PCNA was shown to move to the cell surface and localize at the immune synapse when engaged with NKp44-expressing NK cells[172]. The same group recently developed an antibody against a domain on PCNA that could be exposed on tumour cell surfaces and blocked the NKp44 interaction to effectively potentiated NK cell-mediated responses to several PCNA-expressing tumour cells[175]. The work demonstrates that PCNA does indeed reach the cell surface and can be targeted with specific antibodies to overcome the NKp44/PCNA inhibitory axis.

HHLA2 is a ligand for the inhibitory KIR3DL3 and could therefore be another possible target for checkpoint blockade to boost NK cell responses. HHLA2 expression was found in different regions of tumours to those expressing PD-L1[176,177], although a recent meta-analysis of HHLA2 expression in a variety of cancers suggests that it may be difficult to draw conclusions yet on its prognostic significance[178]. KIR3DL3 is highly polymorphic, but has been minimally detected on NK cells to date[179,180], although it is unclear if expression is induced by the TME. As another complication, HHLA2 is also the ligand for the NK activating receptor transmembrane and immunoglobulin domain-containing protein 2 (TMIGD2)[181,182]. Nonetheless, some HHLA2 antibodies can block the KIR3DL3 interaction without inhibiting TMIGD2-binding, which indicates the potential to develop antibodies that selectively block the inhibitory pathway[183]. Although it is unclear if the KIR3DL3–HHLA2 inhibitory axis will prove to be a viable target cancer therapy, it is worth further exploration.

CD47 is a widely expressed tumour surface antigen that is known to block phagocytosis of tumour cells by engaging with the macrophage receptor SIRPα[184]. SIRPα is also expressed on the surface of NK cells, where it inhibits natural cytotoxicity against mouse MHC-I^{-} CD47^{+} tumour cells[185]. Checkpoint blockade therapeutics targeting the CD47 axis are currently being studied in clinical trials for its effects on antigen presenting cells, but NK cell function may be enhanced as well (NCT02663518, NCT04588324).

Antibodies that induce ADCC responses

A hallmark of NK cell effector function is the ability to kill IgG1- or IgG3-opsonized target cells by ADCC through CD16 (Figure 2). A variety of humanized mAbs have thus emerged

that exploit this form of NK cell cytotoxicity by targeting tumour antigens in a variety of cancers. Many of these antibodies are currently in clinical trials, and a summarized list of several of these, their cognate antigens, prospective tumour targets being evaluated, and stage of testing can be found in Table 3.

A common polymorphism is found in CD16 that significantly affects affinity for Fc and can influence ADCC responses by NK cells. Specifically, the polymorphism at position 158 of phenylalanine (158F) or valine (158V) results in CD16 with low or increased affinity for Fc, respectively[186]. The ADCC-inducing antibodies trastuzumab (anti-HER2) and rituximab (anti-CD20) both have limitations in response rate when used to treat patients that express the low affinity 158F variant on NK cells, which is somewhat more prevalent in humans than the higher affinity variant 158V[187–190]. Accordingly, many current generation ADCC-inducing antibodies are Fc-modified to improve CD16 binding and serum retention. For example, the next generation HER2 antibody margetuximab recently approved for $HER2^+$ breast cancer treatment (NCT02492711) that has five amino acid substitutions in Fc to increase binding to low affinity CD16[191]. Most of these engineered mAbs are also synthesized in a mammalian culture system that prevents the Fc region of the antibody from fucosylation, which also enhances binding to CD16. Further information on current design strategies of antibody engineering can be found in a recent review[192].

Similarly, CD20-directed antibodies analogous to rituximab have gone through several iterations of improved design to treat B cell malignancies, such as obinutuzumab and ublituximab. Obinutuzumab generally provides better outcomes than rituximab, but some comparative clinical trials have found higher incidence of adverse effects from obinutuzumab[193,194], thereby prompting caution. Ublituximab seems to lack the toxicity of obinutuzumab, and has shown a very promising response rate in combination with the PI3K-δ inhibitor umbralisib across patients with NHL and small lymphocytic lymphoma (SLL) or CLL[195]. Importantly, mechanisms other than ADCC may contribute to the antitumour response by anti-CD20 antibodies *in vivo*, such as activating complement (rituximab, ofatumumab, ublituximab), inducing direct cell lysis (obinutuzumab), or initiating antibody-dependent cellular phagocytosis by CD16-expressing macrophages[196,197]. From the perspective of NK-based therapy, it is critical to consider the pleiotropic effects potentially induced by any IgG1- and IgG3-based antibody in addition to stimulating ADCC.

Treatment of MM has also benefited from ADCC-inducing antibodies, such as elotuzumab (anti-SLAMF7) and daratumumab (anti-CD38). CD38 and SLAMF7 are highly expressed on myeloma cells, but also on NK cells. Elotuzumab efficacy in patients with myeloma requires combination therapy with bortezomib, lenalidomide, or pomalidomide, all of which stimulate NK cell function[198]. This strongly suggests that NK cells are crucial in antitumour responses by elotuzumab. Interestingly, although elotuzumab triggers strong ADCC, it can also enhance cytotoxicity through direct co-stimulation of SLAMF7 on the NK cell surface and promote homotypic SLAMF7–SLAMF7 interactions between NK and tumour cells[199,200]. When given in combination with pomalidomide and dexamethasone, elotuzumab has shown a 53% overall response rate in MM (compared with a 26% response rate with the pomalidomide + dexamethasone combination alone)[201]. Daratumumab is effective as a single agent, but the combination of daratumumab, pomalidomide, and

dexamethasone resulted in a 69% overall response rate in an ongoing MM trial, as compared to 46% for pomalidomide + dexamethasone alone[202]. However, daratumumab can also induce NK cell fratricide, which has not been observed with elotuzumab treatment, but may limit daratumumab effectiveness over multiple doses[203].

Several other antibodies previously used to enhance NK cytotoxicity indirectly or through blockade can also function through ADCC. Avelumab is an IgG1 antibody against PD-L1 that can induce ADCC in addition to checkpoint blockade[204,205], which makes it unique compared to other PD-L1 antibodies that are IgG4 or Fc-mutated to reduce binding to FcγRs and thereby limit immune cell activation and increase available levels of circulating antibody. Other antibodies are targeting MICA, which can be shed by some tumours, and this soluble form can suppress NK cells by engaging with NKG2D. MICA antibodies can stabilize MICA expression on tumour cells[206], and they also enhance NK cytotoxicity through ADCC, offering a new option for clinical utility[207,208].

Finally, strategies to stabilize surface expression of CD16 on NK cells and target antigen on tumour cells are under investigation to improve ADCC responses. ADAM17 is a metalloprotease expressed by NK cells that can cleave CD16 from the NK cell surface[209]. ADAM17 inhibitors can enhance ADCC responses by NK cells *in vitro*[209], and promising early results were released from a phase II clinical trial using an ADAM17 inhibitor in combination with rituximab to treat DLBCL (NCT02141451). There is reason for skepticism as to whether ADAM17 inhibitors will effectively enhance ADCC *in vivo*, however, since CD16 cleavage by ADAM17 is important for the natural release of NK cells after ADCC of a target cell, so blocking this cleavage could limit engagement with further target cells[210]. Also, preclinical studies in mice showed that treatment with compounds that block antibody-mediated endocytosis of tumour antigens (Dyngo4a and the approved drug prochlorperazine) significantly improved surface retention of bound ADCC-inducing antibodies and antitumour responses, which required intact Fc and NK cells[211].

Bispecific and trispecific antibodies

Bispecific and trispecific antibodies, which can simultaneously engage with NK activating receptors and tumor antigens, have shown promise in pre-clinical studies and early clinical trials. These molecules have been engineered to engage with CD16 and one or multiple other targets on the tumour cell and NK cell, bringing both into proximity and simultaneously promoting more efficient and/or sustained NK-mediated cytotoxicity (schematics in Figures 2 and 3). Unlike normal mAbs, these constructs can be designed for binding multiple types of tumour antigens or NK receptors simultaneously and can be made with CD16 engaging Fv fragments instead of Fc for stronger and equal binding affinity for 158V and 158F polymorphisms and fewer Fc-mediated off-target interactions (such as becoming fixed through complement or binding other Fc receptors). Success has been achieved with similar systems that direct T cell cytotoxicity, specifically through engaging CD3, and some of these drugs have already received FDA approval[212]. Despite these successes, similar NK cell-specific constructs that trigger CD16 directly can ensure activation of the cytolytic $CD56^{dim}$ NK cells and overcome the low affinity Fc binding of the 158F CD16

polymorphism, whereas constructs that engage CD3 not only stimulate the $CD8^+$ cytotoxic T cell population, but also $CD4^+$ T_H and Treg cells.

The CD16 bispecific antibody that has progressed furthest in the clinic is AFM13, which targets CD30[213], a surface antigen expressed in B and T cell lymphomas. AFM13 has been well tolerated in Phase I and II trials in the treatment of various lymphomas[213] and has been granted orphan drug designation for the treatment of peripheral T cell lymphoma. As of 2021, several active clinical trials employ AFM13 for treatment of refracted or relapsed peripheral T cell lymphoma as a monotherapy (NCT04101331) or to treat HL and NHL in conjunction with chemotherapy (NCT04074746)[214]. AFM24 is another tetravalent bispecific like AFM13, but it targets epidermal growth factor receptor (EGFR) instead of CD30, and it is currently in Phase I/II for the treatment of metastatic colorectal cancer and NSCLC (NCT04259450).

Various other anti-CD16 bispecific antibody systems have succeeded at enhancing tumour cell killing by NK cells, such as homodimeric tribodies with two HER2 or CD20-binding domains[215,216] or the single chain recombinant Fv Bispecific Killer Engagers (BiKEs), which were developed to engage a variety of antigens, including HER2, CD20, and CD30[217]. Tri-specific killer engagers (TriKEs) incorporate two Fv domains targeting a tumour antigen and CD16 with intervening IL-15 that enhances NK cell activity (Figure 3). TriKEs in preclinical development target CD133 to eradicate cancer stem cells[218], CD19 to eliminate Burkitt lymphoma and CLL tumour cells[219], or CLEC12A to eliminate AML cells[220], to name a few examples. A CD33 TriKE is in Phase I/II clinical trials as a monotherapy for the treatment of myelodysplastic syndromes, refractory/relapsed AML or advanced-stage systemic mastocytosis (NCT03214666).

Additional advanced trispecific antibodies are also being engineered to enhance NK cell-mediated antitumour responses. NK cell engagers are innovative trispecific constructs that bind tumor antigen, CD16, and NKp46 or NKp30 and have shown impressive preclinical activity[221]. Another approach combines a CD16/cetuximab bispecific antibody with an Fv domain to bind CD137, as engaging CD137 enhances response to cetuximab[222,223]. Altogether, the potential for engaging multiple NK cell receptors simultaneously makes trispecific antibody constructs an exciting frontier in NK cell immunotherapy, although establishing which activating receptors to engage for optimal boost of antitumour responses will take further research.

Adoptive NK cell therapies

Adoptive transfer of NK cells has come of age with the recent success of chimeric antigen receptor (CAR)-bearing NK cell and cytokine-stimulated NK cell therapies[224]. Most NK-based adoptive transfer therapies harvest NK cells from various sources, enhance their function *ex vivo* through a variety of methods, then transfuse them into a cancer patient. A recent review has detailed these exciting advances[224], so we will only briefly summarize some of the most impactful advances and future directions under development. Active clinical trials are listed in Table 4.

Both autologous and allogeneic NK cells can be sources for adoptive transfer, and whereas autologous NK cells avoid risk of a graft versus host response, KIR mismatch in adoptive transfer of allogeneic haploidentical NK cells can be useful in treating AML, which is sensitive to the NK cell-mediated graft versus tumour response[39,225,226]. This strategy has been further refined through the in vitro differentiation of allogeneic NK cells from induced pluripotent stem cells[227,228]. Allogeneic NK cells can also be derived from sources rich in NK cells, such as cord blood. There is some evidence to suggest that cord blood-derived NK cells are easier to grow and stimulate in culture, but NK cells derived from adult peripheral blood are more naturally cytolytic[229,230]. As the field develops, certain therapeutic strategies may benefit from taking NK cells from distinct sources. In addition, infusions of an *ex vivo* expanded autologous NK cell product, ACP-001, has been recently approved by the FDA for orphan drug designation to treat MM (NCT04558853).

A straightforward approach to enhance NK cell function has involved *ex vivo* stimulation with cytokines before transfusion, historically using IL-2[231], which has fallen out of favour owing to adverse effects. *In vitro* stimulation of NK cells with the combination of IL-12/IL-15/IL-18 generates highly functional long-lived ‘memory-like’ NK cells for adoptive transfer in both preclinical and clinical studies[224]. These ‘memory-like’ NK cells are less sensitive to KIR inhibition and a phase I trial showed encouraging antitumour responses in patients with AML[232,233]. IL-15, IL-12, and IL-18 have recently been shown to stimulate an ER stress response pathway in NK cells to promote MYC-dependent proliferation and oxidative phosphorylation in the mitochondria associated with enhanced antitumour responses[234]. Another recent study used an interesting approach of pre-loading ‘memory-like’ NK cells with the tetravalent anti-CD16/CD30 bispecific antibody AFM13, which binds to NK cells with high affinity through two CD16 targeting Fv domains[213], to facilitate potent responses toward $CD30^+$ lymphomas upon adoptive transfer into a mouse xenograph model[235]. Highly functional and long-lived NK cells can also be stimulated and expanded *in vitro* through transgenic feeder cell lines that express membrane-bound IL-15, IL-21, and/or other stimulatory molecules[236,237]. Interestingly, in NK cells expanded ex vivo in the presence of IL-21-mediated STAT3 signaling, glycolysis is upregulated and oxidative phosphorylation is downregulated (Warburg effect), similar to tumour cells[29]. This reprogramming not only made the NK cells more adaptable to nutrient-deprived metabolic conditions in the TME, but also remarkably enhanced their tumour killing capacity over time when exposed to the TME[29].

As an exciting next generation approach, NK cell adoptive therapies have employed CAR–bearing NK cells. CAR-T cells that recognize CD19 have been approved by the FDA for treating B cell NHL, and cord blood-derived NK cells transduced to express similar CD19-targeting CAR have shown impressive early results in clinical trials for treating B cell lymphomas and leukaemias[238]. Importantly, the therapy did not induce cytokine storm or graft versus host toxicity, and some NK cells persisted for over one year[238]. Other antigen targets for CARs in NK cells are also at varying stages of clinical trials (Table 4). As a whole, CAR-NK cells offer several advantages over CAR-T cells, including lower risk of cytokine release syndrome, which is likely due to their lack of IL-6 production and less enduring production of TNF[239], and a lack of autocrine growth signaling that would perpetuate long-term NK cell survival beyond required antitumour need (such as

IL-2 production by T cells that could enable their long-term persistence). Additionally, NK cells that lose the transgenic CAR can still recognize and kill through their endogenously expressed activating receptors. CAR on NK cells can also overcome inhibitory KIR and NKG2A receptors[182,240]. Many CAR-NK cells are derived from sources previously mentioned[238,239], but increased demand for easily modified ‘off the shelf’ NK cell platforms has included development of CAR-expressing NK cell lines, such as NK-92[241–243]. Although the durability of these NK cellular therapies is still under investigation, many have been shown to be safe and effective.

In addition to introducing CARs, additional genetic modifications are being explored to enhance NK cells for adoptive transfer, many of which are in clinical trials (Table 4). Better tumour infiltration has been demonstrated in preclinical studies through transgenic expression of chemokine or adhesion receptors, such as CXCR3 in myeloma models or CCR7 in lymphoma models[244,245]. ADCC-based therapies may also use NK cells expressing high affinity 158V CD16 (including CD16 modified to prevent proteolysis by ADAM17) in combination with an ADCC-inducing antibody[246,247], which are currently undergoing early clinical trials (NCT04023071, NCT04551885, NCT03853317). Finally, CRISPR/Cas9 gene editing methods are being used to remove negative regulators, such as the IL-15R — a member of the suppressor of cytokine signaling (SOCS) family (CIS) — from iPSC-derived NK cells[248]. Gene editing has also been used to remove CD38 from NK cells and circumvent daratumumab-induced fratricide[249]. The introduction of transgenes, such as CARs, and use of modern gene editing technologies to modify NK cells offer extensive possibilities for building better NK cells for adoptive immunotherapy to treat both haematopoietic and solid tumours.

Conclusions and future directions

After decades of research that informed the basic biology of NK cells, the field has recently evolved to make rapid advances in developing effective immunotherapies that exploit these cells to treat cancer. Cytokines and numerous other factors can be used to broadly potentiate cytotoxicity and cytokine responses by NK cells and are being tested in the clinic. In particular, superagonist forms of IL-15 offer substantial opportunities to induce the activation, proliferation, and survival of NK cells in patients. Antibody-mediated therapies have evolved substantially to reduce NK cell activation threshold by blocking immune checkpoint receptors and to facilitate targeted attack upon bridging tumour surface markers with CD16 and other activating receptors. Adoptive NK cell therapies have also made exciting advances and have proven to be safe and effective in recent clinical trials. Although the durability of these responses is still under investigation, genetic manipulation offers unlimited opportunities to further optimize NK cellular therapies by introducing CARs and targeting receptors and removing inhibitory molecules. The lack of serious cytokine release syndrome or autoimmune responses in these adoptive therapies clearly demonstrates that many inhibitory and exhaustion mechanisms are restraining NK cells from damaging healthy tissues, which offers profound advantages over T cell-mediated approaches.

In fact, this field is still in its infancy, and the future looks bright for development of new ways to further enhance the NK cell immunotherapy platform. Important goals will be to

improve NK cell targeting of solid tumours and enhance their activation, cytolytic capacity, and survival upon reaching the harsh immunosuppressive TME. Because their activation is orchestrated by a wide array of cell surface receptors, therapeutic agents that target these receptors to engage with tumour cell surfaces is key. CARs, as well as advanced antibody engineering and other multimeric receptor engagement technologies provide extensive opportunities to optimally trigger NK cell activation in contact with tumour cells. NK cell-focused combination therapies should provide the next wave of clinical advances in finding the appropriate balance of therapeutics that improves their targeted activation while effectively overcoming their embedded restraint mechanisms.

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Glossary terms:

Opsonization	The binding of antibodies to the surface of a pathogen or tumour cell, which are recognized by Fc receptors on phagocytes to facilitate antibody dependent cellular phagocytosis or by CD16 on NK cells to trigger ADCC.
Tolerize	The process by which normal cells induce tolerance toward NK cell-mediated attack by expressing MHC-I molecules that engage inhibitory receptors, such as KIR.
Fucosylation	The process of covalently attaching the sugar fucose to IgG molecules as a post-translational event, which reduces the affinity of IgG1 or IgG3 to bind CD16 and thereby decreases their potency in stimulating ADCC by NK cells.
Fratricide	Classically defined as killing one’s brother, this term has been used to define the process by which NK cells sometimes aberrantly attack and kill each other.
Feeder cell lines	Cell lines that are used to stimulate the *ex vivo* proliferation of NK cells and are generally chosen for low surface expression of MHC-I and/or engineered to express surface bound cytokines or ligands for NK cell activating receptors.

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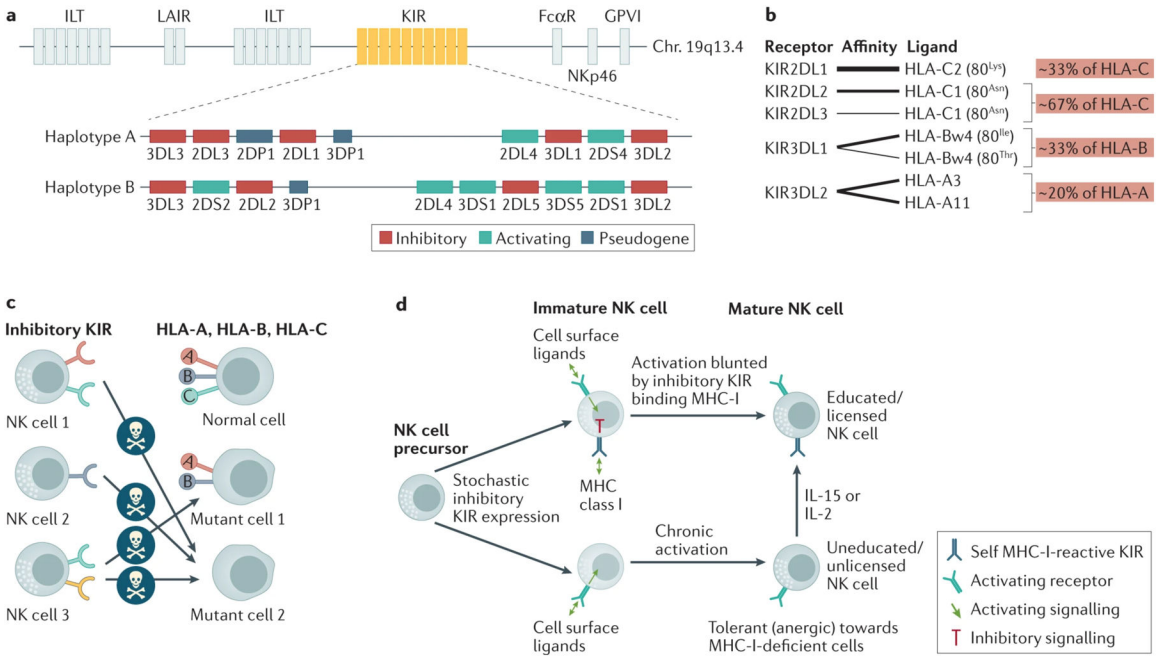

Figure 1. Killer Cell Ig-like Receptors (KIRs) provide diversity, tolerance, and education to human NK cells.

a. Humans inherit different numbers and combinations (haplotypes) of the 14 available killer cell inhibitory receptor (KIR) genes, with only two examples shown. The KIR genes are inherited in close proximity in a locus on chromosome 19. Haplotype A contains combinations of mostly inhibitory KIRs and only two activating KIRs (usually KIR2DL4 and KIR2DS4), while haplotype B contains more activating KIRs. b. Different inhibitory KIRs recognize subsets of the available alleles of the class I human leukocyte antigen complex (HLA). Relative receptor-ligand affinities are represented by thickness of the interlinking lines. Class I HLA subgroups are indicated with their general frequency in the human population. HLA-C alleles can be divided into C1 and C2 subgroups according to either asparagine or lysine at position 80, respectively, and HLA-B alleles can be divided into Bw4 or Bw6 subgroups. c. KIRs are expressed on NK cells in a variegated manner, forming a repertoire that can be tolerized and educated by interaction of at least one KIR with the endogenous ‘self’ class I HLA. A subset of NK cell clones could recognize a cell that has lost only one class I HLA allele (mutant cell #1), whereas if an abnormal cell loses all class I HLA (mutant cell #2), it has lost ligands for all KIR and is susceptible to all NK cells. d. KIR-mediated education during NK cell development. KIRs are stochastically expressed on individual NK cell clones during development. If a KIR recognizes ‘self’ class I HLA (top), it inhibits activation signaling and promotes maturation to a fully competent NK cell (educated), but if no KIR is expressed that recognizes ‘self’ class I HLA, chronic activation signaling results in an hyporesponsive NK cell (uneducated; bottom). Exposure to IL-15 or IL-2 can stimulate an uneducated NK cell into a competent, educated state. Figure 1B has been adapted with permission from REF.[252] and figure 1D has been adapted with permission from REF.[2].

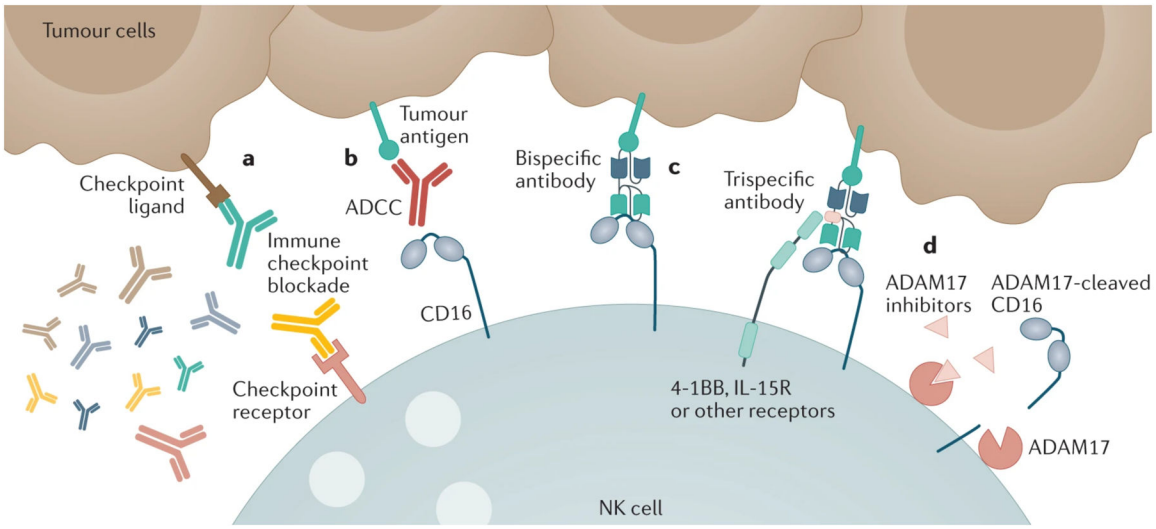

Figure 2. Antibodies in NK cell immunotherapy.

Antibodies can be used to enhance NK cell function in several different ways. a. Immune checkpoint blockade can be used to prevent the engagement of checkpoint inhibitory receptors on NK cells with their ligands on tumours. b. Antibodies targeting tumour antigens can be engaged via their Fc domains with CD16 on the surface of NK cells, triggering ADCC. c. Next generation bi- and tri-specific antibody constructs have also been designed that more efficiently engage with CD16 (and/or other activating receptors) than classic antitumour monoclonal antibodies. d. Finally, it is being investigated whether inhibitors of the metalloprotease ADAM17 can enhance ADCC by preventing the cleavage of CD16. The diversity of therapeutic strategies on display means they can often be used in tandem to activate NK cells through the interaction of multiple signaling pathways, although targeting the NK cell to the tumour is critical.

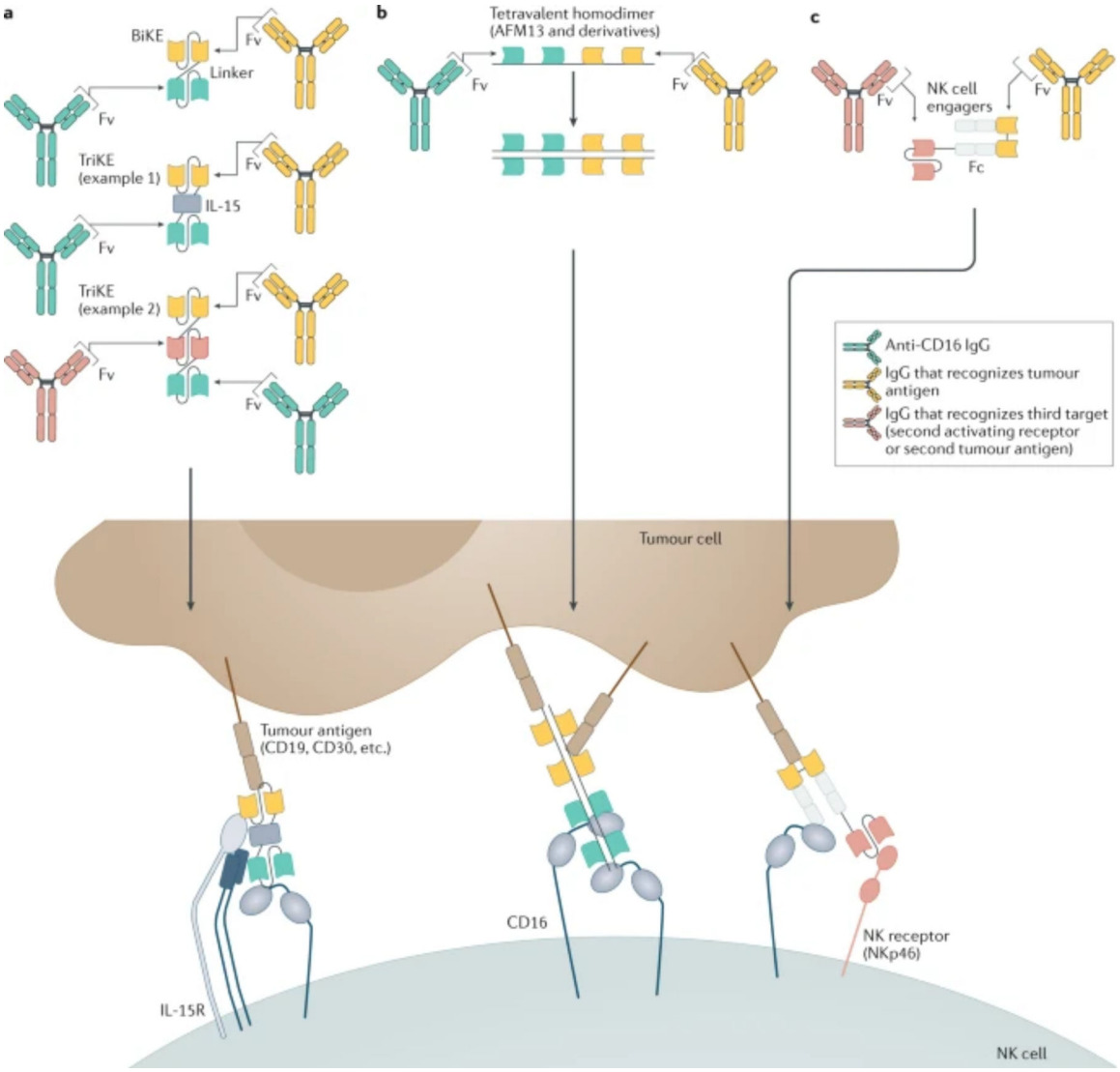

Figure 3. Different strategies for producing bi- or tri-specific antibody constructs.

Many modern antibody constructs do not retain Fc domains and instead engage with CD16 through Fv domains. a. BiKEs and TriKEs are made from various combinations of two or three (respective) Fv fragments from mAbs or ligand molecules linked in a single polypeptide chain, which target some combination of tumor antigen, CD16 and another NK activating receptor. A common TriKE design includes an IL-15 moiety for better stimulation of NK cell activation, survival, and proliferation. b. Other constructs contain multiple Fv domains that form homodimers or homomultimers. Of this category AFM13, a bispecific construct consisting of two anti-CD30 and two anti-CD16 Fv domains, has shown promising results in clinical trials. c. A new innovative type of construct, the NK cell engager, is a complex consisting of different polypeptides that include both Fv and Fc domains. The Fc region of the construct can be modified for efficient CD16 engagement and optimally spaced Fv that optimally engages a NK cell activating receptor (e.g. NKp46) for further potentiation of NK cell stimulation, as well as one tumour antigen.

Table 1 |

Human NK cell receptors and reported ligands

Receptors	Functions	Ligands
NKG2D	activating	MICA, MICB, ULBP1–6
DNAM-1	activating	Nectin-2 (CD112), PVR (CD155)
FcγRIIIa (also known as CD16)	activating	Fc domains of IgG1 and IgG3
NKp80	activating	AICL
NKG2C/CD94	activating	HLA-E
SLAMF7	activating	SLAMF7
KIR2DS1	activating	Group 2 HLA-C
KIR2DS4	activating	Some HLA-C, HLA-A*11, HLA-F
KIR3DS1	activating	HLA-Bw4, HLA-F
TM1GD2 (CD28H)	activating	HHLA2
NKp30 (NCR3)	activating	B7-H6, HLA-B associated transcript 3 (BAT3)/ Bcl2-associated anthogene 6 (BAG6) (soluble/exosomes), galectin-3, heparan sulfate proteoglycans
NKp46 (NCR1)	activating	hemagglutinins, heparan sulfate proteoglycans, vimentin, complement factor P (CFP; properdin, soluble)
NKp44 (NCR2)	activating	21spe-MLL5 (splice variant of MLL5), heparan sulfate proteoglycans, PDGF-DD (soluble), some HLA-DP, hemagglutinins, Nidogen-1, Dengue virus E protein
	inhibitory	PCNA, Nidogen-1 (soluble)
2B4	activating/ inhibitory	CD48
KIR2DL1	inhibitory	Group 2 HLA-C
KIR2DL2, KIR2DL3	inhibitory	Group 1 HLA-C, some group 2 HLA-C, some HLA-B
KIR2DL5	inhibitory	PVR
KIR3DL1	inhibitory	HLA-Bw4 containing alleles of HLA-B and HLA-A
KIR3DL2	inhibitory	some HLA-A (such as A*03 and A*11), HLA-F
KIR3DL3	inhibitory	HHLA2
NKG2A/CD94	inhibitory	HLA-E
NKR-P1A	inhibitory	CLEC2D (LLT1)
LILRB1 (ILT2, LIR1)	inhibitory	HLA-A, HLA-B, HLA-C, HLA-G, HLA-F, S100A9
KLRG1	inhibitory	E-cadherin, N- cadherin, and R-cadherin
CD96 (TACTILE)	inhibitory	Nectin-1 (CD111), PVR
TIGIT	inhibitory	Nectin-2, PVR
PVRIG	inhibitory	Nectin-2
TIM-3	inhibitory	Galectin-9, CEACAM-1, phosphatidyl serine, HMGB1
LAG-3	inhibitory	MHC class II, galectin-3, LSECtin (liver sinusoidal endothelial cell lectin), FGL1
PD-1	inhibitory	PD-L1, PD-L2

Abbreviations: AICL, activation-induced C-type lectin; LAG-3; PVR, PD-1, PD-L1, PVRIG ; TIGIT; TIM-3

Table 2.

Clinical trials involving cytokines that have important roles in NK cell biology

Cytokine agonist or antagonist	Active clinical trials
IL-2 (and agonists ALKS 4230, F42K)	NCT02799095, NCT03861793, NCT04144517, NCT04592653, NCT04830124, NCT05092360
IL-12 (fusions with antibodies, encoding genetic material, and controlled release)	NCT02062827, NCT02483312, NCT03132675, NCT03567720, NCT03823131, NCT04095689, NCT04370587, NCT04471987, NCT04526730, NCT04756505, NCT04911166
IL-15 (hetIL-15, N-803)	NCT01898793, NCT02138734, NCT02452268, NCT02465957, NCT02523469, NCT02782546, NCT02890758, NCT02989844, NCT0322825, NCT03228667, NCT03387111, NCT03520686, NCT03563157, NCT04261439, NCT04290546, NCT04390399, NCT04847466, NCT04898543, NCT04927884, NCT05096663
IL-18	NCT04787042
TGF- β Inhibitors (fresolimumab, galunisertib)	NCT01582269, NCT01682187, NCT02452008, NCT02581787, NCT02672475, NCT02688712, NCT03206177, NCT04605562

Table 3.

Select ADCC-inducing antibodies undergoing clinical trials.

Target	Antibody	Type	Status, selected active clinical trials
HER2	Trastuzumab	Humanized IgG1	FDA approved: breast cancer and gastric/GEJ cancer
	Margetuximab	Fc modified, chimeric IgG1[191]	FDA approved: breast cancer Phase II/III: Gastric/GEJ cancer (NCT04082364)
CD20	Rituximab	Chimeric IgG1	FDA approved: NHL and CLL Phase II: ALL (NCT02199184)
	Ofatumumab	Human IgG1	FDA approved: CLL Phase II: NHL, ALL (NCT02199184)
	Obinutuzumab	Afucosylated, Fc modified, humanized IgG1[250]	FDA approved: CLL and FL Phase II: NHL (NCT03198026), hairy cell leukaemia (NCT03410875)
	Ublituximab	Low fucose, chimeric IgG1[250]	Phase III: CLL (NCT02612311) Phase II: NHL (NCT02793583)
CD19	Tafasitamab	Fc modified, humanized IgG1[251]	FDA approved: R/R DLBCL Phase I: NHL (NCT04661007)
EGFR	Cetuximab	Chimeric IgG1	FDA approved: head and neck cancers, colorectal cancer Phase II: NSCLC (NCT04648189)
GD2	Dinutuximab	Chimeric IgG1	FDA approved: neuroblastoma Phase I: High risk and R/R neuroblastoma (NCT02573896)
PD-L1	Avelumab	Human IgG1	FDA approved: Merkel cell carcinoma, urothelial carcinoma and renal cell carcinoma Phase I/II: Gestational trophoblastic neoplasia (NCT04396223), squamous cell anal carcinoma (NCT03944252)
SLAMF7	Elotuzumab	Humanized IgG1	FDA approved: MM Phase II: R/R MM (NCT03361306)
CD38	Daratumumab	Human IgG1	FDA approved: MM Phase III: SMM (NCT03937635) Phase II: R/R MM (NCT03841565)
	Isatuximab	Chimeric IgG1	FDA approved: MM Phase III: SMM (NCT04270409) Phase II: R/R MM (NCT04287855)

Abbreviations: ALL, acute lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia CNS, central nervous system; DLBCL, diffuse large B-cell lymphoma, FL, follicular lymphoma; GEJ, gastroesophageal junction; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; NSCLC, non-small cell lung cancer; R/R, relapsed/refractory; SMM, smoldering multiple myeloma

Table 4.

Ongoing clinical trials with engineered NK cells

Modification	Phase	Malignancy	Combination Treatments	Clinicaltrial.gov Designation
NKG2D ligand CAR	Phase I	R/R AML, R/R MDS	Monotherapy	NCT04623944
HER2 CAR	Phase I	Glioblastoma	Monotherapy	NCT03383978
158V non-cleavable CD16	Phase I	B cell lymphoma, AML, solid tumors	CD20 or PD-L1 mAbs, chemotherapy, IL-2	NCT04023071, NCT04551885
158V non-cleavable CD16, CD38 KO, contiguously active IL-15R	Phase I	Multiple Myeloma, AML, monocytic leukemia	Daratumumab or elotuzumab, chemotherapy	NCT04614636, NCT04714372
CD19 CAR, 158V non-cleavable CD16, contiguously active IL-15R	Phase I	B cell lymphoma, CLL	Monotherapy or CD20 mAbs	NCT04245722
CD19 CAR / IL-15 production	Phase I/II	B cell malignancies	Chemotherapy	NCT03056339
BCMA CAR	Phase I/II	Multiple myeloma	Monotherapy	NCT03940833
ROBO1 CAR	Phase I/II	ROBO1 positive tumours	Monotherapy	NCT03940820, NCT03931720
		Pancreatic cancer	Monotherapy	NCT03941457
PD-L1 CAR, 158V CD16, IL-2 production	Phase II	Various	Chemotherapy, aldoxorubicin HCl, IL-15 superagonist	NCT04390399, NCT03228667, NCT04847466
CD5 CAR, IL-15 production	Phase I/II	R/R Hematological malignancies	Chemotherapy	NCT05110742

Abbreviations: AML, BCMA, CLL; ICB, immune checkpoint blockade; MDS, PD-L1, ROBO1, Roundabout homolog 1; R/R, relapsed/refractory