nature REVIEWS
DRUG DISCOVERY

Therapeutic approaches to enhance natural killer cell cytotoxicity: the force awakens

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Scientific insights into the human immune system have led to unprecedented breakthroughs in immunotherapy, and drugs and cell-based therapies that have been developed to bolster humoral and T cell immune responses represent an established and growing component of cancer therapeutics. Although NK cells have long been known to have advantages over T cells in terms of their capacity to induce antigen-independent immune responses against cancer cells, their therapeutic potential in the clinic has been largely unexplored. Here, we present different pharmacological and genetic strategies to bolster NK cell antitumour immunity. These approaches, as well as advances in our ability to expand NK cells *ex vivo* and manipulate their capacity to home to the tumour, have now armed investigators with a variety of new strategies to harness the full potential of NK cell-based cancer immunotherapy in the clinic.

NK cell tumour killing

NK cells can mediate cytotoxicity through several distinct mechanisms. Degranulation is the most studied pathway, in which NK cells release cytotoxic granulae upon interaction with target cells; this is controlled by NK cell receptors such as NKG2D, DNAM1 and others, which are counterbalanced by signalling through inhibitory receptors. The 'missing-self' hypothesis, formulated in the 1980s, postulated the lack of self-MHC class I molecules on target cells as a common factor leading to NK cell cytotoxicity. Subsequent research has revealed that the functional capacity of NK cells is 'tuned' by self-MHC class I molecules (known as NK cell education). In humans, KIRs and NKG2A are currently the only known receptors to mediate functional tuning. The Fc receptor CD16 can trigger potent degranulation upon interaction with antibody-coated cells (known as ADCC) without a need for simultaneous co-activation signals. Other routes by which NK cells can kill are through the death receptor pathways TRAIL–TRAILR and FAS–FASL. These receptors induce apoptosis through caspase activation inside target cells and, therefore, mediate cytotoxicity independent of both NK cell education and signalling from receptors controlling NK cell degranulation.

Cellular therapies and *ex vivo* manipulation of NK cells

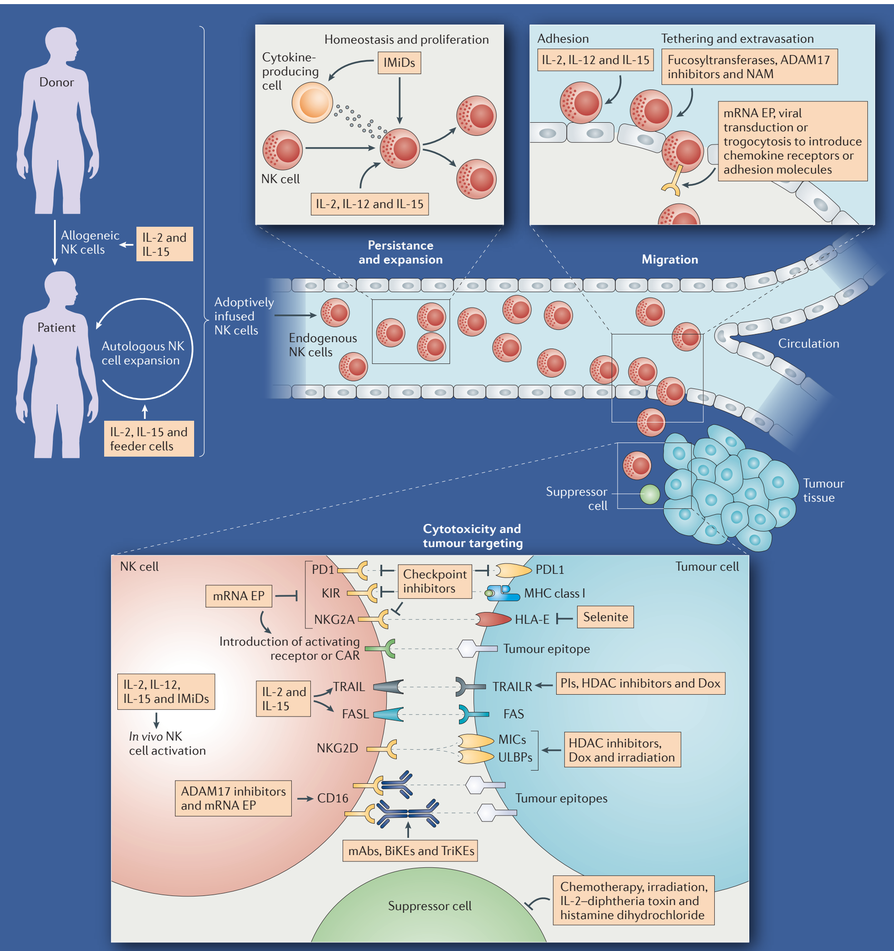
Adoptive transfer of short-term, *ex vivo*, IL-2-activated allogeneic NK cells has been shown to induce clinical responses in patients with AML and multiple myeloma. Administration of IL-2 after adoptive cell transfer can further promote *in vivo* expansion of infused NK cells. However, interest has shifted to the use of IL-15 to avoid expanding T_{reg} cells. Numerous methods have also been developed to expand NK cells *ex vivo*, which enables the use of multiple large-number infusions of highly activated NK cells.

Genetic manipulation of NK cells before adoptive transfer may allow for the optimization of *in vivo* persistence, homing to tumours and tumour cytotoxicity; NK cells engineered to express an anti-CD19 CAR are in clinical trials. Genetic manipulation of primary NK cells using viral vectors is currently inefficient, but new GMP-compliant methods to reprogramme NK cells using mRNA electroporation offer rapid and cost-efficient ways to explore a wide range of genetic approaches to enhance NK cell immunotherapy. A complementary approach is the viral transduction of NK cell lines using adenoviral and lentiviral vectors, enabling stable transgene expression. However, infusions of allogeneic NK cell lines require conditioning of the patient to avoid rapid rejection of the infused cells by the host immune system.

There are also several investigational strategies to manipulate *ex vivo* expanded NK cells with small-molecule drugs. For example, treatment with inhibitors of ADAM17 was shown to augment NK cell ADCC by preventing shedding of the CD16 receptor, and treatment of NK cells with nicotinamide enhances their expression of L-selectin, which is known to be essential for cellular trafficking.

One of the hurdles of using cytokine-activated and *ex vivo* expanded NK cells in patients with haematological malignancies is that NK cells express mostly non-fucosylated ligands for E-selectin, which limits their ability to home to the bone marrow. Preclinical investigations show that *ex vivo* fucosylation of adoptively transferred NK cells improves their antitumour effects in haematological cancers.

A novel, alternative strategy to improve NK cell migration is to alter their phenotype using mRNA EP or by culturing them on feeder cells that express homing receptors (such as CCR7), which are transferred to the NK cell membrane by trogocytosis.



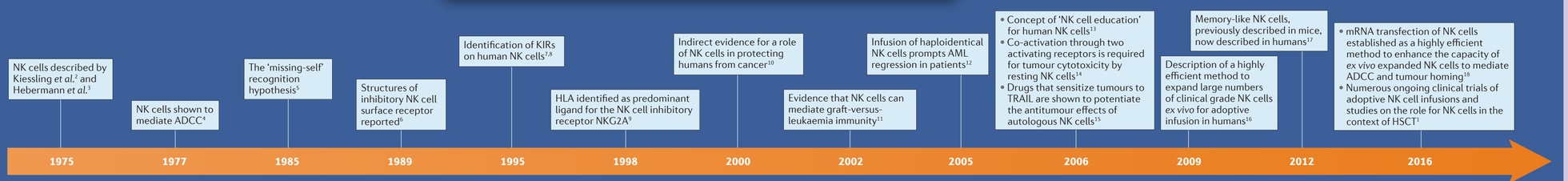
Cytokines to boost NK cell persistence, expansion, cytotoxicity and migration

IL-2 and IL-15, as well as the pro-inflammatory cytokine IL-12, are currently being characterized in terms of their ability to stimulate NK cell antitumour immunity in humans. IL-2 has been shown to promote homeostasis, proliferation and cytotoxicity of NK cells, and rIL-2 (also known as aldesleukin) was the first cytokine used clinically to boost immune responses in cancer patients. However, responses were limited and toxicity substantial. Low-dose IL-2 therapy has been incorporated into clinical trials to support the *in vivo* persistence of adoptively infused NK cells. However, even ultra-low doses of IL-2 have been shown to stimulate the expansion of host T_{reg} cells, suppressing NK cell proliferation and cytotoxicity. New variants of IL-2, constructed to selectively bind to the IL-2Rβ subunit expressed on NK cells rather than the IL-2Rα subunit expressed on T_{reg} cells, are under development.

IL-15 promotes NK cell development, expansion and homeostasis while avoiding T_{reg} cell mobilization. However, scIL-15, the first version of IL-15 to be used in the clinic, had high toxicity. When used following adoptive NK cell infusion in patients with AML, scIL-15 supported both the persistence and proliferation of NK cells. A heterodimeric IL-15–sIL-15Rα complex (known as hetIL-15) is more potent at stimulating NK cell proliferation. Although unlikely to be of great benefit as a single agent, it may be useful in combination with adoptive NK cell infusions or with drugs that induce NK cell tumour cytotoxicity. IL-12 upregulates adhesion molecules and the migration capacity of NK cells. Despite limited efficacy and substantial toxicity, early clinical studies of high-dose IL-12 therapy in patients with cancer showed some antitumour responses. A phase I study of low-dose subcutaneous rIL-12 in healthy individuals reported an improved toxicity profile and provided evidence that this cytokine modulates NK cell migration.

Drugs to augment NK cell cytotoxicity and tumour targeting

- *Immunomodulatory drugs*. The thalidomide derivatives lenalidomide and pomalidomide can decrease the threshold for NK cell activation. Lenalidomide represents a standard therapy for multiple myeloma and myelodysplastic syndromes, and it indirectly augments NK cell cytotoxicity and proliferation by stimulating the release of IL-2 and IFN-γ from surrounding T cells and dendritic cells.
- *Immune checkpoint inhibitors*. The checkpoint protein PD1 (expressed on activated T cells, B cells, monocytes and NK cells) and its ligand PDL1 (expressed by tumour cells) have a central role in tumour recurrence and progression, as signalling through this pathway suppresses lymphocytes, including NK cells. *In vitro*, blockade of PD1 on NK cells augments lysis of autologous tumour cells; preclinical experiments have shown that PD1 blockade boosts NK cell-mediated ADCC and NK-cell tumour trafficking, as well as suppressing T_{reg} cell function. To what extent these mAbs bolster tumour immunity through NK cells, and their potential to enhance the antitumour effects of adoptively infused NK cells, remains to be investigated. PD1 inhibitors are currently in clinical trials in combination with lirilumab (also known as IPH2102), a mAb targeted against KIR. Anti-KIR mAbs such as IPH2101 and IPH2102 were shown to augment NK cell-mediated lysis of tumour cells and ADCC *in vitro*. However, a phase II clinical trial of IPH2101 in patients with multiple myeloma did not establish efficacy. Whether anti-KIR mAbs will show efficacy in other disease settings or in combination with other therapies remains to be determined.
- *mAbs, BiKES and TriKEs*: Tumour-targeting mAbs can induce ADCC through binding of their constant domain to the CD16 receptor on NK cells. However, the degree to which these mAbs mediate NK cell antitumour responses is poorly characterized. BiKEs and TriKEs are engineered molecules that exclusively act through ADCC by crosslinking epitopes on tumour cells with CD16 on NK cells. They are easier to produce than mAbs and bind to a different region of the CD16 molecule, resulting in stronger NK cell ADCC. Several BiKEs and TriKEs are in preclinical investigations.
- *Drugs that sensitize tumours to NK cells*. Proteasome inhibitors, such as bortezomib and the anthracycline doxorubicin, can enhance the susceptibility of tumour cells to NK cell killing by upregulating TRAILR on tumour cells and increasing the activity of caspase 8. Moreover, some proteasome inhibitors and histone deacetylase inhibitors can upregulate NKG2D ligands on the tumour cell surface, and selenite was shown to enhance NK cell killing by reducing the expression of the MHC class I molecule HLA-E on tumour cells.
- *Strategies to inhibit suppressor cells*. NK cell activity can also be enhanced by inhibiting suppressor cells with chemotherapy, irradiation or histamine dihydrochloride, or by killing them with diphtheria toxin conjugated to IL-2.



Class	Drug	Effects on NK cells	Patient populations	Comments
*Clinical studies with drugs that can bolster NK cell antitumour immunity**				
Cytokines	IL-2	↑ Cytotoxicity ↑ Persistence and expansion	Melanoma, RCC, AML, neuroblastoma, breast cancer, ovarian carcinoma, fallopian tube cancer and peritoneal cancer	Some studies combine IL-2 with antitumour mAbs. rIL-2 (aldesleukin) is FDA approved
	IL-15	↑ Cytotoxicity ↑ Persistence and expansion	Melanoma, RCC, lung cancer, SCC and multiple myeloma	scIL-15 and hetIL-15 used‡
	IL-12	↑ Cytotoxicity ↑ Migration	Healthy volunteers	Lower doses improve toxicity profile
IMiDs	Lenalidomide	↑ Cytotoxicity ↑ Persistence and expansion	Multiple myeloma, BCL and neuroblastoma	FDA approved
Checkpoint inhibitors	PD1-specific mAbs	↑ Cytotoxicity	Solid tumours and multiple myeloma	Tested in combination with IPH2102 (lirilumab)‡
	KIR-specific mAbs	↑ Cytotoxicity	Multiple myeloma, AML, melanoma, lung cancer and peritoneal cancer	IPH2101 and IPH2102‡
Tumour-targeting mAbs	CD20-specific mAbs	↑ Cytotoxicity	BCL and multiple myeloma	Rituximab and veltuzumab. FDA approved
	GD2-specific mAbs	↑ Cytotoxicity	Neuroblastoma	Several different GD2-specific mAbs are being evaluated‡
	EGFR-specific mAbs	↑ Cytotoxicity	SCC	Cetuximab used in all studies†
	ERBB2-specific mAbs	↑ Cytotoxicity	Breast cancer	Trastuzumab used in all studies†
Tumour-sensitizing agents	Bortezomib	↑ Cytotoxicity	CLL, RCC, lung cancer, multiple myeloma and sarcoma	Administered before infusion of expanded NK cells to sensitize tumours to NK cell TRAIL‡
T_{reg} cell eradication	Diphtheria toxin IL-2	↑ Cytotoxicity ↑ Persistence and expansion	AML, non-Hodgkin lymphoma and CLL	Used before NK cell infusion and in one study combined with pentostatin and rituximab‡
Clinical studies evaluating adoptively infused NK cells				
Activated, non-expanded NK cells	Autologous NK cells plus IL-2	↑ Cytotoxicity	Melanoma, RCC, lung cancer, nasopharyngeal cancer	Limited number of studies in patients with different tumour types‡
	Autologous NK cells plus IL-15	↑ Cytotoxicity	Neuroblastoma, sarcoma, Wilms tumour and rhabdomyosarcoma	Intended to bolster NK cell tumour immunity more specifically than IL-2 does
	Allogeneic NK cells plus IL-2	↑ Cytotoxicity	AML, multiple myeloma, myelodysplastic syndromes, lymphoma, ovarian carcinoma, melanoma, neuroblastoma, Ewing sarcoma, breast cancer and fallopian tube cancer	Most data published on adoptive NK cell therapy comes from these studies‡
	Allogeneic NK cells plus IL-15	↑ Cytotoxicity	AML and myelodysplastic syndromes	Intended to bolster NK cell tumour immunity more specifically than IL-2 does
Ex vivo expanded NK cells	Autologous NK cells	↑ NK dose and cytotoxicity	CLL, RCC, lung cancer, multiple myeloma, sarcoma, colon cancer, melanoma, neuroblastoma, prostate cancer, ALL and pancreatic cancer	Various expansion methods used, including EBV–LCL and membrane-bound cytokine/4-1BBL feeder cells†
	Allogeneic NK cells	↑ NK dose and cytotoxicity	AML, myelodysplastic syndromes, T-cell lymphoma and multiple myeloma	Various expansion methods used, including membrane-bound cytokines or 4-1BBL feeder cells. Some studies use IL-2 after NK cell infusion‡
Expanded, genetically manipulated NK cells	CD19 CAR mRNA	↑ NK dose and redirected tumour targeting	BCL	Haploidentical NK cells expanded with K562 mbIL-15/4-1BBL feeder cells
NK cell line	NK-92	Off-the-shelf NK cells	AML, multiple myeloma and lymphoma	Dose-escalating clinical trials

Abbreviations
ADAM17, disintegrin and metalloproteinase domain-containing protein 17; ADCC, antibody-dependent cellular cytotoxicity; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BCL, B cell lymphoma; BiKE, bispecific killer engager; CAR, chimeric antigen receptor; CCR7, CC-chemokine receptor 7; CLL, chronic lymphatic leukaemia; CXCR3, CXC chemokine receptor type 3; DNAM1, DNAX acessory molecule 1; Dox, doxorubicin; EBV–LCL, Epstein Barr virus–lymphoblastoid cell line; EGFR, epidermal growth factor receptor; EP, electroporation; FASL, FAS ligand; Fc, crystallizable fragment; GMP, good manufacturing practice; HDAC, histone deacetylase; hetIL-15, heterodimeric IL-15–sIL-15Rα complex; HSCT, haematopoietic stem cell transplantation; IL, interleukin; IL-2R, IL-2 receptor; IMiD, immunomodulatory drug; KIR, killer cell immunoglobulin-like receptor; mAb, monoclonal antibody; mbIL-15, membrane-bound IL-15; MIC, MHC class I polypeptide-related sequence; MHC, major histocompatibility complex; NAM, nicotinamide; NK, natural killer; NKG2, NK group 2; PI, proteasome inhibitor; PD1, programmed cell death protein 1; PDL1, PD1 ligand 1; PSGL1, P-selectin glycoprotein ligand 1; RCC, renal cell carcinoma; rIL-2, recombinant IL-2; SCC, squamous cell carcinoma; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand, TRAILR, TRAIL receptor; T_{reg} cell; regulatory T cell; TriKE, trispecific killer engager; scIL-15, single chain recombinant IL-15; sIL-15Rα, soluble IL-15Rα; ULBP, U16-binding protein. *Only trials studying the effect of drug treatment on NK cells as a primary or secondary end point are listed. Data presented in the tables are from ClinicalTrials.gov. †Phase II or higher.

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