Review

Natural killer cell therapies

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Natural killer (NK) cells are lymphocytes of the innate immune system. A key feature of NK cells is their ability to recognize a wide range of cells in distress, particularly tumour cells and cells infected with viruses. They combine both direct effector functions against their cellular targets and participate in the generation, shaping and maintenance of a multicellular immune response. As our understanding has deepened, several therapeutic strategies focused on NK cells have been conceived and are currently in various stages of development, from preclinical investigations to clinical trials. Here we explore in detail the complexity of NK cell biology in humans and highlight the role of these cells in cancer immunity. We also analyse the harnessing of NK cell immunity through immune checkpoint inhibitors, NK cell engagers, and infusions of preactivated or genetically modified, autologous or allogeneic NK cell products.

2023 heralds the fiftieth anniversary of the pioneering publications that set the stage for the discovery of NK cells[1–3]. Further characterized as a unique cellular entity distinct from other known immune cells and also officially named in 1975[4,5], NK cells are now known to belong to the group of innate lymphoid cells (ILCs)[6]—a family of cells that has been recognized as such since 2008[7]. ILCs are lymphocytes of the innate immune system that do not express the type of diversified antigen receptors found on T cells and B cells[8]. Among the five major ILC subsets, type 1, 2 and 3 ILCs (ILC1, ILC2 and ILC3 cells, respectively) are mostly tissue-resident cells and mirror $CD4^+$ T helper type 1 (T_H1), T_H2 and T_H17 cells, respectively, in terms of cytokine production, whereas NK cells that are present both in the blood and tissues can be considered to be innate counterparts of $CD8^+$ cytotoxic T cells[9]. Over the past five decades, the importance and potential of NK cells has been extensively explored. What began as academic intrigue has evolved into a promising area of immunotherapy, particularly in the fight against cancer.

What are NK cells?

NK cells are effector ILCs that arise from bone marrow progenitor cells[6,10–14]. The total number of NK cells in humans has been estimated to be 2×10^{10} cells (95% confidence interval: 0.5×10^{10}–6×10^{10}), making up around 1% of total immune cell types in the body and 2% of total lymphocytes[15]. At steady state in healthy individuals, NK cells are present mainly in the liver, bone marrow and blood where they constitute around 10% of the total number of peripheral lymphocytes. Their functions are tightly regulated by a repertoire of inhibitory and activating receptors, enabling them to recognize and to directly or indirectly eliminate stressed cells while sparing normal cells. The vast majority of mature NK cells are cytolytic, and all NK cells can produce a number of cytokines, including interferon-γ (IFNγ), growth factors such as FMS-like tyrosine kinase 3 ligand (FLT-3L) and granulocyte–macrophage colony-stimulating factor (GM-CSF), and chemokines, including XCL1 and CCL5[6,10–14].

Human NK cells are usually classified on the basis of the expression of the two surface molecules CD56 (encoded by *NCAM*) and CD16a (encoded by *FCGR3A*)[12]. $CD56^{bright}CD16^-$ NK cells exhibit lower cytotoxicity but produce cytokines, growth factors and chemokines. By contrast, $CD56^{dim}CD16^+$ NK cells are highly cytotoxic due to their expression of granzymes (*GZMA*, *GZMB*) and perforin (*PRF1*), and can also produce cytokines, growth factors and chemokines[16]. More recently, single-cell RNA-sequencing analyses have provided insights into the diversity of NK cells[17,18]. Unsupervised classification algorithms based on gene expression enabled the identification of three major NK cell populations (Fig. 1) and also revealed the presence of several subpopulations within them. Type 1 NK (NK1) cells, which are the most abundant in blood, correspond to $CD56^{dim}CD16^+$ NK cells and, besides the strong expression of CD16 (*FCGR3A*) and cytotoxicity effector molecules (*GZMA*, *GZMB*, *PRF1*), they selectively express additional genes such as *SPON2*, the biological function of which in NK cells remains to be elucidated. The NK2 cell population corresponds to $CD56^{bright}CD16^-$ NK cells. These cells exhibit a characteristic transcriptional signature, including granzyme K (*GZMK*), a characteristic chemokine profile (*XCL1*, *XCL2*), cell surface markers (*CD44*, *SELL*, *KLRC1*) and strong expression of the transcription factor TCF1 (encoded by *TCF7*). Finally, a third major population, tentatively referred to as NK3 cells, comprises mainly $CD16^{dim}$ adaptive $NKG2C^{high}$ (encoded by *KLRC2*) NK cells, including $CD57^+$ cells[19–21]. This subset presents memory-like properties with enhanced functional responses, in a manner resembling memory T cells, after recognition of various viral, bacterial, cytokine or hapten stimuli[22–26]. They exhibit a characteristic cytotoxic signature (*GZMH*), surface markers (*KLRC2*, *CD3E*) and a specific cytokine signature (*IL32* and *CCL5*)[27,28]. The abundance of each of the various NK cell subsets depends on their anatomical localization and pathophysiological conditions. NK1 cells are present at higher concentrations in the bone marrow, spleen and blood, whereas NK2 cells are more prevalent in the lungs, tonsils, lymph nodes and intestines. NK2 cells show greater tissue imprinting compared with NK1 cells and share several common markers with $CD8^+$ tissue-resident memory T cells, notably, increased expression

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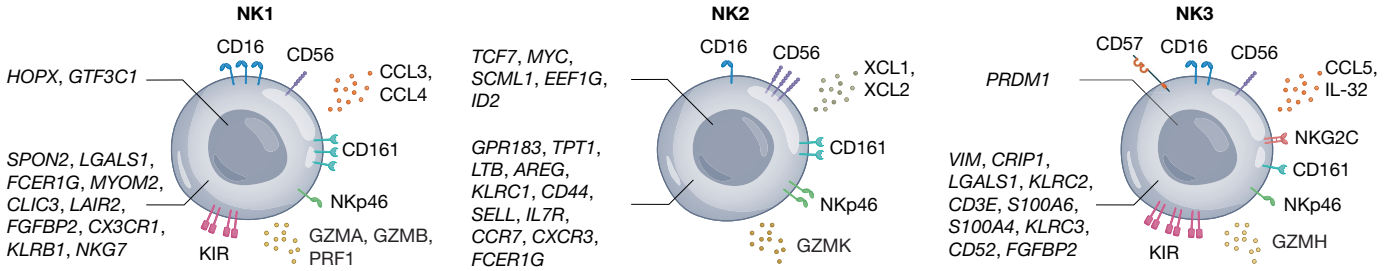

Fig. 1 | Prominent subsets of circulating NK cells in humans. In the peripheral blood of healthy individuals, three dominant NK cell subsets—NK1, NK2 and NK3—are discernible through single-cell transcriptomic analysis. This figure delineates the distinct transcription factors, secreted molecules and cell surface receptors that are characteristic of each subset.

of CD103 (encoded by *ITGAE*) and CD49a (in the lungs and intestines) and CXCR6 (in the bone marrow, spleen and lymph node)[9,14]. Recently, a human pan-cancer atlas of NK cells described tumour-associated NK cells characterized by reduced cytotoxicity and high expression of stress-associated proteins (*DNAJB1* and *HSP* gene family), inhibitory MHC-class-I-specific receptors (members of the *KIR* family) and several transcription factors that control the fitness of NK cells such as NR4A1, EGR3 and KLF6[29].

Another level of complexity in the dissection of NK cell biology resides in the strong similarities with ILC1 cells[30]. For example, NK cells and ILC1 cells that express the activating NK cell receptor NKp46 (encoded by *NCR1*, also known as *CD335*) are dependent upon T-bet (encoded by *TBX21*) and secrete IFNγ [30]. Moreover, NK cells can transition to ILC1-like cells in the presence of environmental cues such as transforming growth factor-β (TGFβ), and these cells have been shown to promote tumour development in some preclinical models[31,32]. Yet, cytotoxic ILC1 cells or ILC1-like cells have also been described to have a role in immunosurveillance of malignancies[33]. More studies are needed to understand the biology of NK cells as compared to ILC1 cells, as distinctive mechanisms involved in the control of NK cells and ILC1 function have already been described[34]. To effectively harness the antitumour properties of NK cells, it is therefore essential to distinguish the phenotype and effector functions of closely related types of ILCs, that is, circulating NK cells, tissue infiltrating NK cells, ILC1 cells and tumour-associated NK cells. In mice, bona fide NK cells and ILC1 cells can be distinguished by the mutually exclusive cell surface expression of CD49b (encoded by *Itga2*) and CD49a (encoded by *Itga1*) respectively[30]. Syndecan-4 is also a useful marker that enables discrimination between mouse ILC1 and NK cells[35]. In humans, it has been proposed that CD200R, CXCR3, CXCR6, DNAM-1 and TRAIL could distinguish ILC1 cells from NK cells[36,37], but the phenotype of ILC1 cells varies according to the tissue in which these cells reside, making the identification of pan-ILC1 markers challenging[38]. At the transcriptomic level, a gene signature with eight cross-species and cross-organ NK-specific markers, *EOMES*, *GZMA*, *IRF8*, *JAK1*, *NKG7*, *PLEK*, *PRF1* and *ZEB2*, has been proposed[35]. Importantly, these eight markers also discriminate human NK cells from the other ILC subtypes and CD4 T cells. By contrast, only three cross-species and cross-organ ILC1-specific markers, *IL7R*, *LTB* and *RGS1*, have been identified, but these three markers were also expressed by other ILC populations and $CD4^+$ T cells.

Recognizing cells in distress

An essential feature of NK cells is their ability to distinguish between normal cells and cells in distress, and to eliminate the latter directly or indirectly[6,10–14,39] (Table 1). NK cells recognize their targets by expressing multiple cell surface receptors[6,10–14,39]. NK cells sense the level of expression of MHC class I (MHC-I) molecules through a variety of MHC-I-specific inhibitory receptors. These include killer cell immunoglobulin-like receptors (KIRs) in humans, lectin-like Ly49 molecules in mice and CD94–NKG2A heterodimers in both species. These MHC-I receptors belong to the large family of inhibitory receptors that mediate their function through intracytoplasmic immune receptor tyrosine-based inhibitory motifs[40,41]. Moreover, NK cells recognize self-molecules that are induced or upregulated on the cell surface of stressed cells[42,43]. The prototypical example of this type of stress-induced self-recognition is the activation of NK cells triggered by cell surface receptors such as NKG2D (encoded by *KLRK1*), NKp46 and NKp30 (encoded by *NCR3*, also known as *CD337*), and which recognize MICA/B and ULBPs[44], ecto-calreticulin[45] and B7-H6[46], respectively, on the surface of stressed cells. The ligands for NKG2D are induced in response to the DNA-damage-response pathway, the integrated stress response, cellular hyperproliferation, activated p53 and heat-shock-induced stress[43,47]. Ligands for other activating receptors are also thought to be dependent on these stress-induced signalling pathways, but some of these ligands remain undefined and the precise mechanisms regulating the expression of the identified ligands are unclear. The NK cell activating receptors associate with adaptors that carry immunoreceptor tyrosine-based activation motifs[40,48]. With inputs of both activating and inhibitory signals, NK cells can recognize and contribute to the elimination of a variety of tumours of all histotypes[49,50]. NK cells can also recognize their cellular targets in the presence of antibodies and trigger antibody-dependent cell cytotoxicity (ADCC), owing to the low-affinity IgG Fc region receptor CD16a, which is

Table 1 | The ten hallmarks of tumour immunity of NK cells compared with T cells

	NK cells	T cells
Natural recognition of cancer cells		
Detection of stressed cells	Yes	Yes
Multiple ligands: tumour-antigen-agnostic activity against a vast array of tumour cells	Yes	No (TCR mediated)
Combat tumour cells with low mutation load	Yes	No
No antigen-specific priming required	Yes	No
No need for MHC-I expression; activity increased in absence of MHC-I expression	Yes	No
Elimination of cancer cells		
Direct killing of tumour cells	Yes	Yes
Production of cytokines and chemokines that shape T cell responses	Yes	Yes
Activity against primary tumours and metastasis	Yes	Yes
Clinical studies have demonstrated		
Efficacy in haematological malignancies	Yes	Yes
Excellent safety profile of cell infusions	Yes	No (graft-versus-host disease)

mainly expressed on the surface of NK1 and NK3 cells. NK-cell-mediated ADCC has been postulated to contribute to the efficacy of therapeutic antibodies such as the anti-CD20 antibody rituximab[51]. After recognition, NK cells can eliminate their cellular targets through two main mechanisms: direct cytotoxicity and production of cytokines. Once activated, NK cells form an immunological synapse with the target cell, enabling the cytotoxic granules released by the NK cell to be directed towards the target cell and not to surrounding bystander cells[52]. Direct cytotoxicity of NK cells is also mediated by the interaction between FAS ligand expressed on NK cells and FAS expressed on the target cells. Indeed, engagement of FAS ligand by FAS leads to target cell death by apoptosis[53]. After the target cell is marked for death, the NK cell detaches and can move on to find another potential target. This ability to engage multiple targets sequentially is referred to as 'serial killing', and improves NK cell immunity. Moreover, the production of pro-inflammatory cytokines such as IFNγ and TNF by NK cells might also exert anti-proliferating, anti-angiogenic and pro-apoptotic effects on cancer cells, which could contribute to their antitumour activity[54].

Why harness NK cells for cancer treatment?

Immunotherapy has undoubtedly revolutionized clinical oncology over the past decade[55,56]. In particular, immune checkpoint inhibitors and CAR-T cells have shown impressive responses across multiple malignancies[55,56]. However, only a subset of patients benefit from these treatments and unmet medical needs remain high. New immunotherapies, in particular, approaches with the potential to circumvent the ability of tumours to evade T cell-directed strategies, are warranted. Given the critical role that innate immune responses have in immunity, harnessing these responses opens new possibilities for tumour control[57] but remains a challenge[58]. In that regard, NK cells have a unique set of antitumour properties that differ from those of T cells (Table 1). NK cells do not require antigen-specific priming and recognize a wide range of cells in distress, regardless of their embryological origin or the type of cell-stress trigger[49,50]. They also exhibit the notable property of controlling the development of metastases by putting metastatic cells into a dormant state through the production of IFNγ or by killing circulating tumour cells before they invade the metastatic niche[59–61]. Importantly, NK cells are not only killer cells that help to suppress tumours, but they are also involved in the generation, shaping and maintenance of adaptive immune responses by promoting, for example, the antigen-presenting function of dendritic cells, such as type 1 conventional dendritic cells at the tumour bed, through the secretion of FLT-3L, XCL1 and CCL5, and by acting directly on T cells through IFNγ[62–64]. In contrast to T cells, infusion of NK cells has been shown to be safe in patients with allogeneic grafts, as NK cells do not mediate graft-versus-host disease[49,50,65,66]. Finally, a very important distinguishing factor between T and NK cells lies in the increase in NK cell function when tumour cells downregulate MHC-I expression on the cell surface. Loss of MHC-I expression is a common T cell immune evasion mechanism[67]. By contrast, as NK cells express inhibitory MHC-I receptors, MHC-I loss contributes to the recognition and efficient elimination of tumour cells by NK cells[68–71]. Thus, several features of NK cell biology make their use an interesting and complementary to other modalities used in oncology, including monoclonal-antibody-based therapies, cell-based therapies or a combination of both (Fig. 2).

Unleashing NK cells

NK cell activity can be restrained by the engagement of checkpoint inhibitors expressed on their surface. Several inhibitory receptors such as NKG2A, lymphocyte activation gene 3 (LAG3), T cell immunoglobulin and mucin domain-containing 3 (TIM-3) and T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT), which mediate inhibition on both NK and T cells, have been described. A number of monoclonal antibodies blocking these immune checkpoints are currently undergoing clinical evaluation. In addition to the anticipated indirect effect on T cell function therapies, both preclinical and clinical data point to their potential impact on unleashing the NK cell compartment (Fig. 2 and Table 2). Here we present studies assessing the therapeutic impact of these four inhibitory checkpoint inhibitors on NK cell functionality. However, more research is warranted to understand the effect of targeting these inhibitory cell surface receptors on NK cells and their potential contribution to therapeutic response.

NKG2A

The CD94–NKG2A heterodimer receptor is expressed by approximately half of NK cells, as well as by a subset of $CD8^+$ T cells. Blocking NKG2A can unleash both T cell- and NK-cell-mediated antitumour immune responses[72,73]. One such antibody is monalizumab, a humanized IgG4 antibody that specifically blocks the interaction between human NKG2A and its cognate non-classical MHC-I molecule HLA-E[72]. Current development efforts for monalizumab are focused on evaluating its efficacy in various combination strategies for lung cancer.

LAG3

LAG3 is expressed on various immune cells, including NK cells, activated and exhausted T cells, B cells, regulatory T cells and dendritic cells[74–77]. LAG3 is a protein that shares similarities with the CD4 molecule and has the ability to interact with MHC-II molecules[78,79]. Moreover, LAG3 can also bind to galectin-3, fibrinogen-like protein 1 and liver sinusoidal endothelial cell lectin[80–84]. The exact mechanism of action of LAG3 is not fully understood at this time. Nevertheless, it is a promising immunotherapeutic target with more than 20 LAG3-targeting therapeutics in clinical trials. Within the class, relatlimab has been approved in combination with the anti-PD-1 antibody nivolumab for the treatment of unresectable or metastatic melanoma. Preclinical studies have shown that blocking LAG3 boosts NK cell tumour immunity[85].

TIM-3

TIM-3 is an inhibitory receptor that is expressed by various immune cells, including T cells, NK cells, regulatory T cells, dendritic cells and macrophages. In healthy adults, NK1 cells and a subset of NK2 cells express TIM-3[86]. Blockade of TIM-3 in combination with anti-PD1 suppress disease progression in MHC-I-deficient tumour-bearing mice[87]. In patients with metastatic melanoma, NK cells are functionally impaired compared with those from healthy individuals. This impairment was reversed by blocking TIM-3[88]. Furthermore, TIM-3 expression is correlated with the disease stage, and blocking TIM-3 reverses the dysfunction of NK cells in patients with lung adenocarcinoma[89]. In multiple myeloma, both peripheral blood and bone marrow NK cells from patients express higher levels of TIM-3 compared with the control individuals[90]. Blockade of TIM-3 restored NK-cell-mediated killing of multiple myeloma tumour cells in vitro and significantly inhibited tumour growth in mouse models of multiple myeloma.

TIGIT

TIGIT is an inhibitory receptor expressed by T cells and NK cells. It binds to its ligands, poliovirus receptor (encoded by *PVR*, also known as *CD155*), nectin-2 and nectin-3[91], triggering inhibitory signalling. TIGIT competes with DNAM-1 (encoded by *CD226*) for binding to PVR and nectin-2[92]. TIGIT signalling reduces NK cytotoxicity and cytokine release. TIGIT-positive NK cells have been detected in various human cancers and tumour-infiltrating lymphocytes isolated from patients

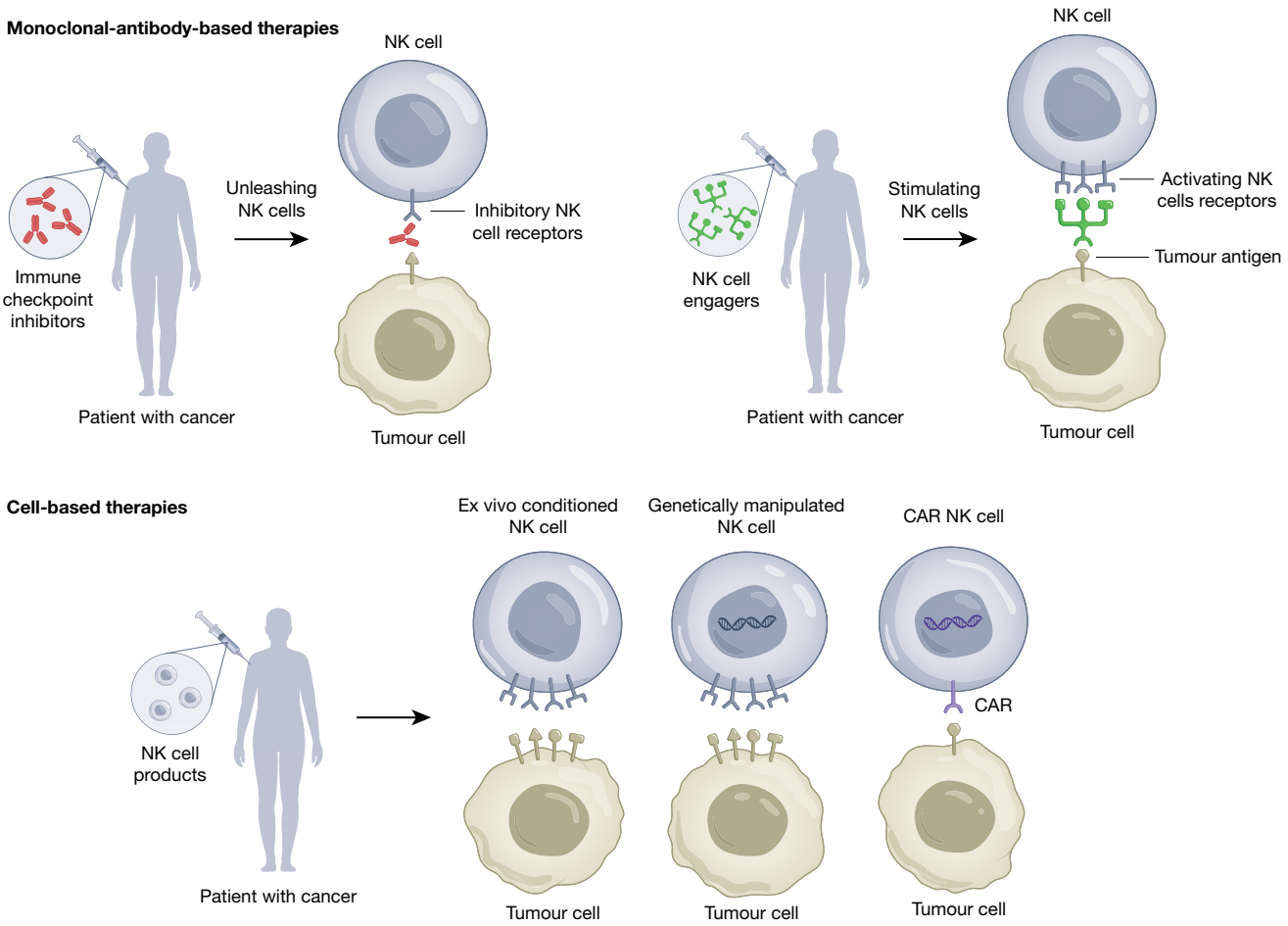

Fig. 2 | NK cell therapies. Two primary therapeutic strategies are being explored to enhance the antitumour efficacy of NK cells: monoclonal-antibody-based therapies (top) and cell-based therapies (bottom). The monoclonal-antibody-based NK therapies encompass the activation of NK cell antitumour immunity using immune checkpoint inhibitors (red antibodies) such as anti-LAG3, anti-NKG2A, anti-TIM-3 and anti-TIGIT monoclonal antibodies, and the augmentation of NK cell antitumour response through monoclonal-antibody-derived tools that stimulate their activating receptors, such as NK cell engagers (light green). The cell-based NK therapies use various sources of NK cell products that are injected into the patients, such as ex vivo conditioned NK cells, genetically manipulated NK cells and CAR NK cells. Activating and inhibitory NK cell receptors (purple) and their cognate ligands expressed on tumour cells are shown (dark green).

with colorectal cancer. Blockade of TIGIT restored NK cell dysfunction and promoted NK-cell-mediated tumour immunity in different mouse models of cancer[93].

Boosting NK cells

Immune cell engagers are bioengineered molecules designed to steer immune cells toward tumours. These engagers primarily consist of multi-armed antibodies that act as bridges between tumour cells and effector cells, facilitating the establishment of an immune synapse. Cell engagers achieve this by targeting tumour antigens and activating receptors expressed on immune effectors. NK cell engagers (NKCEs) have emerged as promising immunotherapies to redirect NK cells and activate their antitumour activity (Fig. 2).

CD16-based NKCEs

NK cells exhibit effector activity through ADCC by recognizing and killing cells opsonized with IgG1 and IgG3 antibodies through CD16a. A common polymorphism in CD16, either phenylalanine (Phe158, low affinity) or valine (Val158, high affinity), affects its affinity for the Fc portion of antibodies and can influence NK-cell-mediated ADCC[94].

Various strategies have been used to enhance NK-cell-mediated antitumour potential[95]. These include glycoengineering the Fc region of cytotoxic antibodies to improve their binding to CD16a[96,97], as well as amino acid substitutions[98]. Another approach involves the development of CD16-engaging Fv fragments, which directly target CD16a on NK cells along with the tumour antigen. This approach bypasses the CD16 Phe/Val158 polymorphism, minimizes off-target interactions with complement or other Fc receptors, and inhibits the displacement of the Fc portion of the therapeutic monoclonal antibodies by the high concentration of circulating antibodies. AFM13, a bispecific NKCE targeting CD30 and CD16, has been evaluated as a monotherapy in a registrational phase 2 trial for CD30-positive relapsed or refractory lymphomas[99]. However, despite initial promising data as a monotherapy, the development of this drug is now focused on combinations, mainly with allogeneic NK cells. Other bispecific antibodies targeting EGFR (AFM24) and CD123 (AFM28) are currently undergoing phase 1/2 and phase 1 trials, respectively (Table 2). Moreover, several antibodies targeting CD16 and tumour antigens such as CD19, CD20, CD33, CD133 and EPCAM have demonstrated efficacy in preclinical studies[100–103].

Trispecific natural killer engagers have been developed by incorporating an IL-15 cytokine element linking the two antibody domains[104].

Table 2 | Clinical landscape of NK-cell-targeting approaches in cancer therapy

		Phase 1	Phase 2	Phase 3	Approved
Monoclonal-antibody-based therapies					
		Target molecules			
Checkpoint inhibitors				TIGIT NKG2A TIM3	LAG3
NKCEs	NKp46	CD20 BCMA CD123			
	CD16	CD123 EGFR	CD30*		
	NKG2D	CD33 BCMA EGFR HER2 c-MET			
Cell-based therapies					
NK cells		Allogeneic NK cells (iPS cell-, UCB-, placenta-, PBMC-derived; >10) Autologous NK cells (×2)	Allogeneic NK cells (UCB-derived and PBMC-derived ×2) Autologous NK cells (×2)		
CAR NK cells		BCMA HER2 CD123 NKG2D ligands	PD-L1 CD19		

The landscape shows the highest level of current clinical development for each target, broken down by approaches targeting NK cells (checkpoint inhibitors, NKCEs, allogeneic or autologous NK cell infusions and CAR NK cells). The analysis is based on active industry-sponsored clinical trials published at https://clinicaltrials.gov/ as of October 2023, from drug candidates in phase 1 clinical trials to approved molecules. The asterisk indicates that AFM13, the bispecific NKCE targeting CD30 and CD16, was tested in combination with allogeneic NK cells derived from umbilical cord blood (UCB) in patients with CD30-positive lymphomas. iPS cell, induced pluripotent stem cell; PBMC, peripheral blood mononuclear cell.

IL-15 provides activation, proliferation and survival signals to NK cells[105]. GTB-3550 (anti-CD16, anti-CD33 and IL-15) showed a clinical benefit but a second-generation camelid nanobody CD33-targeting trispecific natural killer engager (GTB-3650) has been developed with improved tissue penetration[106]. Furthermore, GTB-7550, targeting CD19 (anti-CD16, anti-CD19 and IL-15), has shown enhanced NK cell proliferation and function against multiple B cell malignancies in vitro and is currently undergoing preclinical testing[107]. Note that the expression of CD16 is downregulated in the tumour microenvironment (TME) through its shedding[108], which may limit the benefit of these molecules.

NKG2D-based NKCEs

NKG2D is a cell surface activating receptor expressed on all NK cells in humans and mice, as well as on the cell surface of all $CD8^+$ T cells in humans. The chronic binding of NKG2D to its cognate ligands on the cell surface can lead to desensitization of NK cells, impairing their effector functions[109]. An NK cell engager targeting NKG2D and the multiple-myeloma-associated antigen CS1 has demonstrated efficacy in a preclinical mouse model of multiple myeloma[110]. Another bispecific NKCE has been developed to target both NKG2D and HER2, promoting cytotoxicity of NK cells in vitro[111]. Furthermore, trispecific NK cell engager therapies targeting HER2 (DF1001), BCMA (DF3001), CD33 (DF2001) and EGFR (DF9001) on tumour cells are currently being evaluated in phase 1/2 or phase 1 clinical trials for several haematologic and solid tumours (Table 2). Early signs of clinical activity without dose-limiting toxicities have been reported for DF1001[112]. Moreover, several molecules have been designed fusing an NKG2D ligand (such as MICA, ULBP1 or ULBP2) with a single-chain variable fragment targeting a tumour antigen. These molecules directed against antigens such as BCMA, CEA, CD19, CD24, CD138 or VEGFR2 have demonstrated antitumour activity in both in vitro and in vivo preclinical models[113,114].

Natural cytotoxicity receptor-based NKCEs

NKCEs have been developed to target activating receptors on NK cells with a preferential expression on NK cells, such as the natural cytotoxicity receptors NKp46 and NKp30. In contrast to CD16a and NKG2D, which are expressed by myeloid cells and T cells, respectively, and of which the cell surface expression is downregulated in cancer conditions[115], NKp46 exhibits stable expression in various cancers. Although it is expressed by ILC1 cells, subpopulations of ILC3 cells and discrete T cell subsets, NKp46 is the most preferentially expressed activating cell surface receptor for NK cells[116,117]. The trispecific antibody-based NK cell engager therapeutic (ANKET) platform consists of an antigen-binding antibody fragment that engages NKp46 on NK cells, another antibody fragment that binds to a tumour antigen and an Fc fragment that binds to Fcγ receptors, such as CD16a on NK cells[115]. In vitro studies have shown that NKp46-ANKET induces potent NK cell activation and promotes NK-cell-mediated lysis of tumour cells. Across a variety of haematological and solid tumour models, NKp46-ANKET has been shown to effectively increase the recruitment of NK cells to the tumour site and control tumour growth[115]. An NKp46-ANKET targeting CD123 (NKp46-ANKETCD123 also known as SAR'579/IPH6101) is currently being evaluated in a phase 1/2 clinical trial for the treatment of relapsed/refractory AML[118] (Table 2). Moreover, NKp46-ANKET targeting of CD19 or CD20 has shown promising preclinical results in promoting tumour cell killing in models of paediatric B cell precursor acute lymphoblastic leukaemia[119]. A tetraspecific NKp46-ANKET has also been developed, incorporating a variant of interleukin-2 (IL-2v) that stimulates the IL-2 receptor complex without binding to IL-2Rα (CD25) to prevent regulatory T cell activation and endothelial cell binding[120]. This tetraspecific NKp46-ANKET, targeting CD20, has demonstrated preferential NK cell activation and proliferation compared with T cells in vitro and massive NK cell infiltration at the tumour bed in preclinical models. Additional multifunctional NK cell engagers targeting NKp46 and alternative tumour antigens have been generated. For example, CYT-303 targets NKp46 and glypican 3, which is overexpressed in hepatocellular carcinomas, and has shown antitumour activity[121]. Another NK cell engager, CYT-338, targets NKp46 and CD38 and have demonstrated efficacy against multiple myeloma in vitro[122].

NKp30 is a type I transmembrane activating receptor that is expressed on NK cells, subsets of ILC2 cells, ILC3 cells, and $CD8^+$ αβ and γδ T cells in humans[123,124]. NKp30 binds to the surface molecule B7-H6 and the nuclear factor HLA-B-associated transcript 3 (BAT3) expressed in diverse tumour cell lines[125]. However, in tumour contexts, the soluble form of its ligand, B7-H6, has been associated with the downregulation of NKp30 on NK cells[126]. This downregulation could limit the potential of NKp30 as a target for bispecific antibodies. NKp30-NKCE targeting EGFR or BCMA has shown preclinical efficacy.

NKCEs have entered the clinic quite recently and, with the exception of AFM13, all molecules are still in phase 1 (Table 2). In contrast to T cell engagers, which can be associated with adverse events such as neurotoxicity and cytokine release syndrome (an acute systemic inflammatory syndrome characterized by fever and multiple-organ dysfunction), NK cell engagers aim to provide a safer alternative, which ongoing clinical trials seem to have confirmed so far. While T cell engagers have demonstrated single-agent activity in several haematologic malignancies, both NK cell and T cell engagers face a common challenge in effectively controlling solid tumours. Of the ten NKCEs in the clinic for which the tumour targeting antigens have been disclosed, three are directed against solid tumours (Table 2).

NK cells as drug products

In addition to monoclonal-antibody-based approaches, the use of NK cells as drug products constitutes a rapidly evolving sector of oncological biotherapy. This paradigm encompasses a spectrum of clinical strategies, including both autologous and allogeneic applications, leveraging cellular progenitors from diverse biological origins such as placental tissues, umbilical cord blood, peripheral blood and induced pluripotent stem cell-derived lineages. These approaches further diverge into distinct modalities, spanning from in vitro pre-activation techniques to cutting-edge genomic editing interventions[49,50,127,128]. Several cancer conditions and oncological treatments, notably chemotherapy, are known to attenuate both the abundance and the operative capacity of patient's endogenous NK cells. This depletion underscores the therapeutic rationale for adoptive NK cell transfer, a strategy to enhance the efficacy and resilience of NK cells within the TME. Allogeneic NK cell infusions have emerged as particularly advantageous[49,50,127,128]. Their attributes include immediate availability, robust antitumoural potency against a wide spectrum of neoplastic targets and absence of graft-versus-host disease. Many ongoing clinical trials are investigating the therapeutic efficacy of allogeneic NK cells across a variety of malignancies, underscoring both the potential and the burgeoning research interest in this domain. The favourable safety profile of allogeneic NK cell infusions has catalysed the development of ready-to-use off-the-shelf therapeutic products. However, allogeneic NK cell infusions necessitate preconditioning regimens, currently comprising agents such as fludarabine and cyclophosphamide to mitigate rejection risks[49,50,127,128]. Conversely, autologous NK cell therapy, which harnesses a patient's own cellular resources, obviates the need for such conditioning treatments. Thus, autologous NK cells could have a role in clinical situations in which patients are not eligible for an allogeneic product (for example, in the context of consolidation treatment or minimal residual disease), because the latter would require harsh conditioning. Nonetheless, despite advancements in feeder-cell-free expansion protocols and the attainment of significant proliferative yields—typically 1,000- to 2,000-fold within a 2 to 3 week timeframe—the production of autologous NK cells remains time intensive[129]. This temporal demand represents a logistical challenge in the clinical deployment of autologous NK-cell-based interventions.

Enhancing NK cell performance through ex vivo conditioning

A crucial element of current adoptive NK cell therapy is administering large doses of robustly stimulated NK cells. Ex vivo cytokine stimulation is a widely used method for activating NK cells and facilitating their large-scale expansion. This enables their formulation and cryo-preservation, rendering them ready for infusion as a therapeutic product. Although IL-15 and IL-21 enhance NK cell proliferation and cytotoxicity, their effects vary based on dose, stimulation sequence and duration. Specifically, IL-15 enhances NK cell longevity and antitumour activity by boosting the metabolic fitness of NK cells[130]. IL-21 increases the NK cell ability to identify and kill tumour cells by raising activating receptor expression and promoting the production of key anti-tumour mediators such as IFNγ and TNF. Notably, sustained conditioning of NK cells with TGFβ during ex vivo expansion can render them more resilient in the TME[131]. Among the methods to stimulate the ex vivo expansion of NK cells, feeder cell methods using membrane-bound IL-15 (mbIL-15) and another leveraging soluble IL-12, IL-15 and IL-18 are notable. The latter produces NK cells in a cytokine-induced memory-like (CIML) state, reminiscent of viral reactivation in T cell memory[132]. Indeed, memory capacities of NK cells have been documented[22] and their reactions against tumours could possibly be substantially and sustainably amplified[133]. Early clinical trials using these cytokine-activated NK cells appear promising both in terms of safety and efficacy[134,135]. Expansions using feeder cells with mbIL-21 (FC21) have also been successful, yielding a 1,000-fold increase in potent NK cells within 2 weeks. Such cells display enhanced cytotoxicity against diverse tumours[136,137]. The adoption of a cell-free method using particles derived from plasma membranes bearing mbIL-21 (PM21) has further expanded the potential of NK cell therapies to better enable manufacturability and safety. A key feature of these NK cell methods is that they allow for cryo-preservation to enable clinical delivery. Importantly, FC21-NK or PM21-NK cells, which result from these methods, align well with NKCEs due to their co-expression of NKp46 and CD16[138]. Adoption of these methodologies awaits results from clinical trials.

Enhancing NK cell function through genetic engineering

Despite their remarkable abilities, the effectiveness of NK cells in controlling tumour growth can be limited by immunosuppressive factors in the TME[139–141]. Advances in molecular engineering and gene editing will potentially help to overcome some of these limitations. A primary concern is TGFβ, which is overproduced in many cancers and dampens NK cell function. Introducing dominant-negative TGFβ receptors make NK cells resistant to these effects[142]. Hypoxia in the TME can hamper NK cell function[139–141]. Cells engineered to express a high-affinity CD16a receptor or IL-2 exhibit tolerance to hypoxic conditions comparable to those encountered in the TME[143]. Moreover, strategies to optimize the metabolism of NK cells have been shown to enhance their function. For example, deleting or reducing the cytokine-inducible SH2-containing protein (CISH) in NK cells enhances their metabolic fitness and anti-tumour response, offering another route for immunotherapy enhancement[144,145]. It has also been shown that MYC expression acts as a metabolic rheostat that regulates NK cell growth and effector responses[146]. Thus, ectopic MYC expression in NK cells could help promote cell survival and self-renewal and improve NK cell anti-tumour activity and persistence of NK cells in the TME.

The ability to target NK cells to tumours can be further enhanced by engineering them to express CARs that can recognize specific antigens on tumour cells, and trigger NK cell activation and cytotoxicity independent of the native NK cell receptors[147]. Several clinical trials are underway to address the efficacy of CAR NK cells against human tumours[148]. Promising results were reported from a first-in-human phase 1/2 clinical trial of a CAR NK designed to target CD19 for the treatment of B cell lymphoma[149]. In this case, the NK cells were also engineered to produce IL-15 to stimulate proliferation and persistence.

Driving durable responses through combination strategies

As alluded to earlier, NK cell therapies have achieved some success in haematologic malignancies, but their efficacy against solid tumours remains largely unexplored. As we deepen our understanding of tumour cell evasion mechanisms and develop therapies, the potential for combination strategies to magnify NK cell function becomes clear. These combinations aim to harness both innate and adaptive immunity for optimal anti-tumour activity. Current investigational practices combine NK cell therapies with cytotoxic antibodies or checkpoint inhibitors[49,50,127,128]. For example, allogeneic NK cells paired with the anti-PD-1 antibody pembrolizumab have demonstrated promising results in advanced biliary tract cancer, surpassing previous monotherapy outcomes[150]. An emerging method involves combining NK cell therapy with NKCEs, leading to both direct and NKCE-mediated tumour attacks. For example, the AFM-13 and CIML-NK cell combination is undergoing clinical evaluation and has shown promisingearly signs of activity[134]. Oncolytic viruses, which target and eliminate cancer cells while stimulating immunity, can further potentiate NK cell therapy[151]. Their combination might amplify NK cell tumour infiltration and activity.

Lastly, the combination of NK cell therapies and targeted therapies such as MEK and CDK4/6 inhibitors[152], BH3 mimetics[153], and radiotherapy[154], represent promising oppotunities for therapeutic exploration. Depending on the treatment, they can trigger immunogenic cell deaths, making tumours more recognizable to the immune system. Moreover, targeted therapies may reduce the presence of immunosuppressive cells, fostering NK cell function. Together, these multifaceted approaches could herald a new era of enhanced tumour control.

Conclusions and future directions

Beyond the potential of four immune checkpoint inhibitors to activate both T cells and NK cells (Table 2), considerable advancements are evident in NK cell therapies. As it stands, the most advanced of these therapies are in phase 2 clinical trials, featuring at least one NK cell engager and seven NK cell products, of which two are CAR NK cells (Table 2). Over 40 additional clinical trials are in phase 1, with numerous assets under exploration at the preclinical stage, signal a burgeoning interest in harnessing NK cells for cancer therapy and a shift towards clinical validation.

NK cell therapy holds promise to improve oncology treatments. Achieving this vision demands progress in understanding NK cell biology, technology enhancements, efficient manufacturing methods and clear regulatory guidelines. With a plethora of immuno-oncology options available, the challenge lies in determining the best combinations partners for NK cell therapy tailored to specific patient needs and therapeutic scenarios. The broad tumour-recognition ability of NK cells increases demand for scalable, cost-effective manufacturing solutions. Maintaining cell viability and potency post-cryopreservation is crucial. This capability enables centralized, off-the-shelf production and wider distribution to clinics.

NK cell therapies that do not cause infusion-related complications may enable potential re-dosing or the possibility to use adoptive NK cells either as an induction therapy to lead to complete response followed by a bridging therapy with well-tolerated treatments, or as maintenance therapy. To optimize pharmacokinetic persistence and anti-tumour effects, the need for conditioning regimens is a frequent topic of debate. The resolution of this debate will probably require clinical studies to explicitly test the requirement of conditioning. The use of a conditioning chemotherapeutic regimen would be optimal if it could also have synergistic antitumour effects. A key element to assess such questions will be correlative studies to track adoptive versus endogenous NK cells to enable assessment of whether the use of conditioning makes a difference in the pharmacokinetics of the adoptive NK cells. Such studies will be key for back-translational knowledge on how to design future cell therapeutics.

Augmented NK cells

To amplify the therapeutic index of NK-cell-based treatments, there is a need to promote NK cell trafficking to the tumour sites, their metabolic profile and their effector capacity (both direct cytotoxicity and soluble factor production) (Box 1). This can be realized through specific drugs or advanced NK cell products. With regard to next-generation NK cell products, techniques such as gene editing (for example, targeting CISH[155] or CD38[156]) or pretreatment with agents like nicotinamide[157] can be used. As CD38 is an ectoenzyme that consumes NAD^+, inhibition of CD38 or supplementation of nicotinamide leads to NAD^+ accumulation and boosts NK cell metabolic fitness and effector functions[156,157]. Moreover, growing evidence highlights the importance of epigenetic regulation in immune cell differentiation and function. Exhausted $CD8^+$ T cells display unique epigenetic modifications that set them apart from functional memory $CD8^+$ T cells. Along this line, the histone-methyltransferase enhancer of zeste homologue 2 (encoded by *EZH2*), is known to influence NK cell differentiation and function[158], prompting exploration of how harnessing NK cell epigenetic regulation might contribute to the efficacy of NK cell therapies.

Box 1

Ten challenges to optimize NK cell efficacy

Addressing NK cells to the tumour:
- Enhance NK cell homing to the tumour site
- Overcome the physical barrier
- Optimize NK cell recognition of tumour cells

Enhancing NK cell cytotoxicity and viability:
- Determine the requirements for NK cell persistence at the tumour bed
- Define NK cell fitness in patients with cancer
- Investigate NK cell metabolic adaptations in the TME
- Improve activation signalling

Treatment optimization and standardization:
- Explore synergies of treatment combinations
- Develop real-time monitoring techniques
- Standardize protocols

NK cell persistence

The persistence of NK cells in the host is an important consideration in immunotherapy. The long-term persistence of CAR-T cells, spanning months to years, was initially postulated to contribute to sustained responses[159]. However, it remains to be explored whether the peak of cell expansion can also be a determinant of efficacy. Thus, how long NK cells should reside in the host to be effective, and how the duration could be extended to enhance the clinical efficacy remain to be clarified (Box 1). Robust pharmacokinetic studies during ongoing clinical studies and associated back-translational studies will be very important to better understand the immunological persistence and how it can be further improved.

Building on NK cell hallmarks

The ability of NK cells to identify and target stressed cells offers expansive therapeutic possibilities. Indeed, their ability to detect surface molecules on cells infected by intracellular pathogens or those in other stress conditions could be used to deploy NK cell therapies beyond the realm of cancer treatment. Whether these therapies are antibody based, solely cell based or a mixture of the two, they are poised to be explored in therapeutic areas such as infection, inflammation, ageing and metabolic disorders. Along this line, the development of autologous, non-genetically-modified NK cell products in Alzheimer's disease and of allogeneic NK cell infusions alone or in combination with rituximab in lupus nephritis have been launched. Moreover, as follicular T cells promote autoimmunity and express high levels of PD-1, CAR NK cells expressing the extracellular domain of PDL-1 have been generated and showed efficacy in preclinical settings[160]. Finally, it is noteworthy that the phenotypic similarities between NK and ILC1 cells and their potential functional differences have prompted investigations into whether some of the NK cell therapies, either monoclonal-antibody based or cell based, can involve ILC1. Regardless of this important clarification, the progress in the application of NK cells underscores their versatility and importance for the advancement of modern medicine.

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