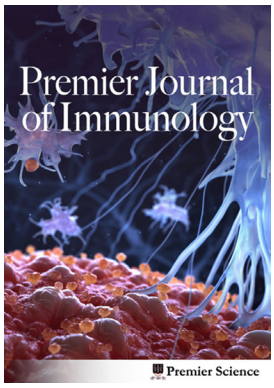

Engineering Natural Killer Cells for Cancer Immunotherapy: Advances, Challenges, and Future Perspectives

Sumit Sharma

ABSTRACT

Cancer is still one of the most common causes of death around the world, and problems with traditional treatments, such as toxicity, resistance, and relapse, have led to the creation of immunotherapeutic strategies. Among these, natural killer (NK) cells have emerged as a promising platform due to their innate ability to recognize and eliminate tumor cells without prior sensitization and without major histocompatibility complex (MHC) restriction. This review presents a full picture of the latest developments in NK cell biology and engineering for cancer immunotherapy. It describes the main ways in which NK cells can be activated, kill other cells, and change the immune system, as well as their main sources, which are peripheral blood, cord blood, NK-92 cell lines, and NK cells created from induced pluripotent stem cells.

Recent advancements in genetic engineering, notably chimeric antigen receptor (CAR)-NK cells, cytokine armoring, and CRISPR-based gene editing, have markedly improved NK cell specificity, durability, and antitumor efficacy. Emerging methodologies, including synthetic biology, nanotechnology-based delivery systems, and metabolic reprogramming, are enhancing resistance to the immunosuppressive tumor microenvironment (TME). Preliminary clinical trials indicate promising efficacy and a favorable safety profile relative to CAR-T cell therapies, especially in hematological malignancies.

Even with these improvements, problems still remain, such as limited in vivo persistence, poor tumor infiltration, and complicated manufacturing processes. Continued integration of advanced engineering strategies and clinical optimization is expected to position NK cell-based therapies as a key component of next-generation cancer immunotherapy.

Keywords: CAR-NK immunotherapy, Crispr-engineered nk cells, Cytokine-armored nk cells, iPSC-derived nk platforms, Tumor microenvironment resistance strategies.

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Introduction

Cancer is one of the major causes of death in the world. Globally, approximately 20 million new cancer cases and 9.7 million cancer-related deaths were recorded in 2022, with projections indicating further growth in the coming decades.[1] Traditional therapies (radiation therapy, chemotherapy, and surgery) have low success rates because of drug resistance, recurrence, and off-target side effects. This has led to increased interest in immunotherapy.[2] Chimeric antigen receptor (CAR) T cells have transformed the treatment of hematologic cancer, and their application is hampered by serious toxicities (cytokine release syndrome [CRS], neurotoxicity), lengthy production times, and the required patient-specific products. In addition to this, CAR-T has not been effective in solid tumors.[3]

Natural killer (NK) cells can be used in addition to immunotherapeutics. NK cells, being innate lymphocytes, are capable of identifying and destroying tumor cells without first being antigen-primed.[4] They do not need to be HLA-compatible, and off-the-shelf allogeneic therapies can therefore be administered without inducing graft-versus-host disease (GvHD). NK cells also release cytokines (e.g., IFN-γ and TNF-α) to activate other immune cells. Recent clinical trials indicate that CAR-NK therapies are effective in terms of high response rates in blood cancers, with minimal toxicity.[5–7] As an example, in one trial, use of cord-blood-derived CAR-NK cells yielded a response rate of 73% in the case of leukemia.[8]

This review provides a summary of recent developments in NK cell biology and engineering. We first describe the mechanisms and sources of NK cells. We then discuss key engineering solutions, genetic editing (including CARs), synthetic biology, and biomaterial-based strategies, that can help to improve the performance of NK cells. We further address the use of engineered NK cells to overcome the suppressive tumor microenvironment. Lastly, we discuss clinical trials, challenges, and future directions of research.

Search Strategy and Scope

This is a structured narrative review. Literature was identified through systematic searches of PubMed, Embase, and ClinicalTrials.gov. Searches used Medical Subject Headings (MeSH) and free-text terms including "natural killer cell," "CAR-NK," "NK cell engineering," "CRISPR NK cell," "iPSC-derived NK," "tumor microenvironment NK," and "NK cell immunotherapy." The search covered the period January 2018 to April 2026, with key earlier studies included where foundational. Clinical trials were identified via ClinicalTrials.gov using "NK cell" and "CAR-NK" filtered to interventional oncology studies Phase I or higher. Inclusion criteria were as follows: primary data, systematic, or high-quality narrative reviews on NK cell biology, engineering, or clinical oncology application. Conference abstracts and non-oncology studies were excluded.

Biology of Natural Killer Cells

NK cells are developed by the common lymphoid progenitor in the bone marrow and pass through a multistage developmental program, which involves the expression of lineage-defining transcription factors and surface receptors (Figure 1). In humans, they are traditionally sub-categorized into the CD56*bright*CD16*low/−* and CD56*dim*CD16+ subsets, which vary in their trafficking properties and effector

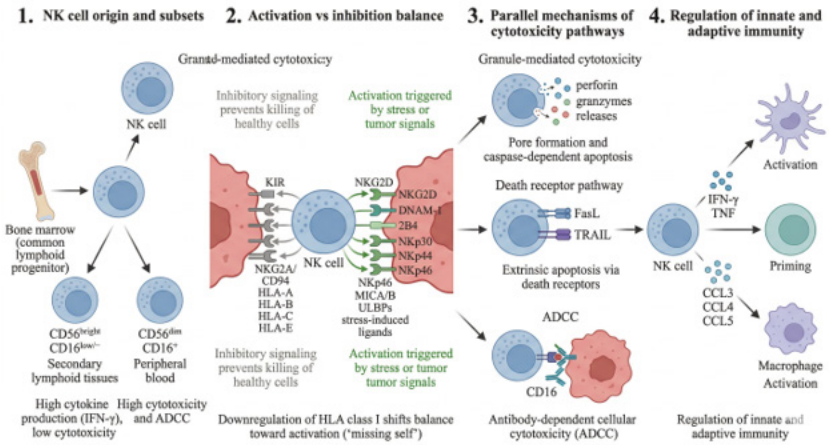

Fig 1 | Biology and mechanisms of action of natural killer (NK) cells in cancer immunotherapy. The figure depicts NK cell developmental subsets, the balance of activating (NKG2D, DNAM-1, NCRs) and inhibitory (KIR, NKG2A/CD94) receptors, cytotoxic mechanisms (perforin/granzyme, FasL/TRAIL, ADCC via CD16), and immunomodulatory cytokine/chemokine output. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; FasL, Fas ligand; IFN-γ, interferon-gamma; KIR, killer immunoglobulin-like receptor; MHC, major histocompatibility complex; NCR, natural cytotoxicity receptor; and TRAIL, TNF-related apoptosis-inducing ligand. Figure is original and created by the authors

functionality.[9,10] CD56*bright* cells are concentrated in the secondary lymphoid tissues, and high levels of cytokines (IFN-γ) are produced; cytotoxicity is relatively weak in the baselines of CD56*bright* cells, as compared to CD56*dim* cells, which are the most prevalent in peripheral blood and also have high levels of natural cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC). More recent single-cell transcriptomic studies have found further NK subpopulations such as adaptive or memory-like NK cells, which are generated following viral infections and exhibit more effective functions and persistence.[11]

NK cell activity is regulated by an active balance between signals of activation and inhibition. KIRs and heterodimer NKG2A/CD94 that bind HLA-E are examples of inhibitory receptors, and their interactions with the HLA-A, HLA-B, and HLA-C molecules recruit phosphatases to suppress proximal signaling and prevent improper killing of healthy cells expressing normal amounts of HLA class I.[12] On the other hand, NKG2D, DNAM-1, 2B4, and natural cytotoxicity receptors (NCRs) NKp30, NKp44, and NKp46 receptors, which are activated by stress-induced ligands (e.g., MICA/B, ULBPs) or viral/tumor antigens and linked to ITAM-containing adaptor molecules, activate NK cell cytotoxicity.[13] Downmodulation of HLA class I on tumor or virus-infected cells places this balance in favor of activation, enabling NK cells to kill targets that cytotoxic T lymphocytes cannot.[9]

NK cells use a number of complementary systems to kill target cells. The prevailing mechanism is polarized discharge of cytotoxic granules containing perforin and granzymes at the immunological synapse, resulting in the creation of a pore in the target cell membrane and caspase-dependent apoptosis.[13] NK cells also secrete death ligands like Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), which interact with cognate death receptors on tumor cells to induce extrinsic apoptotic signaling.[11] Moreover, NK cells induce ADCC by means of FcγRIIIa (CD16), a receptor of the Fc portion of tumor-bound antibodies and targeted cytotoxicity by the NK cells, and it is used by many therapeutic monoclonal antibodies and bispecific engagers.[14]

In addition to direct killing, NK cells secrete cytokines (IFN-γ, TNF-α) and chemokines (CCL3, CCL4, CCL5), which regulate innate and adaptive immunity, inducing the development of dendritic cells, priming of T cells, and activation of macrophages (Figure 1). The conceptual basis of this engineering approach is detailed knowledge of the ontogeny of NK, the biology of the receptors, and mechanisms of action, which can be used to increase specificity, persistence, suppressive resistance, and synergy with other immune system components.[11]

Engineering Strategies for NK Cells

Genetic Engineering

Genetic engineering is also the foundation of NK-cell improvement, and CAR-NK cells represent the most advanced implementation. CAR constructs are typically based on a single-chain variable fragment (scFv) that recognizes a tumor antigen, a hinge and transmembrane domain, and NK-biology optimized intracellular signaling domains (e.g., 4-1BB, CD28, 2B4).[15] CAR signaling has to be able to combine with endogenous activating and inhibitory receptors in NK cells, and it is now being found that activation and persistence can be further augmented by the addition of NK-specific adaptors (such as DAP10, DAP12).[16] The choice of a co-stimulatory domain significantly influences CAR-NK performance: 2B4 engages SAP-family adaptors and amplifies granule exocytosis; 4-1BB promotes mitochondrial biogenesis and metabolic fitness, supporting longer persistence; and DAP10 and DAP12 couple directly to PI3K and Syk/ZAP70 pathways and, when included as accessory signaling modules, augment activation synergistically. 2B4-DAP10 or DAP12 combinations are generally preferred for short-term cytotoxicity, while 4-1BB-containing constructs are advantageous when longer persistence is required.[15,16] The most popular method to insert CARs into primary NK cells, cord blood-derived NK cells, and NK-92 lines is the use of viral vectors (retroviral, lentiviral), but non-viral transposon systems and mRNA electroporation are under development to enhance the safety and flexibility of manufacture.[13,14] Compared with viral vectors, lipid nanoparticle (LNP)–mRNA delivery eliminates insertional mutagenesis risk and simplifies GMP production at the cost of a shorter expression duration; transposon-based systems (e.g., Sleeping Beauty, PiggyBac) bridge this gap by enabling stable integration at lower insertional risk.[13]

CRISPR-Cas9 gene editing can result in systematic modulation of NK-cell-inhibitory pathways and

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metabolic checkpoints. Preclinical gene knockout studies of genes that encode inhibitory receptors or negative regulators downstream (e.g., CBLB, PD-1, CIS) have demonstrated promotion of NK-cell activation, persistence, and control of tumors.[17] Alteration of signaling elements that limit cytokine responsiveness, or interference with adenosine A2A receptors and TIGIT, has also been considered to overcome TME-mediated inhibition. Simultaneously, it is important to use engineering to minimize fratricidal killing and self-tolerance, for example, by modulating HLA-E expression or precisely pairing NKG2A and HLA edits to prevent uncontrolled autoreactivity (Figure 2).[9,12,16]

Cytokine Engineering

Cytokine supplementation, especially interleukin-2 (IL-2), IL-15, and IL-21, is necessary to expand and increase the survival of NK cells, although administration of high doses of cytokines systemically induces devastating toxicities like vascular leak syndrome. To avoid this, cytokine engineering schemes conjugate cytokine signaling directly to the NK cell.[11,14] CAR-NK cell transgenic expression of membrane-bound or secreted IL-15 maintains proliferation and antitumor activity in vivo with no exogenous cytokine, and has been integrated into a few products in clinical stages.[15,18] Others genetically activate CAR cassettes and IL-15, plus its receptor α-chain, to recreate a local autocrine loop; some others genetically activate IL-21 or modified cytokine receptors that are sensitive to low cytokine levels.[19]

Cytokine-armed NK cells can also reprogram the TME by attracting native immune effectors and neutralizing suppressive myeloid ones.[20,21] As an illustration, the expression of IL-12 or IL-18 stimulates Th1-polarizing inflammation, increases antigen presentation, and can transform more inflamed NK- and T-cell-permissive niches (although, again, strictly regulated to prevent systemic toxicity).[22] To achieve tunable proliferation and safety, synthetic cytokine switches have been considered where engineered NK cells have been designed to respond selectively to small-molecule drugs or tumor cues (Figure 2).[20]

Synthetic Biology Approaches

Synthetic biology is based on the classical CAR design, but also has logic gating, controllability, and safety. Logic-gated CARs, including AND/OR/NOT circuits, combine signals of a variety of antigens or microenvironmental markers to enhance specificity and minimize on-target/off-tumor toxicity.[23,24] As an example, dual-CAR NK cells can be co-engaged with a tumor-restricted antigen and a stress-induced ligand, or they can be vetoed using inhibitory CARs of antigens of normal tissues, thereby enhancing the ability to distinguish between malignant and normal cells.[25,26]

Switchable CAR systems also improve the level of control by decoupling antigen recognition and signaling using modular adaptors. Here, NK cells are equipped with a universal CAR that binds a tagged epitope on soluble adaptor molecules, and the same NK product can be redirected to other antigens by changing the adaptor (e.g., CD19, HER2, EGFR), and the same NK product can be titrated or silenced by changing adaptor dosing.[27,28] Safety switches, such as inducible caspase-9 (iCasp9) suicide genes or

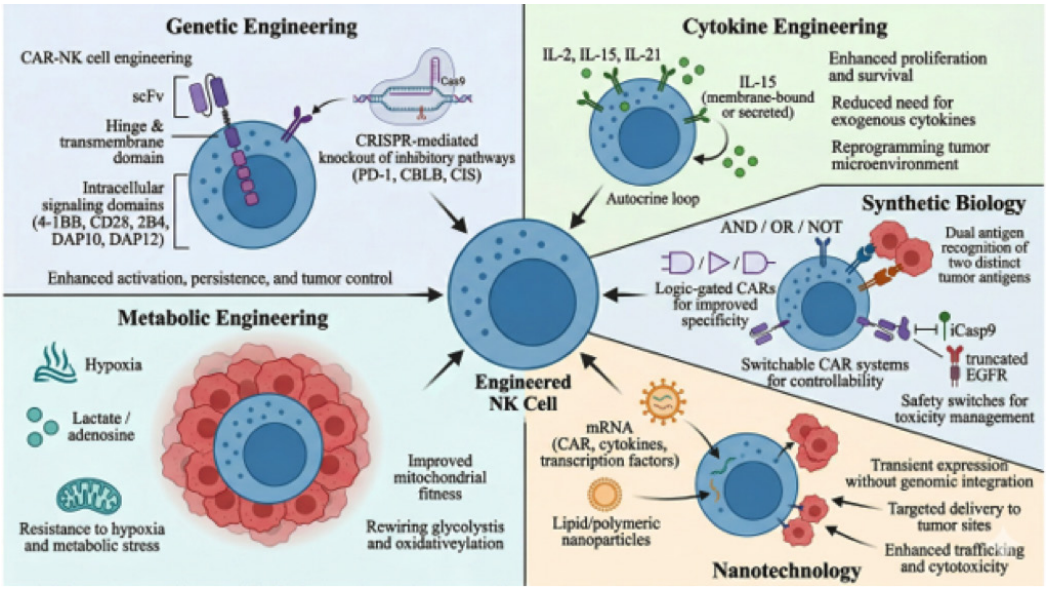

Fig 2 | Engineering strategies to enhance natural killer (NK) cell-based cancer immunotherapy. The figure illustrates CAR design with co-stimulatory domain variants (2B4, 4-1BB, DAP10/12), cytokine armoring (IL-15, IL-21), CRISPR-based gene editing, synthetic biology logic-gated circuits, safety switches (iCasp9), LNP-mRNA nanoparticle delivery, and metabolic reprogramming. Abbreviations: CAR, chimeric antigen receptor; CRISPR, clustered regularly interspaced short palindromic repeats; iCasp9, inducible caspase-9; IFN-γ, interferon-gamma; IL, interleukin; LNP, lipid nanoparticle; mRNA, messenger RNA; TGF-β, transforming growth factor-beta; and TME, tumor microenvironment. The figure is original and created by the authors

elimination markers (e.g., truncated EGFR), allow efficient ablation of engineered NK cells in case of unwanted toxicity, an especially valuable safety mechanism in first-in-human experiments (Figure 2).[29]

Nanotechnology and Nucleic Acid Delivery

Nanotechnology is a potent approach that can be used to regulate NK cells and their microenvironment without long-lasting integration into the genome. mRNA encoding CARs, cytokines, or transcription factors can be delivered into NK cells using lipid nanoparticles, polymeric carriers, or inorganic nanomaterials to stimulate transient expression, thereby decreasing the risk of insertional mutagenesis while promoting strong short-term activity.[30] Local nanoparticle delivery to tumor sites can focus payloads that induce or enlist endogenous NK cells, such as through IL-15 superagonists or by upregulation of NKG2D ligands, and can be used together with adoptively transferred engineered NK products (Figure 2).[31,32]

NK cells can also be shielded by nanoparticles to inhibitory cues, or TGF-β, adenosine pathways, or metabolic checkpoints can be co-delivered by nanoparticles. There is an early indication that NK cells covered or loaded with nanoparticles can be improved in terms of trafficking, resistance to oxidative damage, and increased cytotoxicity with respect to solid tumors. These platforms are highly modular and can be combined in strategies, such as co-packaging of imaging agents, so as to allow non-invasive monitoring of NK-cell biodistribution (Figure 2).[30]

Metabolic Engineering

Hypoxia, nutrient depletion, and the build-up of immune-suppressive metabolites including lactate and adenosine, which damage NK cell effector activity, are the hallmarks of the TME.[33,34] Hypoxia-inducible factor-1α (HIF-1α), under hypoxic conditions, suppresses the expression of activating receptors (e.g. NKG2D, NKp30, NKp46), decreases the release of granzyme B and IFN-γ, and stimulates the expression of TGF-β and adenosine, all of which inhibit NK cytotoxicity. The objective of metabolic engineering is to increase the resilience of NK cells by rewiring the pathways involved in glycolysis, oxidative phosphorylation, and mitochondrial fitness.[35,36]

Preclinical studies have demonstrated that the regulation of mTOR or induction of mitochondrial biogenesis or augmentation of fatty acid oxidation can sustain NK cell fitness in nutrient-depleted and hypoxic conditions, and preserve the ability to eliminate solid tumors. CRISPR-engineered NK cells are also being studied in terms of overexpression of transcription factors that facilitate effector metabolism or deletion of negative regulators of metabolic reprogramming.[16,37] Together with CAR design, metabolic engineering, and nanoparticle-mediated drug delivery, these approaches to cytokine armoring, multi-pronged, can be used to overcome TME barriers and extend NK-cell activity in vivo (Figure 2).[16]

CAR-NK Cell Therapy

CAR-NK cells have been used to adapt the concept of CAR, which has been developed on T cells, to the NK-cell platform to integrate innate and engineered tumor recognition. The most common CAR that is found in NK cells is scFv binding of a surface antigen, followed by a hinge and transmembrane domain with one or more intracellular signaling domains; in NK cells, these can include NK-specific co-stimulatory motifs such as 2B4, 4-1BB, or DAP12. When antigens interact with CAR, the clustering of CAR results in phosphorylation of ITAMs, downstream kinase activation, calcium release, degranulation, and cytokine discharge, which causes lysis of the target cell, and endogenous NK receptors also scan stress ligands and missing self.[13,15]

CAR-NK therapy has been explored in a wide variety of CAR targets. CD19 has by far been the most widely researched antigen in hematologic malignancies, and CAR-NK products based on cord blood, peripheral blood, and NK-92 cells have shown efficacy in B-cell leukemias and lymphomas in preclinical models and in initial phase trials. Other B-cell and plasma-cell antigens, such as CD20, CD22, and BCMA, are being actively developed, and myeloid markers, such as CD33 and CD123 in acute myeloid leukemia, are under active development.[38,39] In the case of solid tumors, other targets include HER2, EGFR, and its mutant EGFRvIII, mesothelin, NKG2D ligands, and PSMA; preclinical experiments have demonstrated successful lysis of glioblastoma, breast, ovarian, pancreatic, and prostate tumor cells in cell culture and in xenografts.[40]

CAR-NK cells have a number of conceptual and clinical advantages compared to CAR-T cells. NK cells are by nature alloreactive, but do not induce GvHD, thus allowing allogeneic donors and standard cell banks without any HLA compatibility. Initial clinical trials of cord-blood-derived CAR-NK cells co-expressing IL-15 and an iCasp9 suicide switch achieved an overall response rate of 73% (8 of 11 patients) in heavily pre-treated B-cell malignancies (NCT03056339), with no CRS, no ICANS, and no GvHD reported.[8] This enhanced safety profile is likely due to NK-cell biology and reduced in vivo persistence, while still permitting adequate exposure to induce tumor clearance in diseases where long CAR-T persistence is not a strict requirement.[40] These safety observations should nonetheless be interpreted with caution, given the small sample sizes and the limited median follow-up.

CAR-NK therapy is developing in a dynamic clinical environment. Products that are under trials in relapsed/refractory B-cell malignancies include NKX019, a CD19-directed CAR-NK cell therapy (NCT04887871), and products in advanced solid tumors include HER2-, EGFR-, and mesothelin-directed CAR-NK cells.[41] Most of these products include IL-15 expression or other armoring approaches to strengthen persistence, and they are produced using cord blood, peripheral blood donors, NK-92 lines, or iPSC-derived NK cells to facilitate off-the-shelf application. Although long-term follow-up is still scarce and obstacles like suboptimal growth, solid tumor trafficking, and antigen escape

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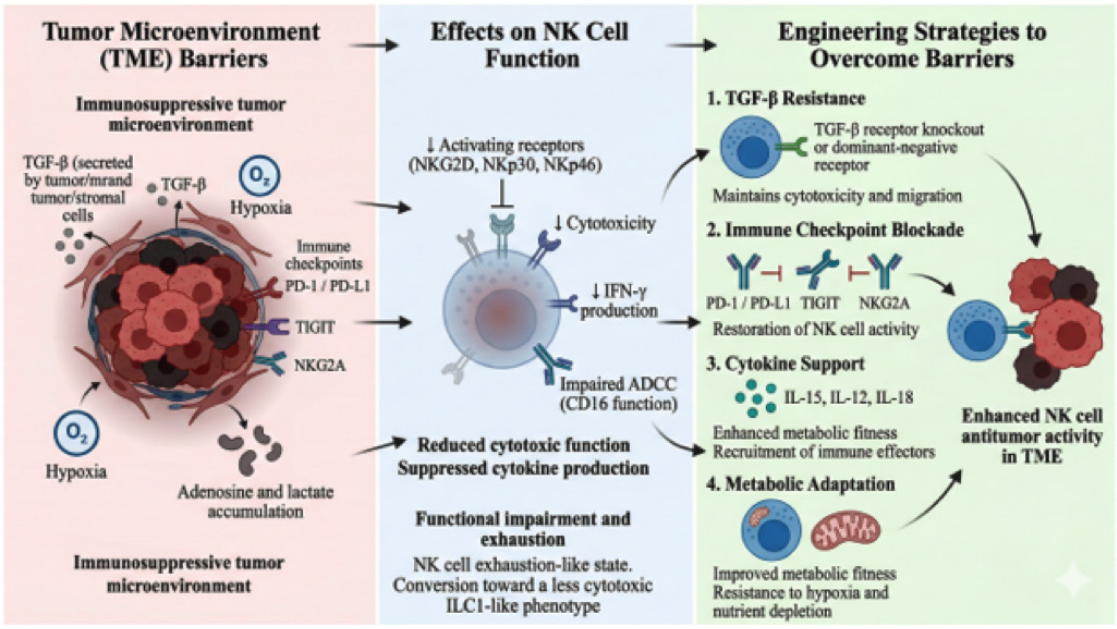

Fig 3 | Overcoming the tumor microenvironment (TME): barriers and engineering strategies in NK cell immunotherapy. The figure depicts TME-mediated suppressive mechanisms (TGF-β, hypoxia/HIF-1α, PD-L1/TIGIT/NKG2A, adenosine, lactate) and corresponding engineering countermeasures. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; HIF-1α, hypoxia-inducible factor-1 alpha; IFN-γ, interferon-gamma; NK, natural killer; TGF-β, transforming growth factor-beta; TIGIT, T cell immunoreceptor with Ig and ITIM domains. The figure is original and was created by the authors

still exist, initial indications show the potential of CAR-NK therapy and justify further optimization efforts.[42]

Overcoming the Tumor Microenvironment

Significant challenges to NK-cell immunotherapy by the TME are immunosuppressive cytokines, metabolic stress, and inhibitory checkpoint pathways. TGF-β, a product of tumor cells and stromal elements, inhibits activating receptors like NKG2D, affects CD16-based ADCC, decreases IFN-γ production, and even induces the transdifferentiation of NK cells to less cytotoxic ILC1-like phenotypes.[43,44] Hypoxia, which is a characteristic of most solid tumors, causes HIF-1α-dependent alterations in both tumor and immune cells, which decrease the expression of NKG2D ligands, degrade granzyme B, and increase the accumulation of adenosine and lactate, further impairing NK-cell activity. Simultaneously, this interaction of inhibitory checkpoints (PD-1/PD-L1, TIGIT, and NKG2A) strengthens exhaustion-like conditions and restricts persistent cytotoxicity (Figure 3).[12,45]

Strategies in engineering are meant to make NK cells resistant to such suppressive cues. TGF-β-resistant NK cells—generated by knockout of TGF-β receptors or expression of dominant-negative receptor forms—maintain cytotoxicity and migratory abilities even in high-TGF-β conditions in preclinical models.[9,43] Monoclonal antibodies targeting PD-1, PD-L1, TIGIT, or NKG2A as blockade to restore NK-cell activity have been demonstrated, and combinations of CAR-NK therapy with checkpoint inhibitors are in preclinical trials. Arming NK cells with IL-15, IL-12, or IL-18 can counteract functional impairment in the TME, enhance metabolic fitness, and attract new immune effectors, but the amounts of cytokines should be carefully maintained to prevent off-tumor inflammation (Figure 3).[14,44]

KIR/HLA matching is an important consideration for allogeneic NK cell products. KIR–ligand mismatch—where donor NK cell KIRs do not recognize recipient HLA allotypes—releases inhibition and enhances alloreactivity, which is therapeutically beneficial. Conversely, KIR–HLA matching risks re-inhibition by residual host HLA expression. For iPSC-derived NK products, the absence of KIR expression at early differentiation stages may reduce HLA-dependent inhibition but also limits ADCC via CD16. Donor selection based on KIR haplotype and HLA allotype analysis is therefore an important strategy for optimizing potency in allogeneic products.[9,12]

Sources of NK Cells

Several cellular sources are currently being utilized in NK-cell–based immunotherapy, each having certain strengths and weaknesses of scalability, safety, and clinical application. Healthy donor peripheral blood NK cells are somewhat mature, and have a high expression of KIR and CD16, but their blood concentrations are low, so ex vivo expansion is required, which may cause a donor-to-donor effect and result in phenotypic drift.[11,17] Another allogeneic source is umbilical cord blood (CB); CB-based NK cells have a high propensity to proliferate, low GvHD risk, and wide availability in the form of cord blood banks, but tend to have a more immature phenotype, with reduced KIR and CD16 expression and functional heterogeneity among donors.[14]

One of the examples of the NK-like cell line that is easy to expand and highly susceptible to genetic engineering is the IL-2-dependent NK-92 cell line derived from a patient with non-Hodgkin lymphoma, and can be efficiently used to develop CAR-NK cells.[44] Nevertheless, since the NK-92 cells are of malignant origin, they need to be irradiated before infusion to stop engraftment and uncontrolled proliferation, which curtails in vivo survival and requires regular dosing. The NK cells generated by the use of iPSCs have the benefits of clonal uniformity, unlimited self-renewal, and the possibility of producing high quantities of standardized NK cells using a single master cell bank, resulting in industrial-scale, off-the-shelf production. iPSC-NKs can be prepared at the pluripotent level to include CARs, cytokine support, and safety switches, and differentiated into homogeneous NKs; nevertheless, differentiation regimens are complicated, and strict quality control is necessary to avoid the remaining pluripotent cells, as well as to guarantee uniformity in functionality (Table 1).[46]

Clinically, the longest history of activity in preclinical trials involves peripheral blood and CB-derived NK cells, which have good safety profiles and signs of activity, whereas NK-92 and iPSC-NK platforms have become more popular as scalable, highly editable sources of next-generation CAR-NK products.[40,46]

Clinical Trials and Applications

Engineered NK cells have so far been used in hematologic malignancies, and most of the clinical experience is from this setting. Initial clinical evaluations of CAR-NK cells derived using cord blood targeting CD19 in relapsed/refractory B-cell leukemias and lymphomas (NCT03056339) showed high response rates, complete remissions, and minimal toxicity—no CRS, no ICANS, and no GvHD, despite use of allogeneic products.[8] Other trials are also being pursued, including CD19-directed CAR-NK cells such as NKX019 (NCT04887871), and NK products targeting CD22, CD20, and BCMA, all of which indicate that CAR-NK therapy is feasible, safe, and preliminarily effective across a range of B-cell and plasma-cell neoplasms.[39]

Solid tumors constitute a more difficult frontier and are also becoming the target of CAR-NK approaches. NK-92-derived and primary NK-cell products against HER2 in glioblastoma and breast cancer, EGFR/EGFRvIII in glioblastoma, and NKG2D ligands in multiple carcinomas, and other antigens including mesothelin, PSMA, 5T4, AXL, and CLDN6 are under clinical trials. Although objective responses have so far been modest and in most instances short-lived, the safety profile is favorable and the lessons in these trials are guiding incorporation of armoring strategies, better trafficking, and combination regimens.[15,19,40]

In all indications, there is a uniform theme of superior safety of CAR-NK over CAR-T, with lower incidence and severity of CRS and neurotoxicity, and no reported GvHD in allogeneic NK sources. However, these safety observations are based on small sample sizes and limited follow-up duration (typically under 12 months in published trials); formal safety comparisons with CAR-T require larger prospective studies. This safety margin enables outpatient administration and combination with other immunotherapies, and can expand access and decrease the use of healthcare resources in comparison with the existing CAR-T paradigms (Table 2).[40,47]

Challenges and Limitations

Although there has been tremendous advancement, engineered NK-cell therapies have a number of notable challenges. One of the major shortcomings is the comparatively limited in vivo persistence, especially in primary NK cells and irradiated NK-92 products, and this may limit longer-term duration of responses in diseases with long-term immune surveillance requirements. Arguably, NK survival can be extended by cytokine armoring and optimization of conditioning regimens, but such an approach has to be balanced with the risks of systemic inflammation and off-tumor effects. The second significant challenge is restrictive trafficking and infiltration into solid tumors, where physical obstacles, aberrant vasculature, and chemokine differences lower the NK concentration in tumor locations.[15,44,45]

Table 1 | Comparison of NK cell sources for immunotherapy

Source	Scalability	Persistence	ADCC/KIR	GvHD Risk	Key Advantage	Key Limitation
Peripheral blood (PB)	Moderate—ex vivo expansion required	Days–weeks	High (KIR+, CD16+)	Low	Mature phenotype; high innate cytotoxicity; ADCC-competent via CD16	Low blood frequency; donor-to-donor variability; phenotypic drift on expansion
Cord blood (CB)	High (proliferative capacity)	Days–weeks	Low (immature; KIR–, CD16–/low)	Very low	Cord blood bank availability; low GvHD risk; off-the-shelf potential	Immature phenotype; reduced ADCC; donor heterogeneity in function
NK-92 cell line	Very high—easy GMP-grade expansion	Hours–days (irradiated product)	Absent/low (CD16–, KIR–)	Low	Highly amenable to genetic engineering; reproducible; scalable	Requires irradiation before infusion; no ADCC; short in vivo survival; repeated dosing needed
iPSC-derived NK	Unlimited (single master cell bank)	Variable (cytokine-dependent)	Low initially; improvable by engineering	Very low	Clonal uniformity; CAR/cytokine/safety switch engineering at pluripotent stage; industrial scale	Complex differentiation; rigorous QC; residual iPSC testing required; no KIR at early stages

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CB, cord blood; GvHD, graft-versus-host disease; iPSC, induced pluripotent stem cell; KIR, killer immunoglobulin-like receptor; NK, natural killer; and QC, quality control.

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Table 2 | Selected CAR-NK cell clinical pipeline (as of 2026)

Product/Source	Target Antigen	Armoring	Indication	Phase	NCT Number	Key Outcome/Safety Signal
CB-derived CAR-NK (Liu et al., 2020)[8]	CD19	IL-15; iCasp9 suicide switch	R/R B-cell ALL and NHL	I/II	NCT03056339	ORR 73% (8/11 patients); complete remissions in majority; no CRS, no ICANS, no GvHD reported
NKX019 (CB-derived)	CD19	IL-15	R/R B-cell lymphoma	I/II	NCT04887871	Ongoing; preliminary data indicate favorable safety profile
iPSC-derived CAR-NK	CD19/CD22	IL-15; multi-antigen targeting	B-cell malignancies	I	Multiple (NCT ongoing)	Ongoing; safety and feasibility data expected
NK-92-derived CAR-NK	HER2	None (irradiated)	GBM; HER2+ breast cancer	I	NCT03383978	Safe; modest and short-lived activity; no dose-limiting toxicities
PB-derived CAR-NK	EGFR/EGFRvIII	IL-15	Glioblastoma	I	NCT (ongoing, multiple)	Early-phase; favorable safety signals; efficacy data maturing
CB- or iPSC-derived	Mesothelin	IL-15; armoring strategies	Ovarian cancer; NSCLC	I	Multiple (recruiting)	Recruiting; no safety or efficacy data available yet

Abbreviations: ALL, acute lymphoblastic leukemia; CB, cord blood; CRS, cytokine release syndrome; GBM, glioblastoma multiforme; GvHD, graft-versus-host disease; ICANS, immune effector cell-associated neurotoxicity syndrome; iCasp9, inducible caspase-9; IL, interleukin; iPSC, induced pluripotent stem cell; NHL, non-Hodgkin lymphoma; NK, natural killer; NSCLC, non-small-cell lung cancer; ORR, overall response rate; PB, peripheral blood; and R/R, relapsed/refractory.

The complexity, cost, and standardization of manufacturing of autologous or donor-derived NK products are still non-trivial, particularly when it comes to individualized collection, activation, expansion, genetic modification, and quality control in Good Manufacturing Practice (GMP). Even though allogeneic and iPSC-based systems offer advantages of economies of scale, they come with a set of regulatory and technical challenges, such as genetic stability, stable differentiation, and intensive safety measures. Lastly, antigen escape, TME-mediated immunity, and possible on-target/off-tumor toxicity remain concerns that require keen target selection, use of safety switches, and rational combination approaches.[48] Manufacturing costs for GMP-grade NK products remain a significant barrier to broad clinical access; while allogeneic platforms are expected to reduce per-patient costs through economies of scale relative to autologous CAR-T, substantial costs of GMP expansion, viral vector production, quality release testing, and cold-chain logistics persist. Regulatory pathways for allogeneic and iPSC-based NK products are evolving across jurisdictions (FDA, EMA), and early regulatory engagement regarding genetic stability, insertional safety, and long-term follow-up requirements is advisable.[46,48]

Pharmacovigilance and Long-Term Safety Monitoring

Long-term safety monitoring is an emerging priority for engineered NK cell programs. Key pharmacovigilance considerations include testing for replication-competent retrovirus or lentivirus (RCR/RCL) from vector-transduced products as mandated by regulatory agencies; genotoxicity assessment through integration site analysis, particularly for lentiviral vectors with strong enhancers; monitoring for unexpected in vivo expansion or transformation; and evaluation of off-tumor NK activity against normal tissues expressing low levels of targeted antigens. Safety switches—notably iCasp9, activated by the small-molecule dimerizer AP1903/rimiducid—have been clinically validated in NK cell trials[8,29] and are increasingly regarded as the standard of care in first-in-human engineered cell therapy trials. Structured adverse event reporting aligned with FDA and EMA guidance for advanced therapy medicinal products (ATMPs) and long-term patient registries are recommended for all clinical-stage NK cell programs.

Future Perspectives

The convergence of stem cell biology, gene editing, synthetic biology, and data-driven design is likely to influence the future development of engineered NK-cell therapies. Manufacture of uniform and multi-engineered NK products with CARs and cytokine support, metabolic improvements, and safety circuits using a limited number of master cell lines is likely to be feasible when using iPSCs, and this approach is likely to be less expensive and can simplify distribution to any part of the world. The precision of the multiplexed changes in gene-editing technologies, including CRISPR, base editing, and prime editing, will enable improvement of effector functionality, persistence, and resistance to inhibition by the TME.[17]

Machine-learning and artificial intelligence methods are being applied to CAR design and antigen selection. Specific applications include computational optimization of scFv paratope sequences for improved affinity and specificity, ML-guided promoter selection for transgene expression in NK cells, antigen co-expression mining from multi-omic tumor data sets to identify synergistic CAR target pairs, and prediction of off-tumor cross-reactivity from structural homology models. Various combinations of engineered NK cells with checkpoint inhibitors, oncolytic viruses, radiation, or targeted agents are under investigation in order to boost tumor immunogenicity, enhance trafficking, and curb antigen escape. Combination with nanomedicine, such as co-delivery of nanoparticles that carry cytokines, small-molecule inhibitors, or metabolic modulators, can additionally enhance the fitness and activity of NK cells in unfavorable TMEs.[44]

Biomarker-based patient selection, monitoring of responses through circulating tumor DNA and immune profiling, and adaptive dosing approaches will

be essential to make the most out of every situation and reduce risk. All these innovations can make engineered NK cells the key element of next-generation, multi-modal immunotherapy of cancer.

Conclusion

Another promising supplement to, or even an alternative in certain circumstances, to CAR-T therapy of cancer is the use of engineered NK cells, especially CAR-NK cells. Their inherent capacity to identify and eliminate malignant cells independently of MHC and their good safety profile, in addition to their compatibility with allogeneic, off-the-shelf production, are some of the major weaknesses of existing T-cell-based technologies that they address. Genetic engineering, cytokine armoring, synthetic biology, nanotechnology, and metabolic reprogramming are gradually enhancing the potency, persistence, and TME resistance of NK-cell products against hematologic malignancies and solid tumors.

There are still considerable obstacles to be overcome, such as improving in vivo persistence, effective trafficking, and invasion of solid tumors, controlling the cost and manufacturing complexity, and antigen escape and immune suppression. However, the fast-paced preclinical and clinical experience, along with the enabling transformative technologies, including iPSC systems and AI-controlled design, can be considered as a solid indication of engineered NK-cell therapies as part of the treatment of cancer in the future.

List of Abbreviations

ADCC, antibody-dependent cellular cytotoxicity
ATMP, advanced therapy medicinal product
BCMA, B cell maturation antigen
CAR, chimeric antigen receptor
CB, cord blood
CRS, cytokine release syndrome
CRISPR, clustered regularly interspaced short palindromic repeats
DNAM-1, DNAX accessory molecule-1
EMA, European Medicines Agency
FasL, Fas ligand
FDA, US Food and Drug Administration
GMP, good manufacturing practice
GvHD, graft-versus-host disease
HIF-1α, hypoxia-inducible factor-1 alpha
HLA, human leukocyte antigen
ICANS, immune effector cell-associated neurotoxicity syndrome
IFN-γ, interferon-gamma
IL, interleukin
iPSC, induced pluripotent stem cell
iCasp9, inducible caspase-9
ITAM, immunoreceptor tyrosine-based activation motif
KIR, killer immunoglobulin-like receptor
LNP, lipid nanoparticle
MHC, major histocompatibility complex
mRNA, messenger RNA
NCR, natural cytotoxicity receptor
NK, natural killer
NKG2D, natural killer group 2D
ORR, overall response rate
PB, peripheral blood
PD-1, programmed cell death protein-1
PD-L1, programmed death-ligand 1
scFv, single-chain variable fragment
TGF-β, transforming growth factor-beta
TIGIT, T cell immunoreceptor with Ig and ITIM domains
TME, tumor microenvironment
TNF-α, tumor necrosis factor-alpha
TRAIL, TNF-related apoptosis-inducing ligand

References

1 Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. https://doi.org/10.3322/caac.21660

2 Debela DT, Muzazu SG, Heraro KD, et al. New approaches and procedures for cancer treatment: current perspectives. *SAGE Open Med*. 2021;9:20503121211034366. https://doi.org/10.1177/20503121211034366

3 Baughan SL, Folsom T, Johnson M, Kreuger J, Webber B, Moriarity BS. Perspective: IL-15 cytokine-armored NK cells as ready-to-use immunotherapy for diverse malignancies: therapeutic potential and toxicity risks. *Front Immunol*. 2025;16:1704404. https://doi.org/10.3389/fimmu.2025.1704404

4 Mace EM. Human natural killer cells: form, function, and development. *J Allergy Clin Immunol*. 2023;151(2):371–385. https://doi.org/10.1016/j.jaci.2022.09.022

5 Siegler EL, Zhu Y, Wang P, Yang L. Off-the-shelf CAR-NK cells for cancer immunotherapy. *Cell Stem Cell*. 2018;23(2):160–161. https://doi.org/10.1016/j.stem.2018.07.007

6 Peng L, Sferruzza G, Yang L, Zhou L, Chen S. CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors. *Cell Mol Immunol*. 2024;21(10):1089–1108. https://doi.org/10.1038/s41423-024-01207-0

7 Ebrahimiyan H, Tamimi A, Shokoohian B, et al. Novel insights in CAR-NK cells beyond CAR-T cell technology; promising advantages. *Int Immunopharmacol*. 2022;106:108587. https://doi.org/10.1016/j.intimp.2022.108587

8 Liu E, Marin D, Banerjee P, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N Engl J Med*. 2020;382(6):545–553. https://doi.org/10.1056/NEJMoa1910607

9 Page A, Chuvin N, Valladeau-Guilemond J, Depil S. Development of NK cell-based cancer immunotherapies through receptor engineering. *Cell Mol Immunol*. 2024;21(4):315–331. https://doi.org/10.1038/s41423-024-01145-x

10 Takahashi E, Kuranaga N, Satoh K, et al. Induction of $CD16^{+}$ $CD56^{bright}$ NK cells with antitumour cytotoxicity not only from $CD16^{-}$ $CD56^{bright}$ NK Cells but also from $CD16^{-}$ $CD56^{dim}$ NK cells. *Scand J Immunol*. 2007;65(2):126–138. https://doi.org/10.1111/j.1365-3083.2006.01883.x

11 Shin E, Bak SH, Park T, et al. Understanding NK cell biology for harnessing NK cell therapies: targeting cancer and beyond. *Front Immunol*. 2023;14:1192907. https://doi.org/10.3389/fimmu.2023.1192907

12 Zhang Y, Guo F, Wang Y. Hypoxic tumor microenvironment: destroyer of natural killer cell function. *Chin J Cancer Res*. 2024;36(2):138–150. https://doi.org/10.21147/j.issn.1000-9604.2024.02.04

13 Rezvani K, Rouce R, Liu E, Shpall E. Engineering natural killer cells for cancer immunotherapy. *Mol Ther*. 2017;25(8):1769–1781. https://doi.org/10.1016/j.ymthe.2017.06.012

14 Rossi F, Fredericks N, Snowden A, Allegrezza MJ, Moreno-Nieves UY. Next generation natural killer cells for cancer immunotherapy. *Front Immunol*. 2022;13:886429. https://doi.org/10.3389/fimmu.2022.886429

15 Moscarelli J, Zahavi D, Maynard R, Weiner LM. The next generation of cellular immunotherapy: CAR-NK cells. *Transplant Cell Ther*. 2022;28:650. https://doi.org/10.1016/j.jtct.2022.06.025

16 Pan W, Tao T, Qiu Y, Zhu X, Zhou X. Natural killer cells at the forefront of cancer immunotherapy with immune potency, genetic engineering, and nanotechnology. *Crit Rev Oncol*

Hematol. 2024;193:104231. https://doi.org/10.1016/j.critrevonc.2023.104231
17 Romanini N, Netsrithong R, Themeli M, Tazzari M. Challenges and opportunities of human iPSC-derived NK as "Off-the-shelf" cellular therapies. *J Exp Clin Cancer Res*. 2025;44(1):327. https://doi.org/10.1186/s13046-025-03558-6
18 Li H, Song W, Li Z, Zhang M. Preclinical and clinical studies of CAR-NK-cell therapies for malignancies. *Front Immunol*. 2022;13:992232. https://doi.org/10.3389/fimmu.2022.992232
19 Lin CZ, Xiang GL, Zhu XH, Xiu LL, Sun JX, Zhang XY. Advances in the mechanisms of action of cancer-targeting oncolytic viruses. *Oncol Lett*. 2018;15:4053–4060. https://doi.org/10.3892/ol.2018.7829
20 Parihar R, Dierksheide J, Hu Y, Carson WE. IL-12 enhances the natural killer cell cytokine response to Ab-coated tumor cells. *J Clin Invest*. 2002;110(7):983–992. https://doi.org/10.1172/JCI0215950
21 Gaggero S, Witt K, Carlsten M, Mitra S. Cytokines orchestrating NK-myeloid cell crosstalk in the TME: implications for natural killer cell-based cancer immunotherapy. *Front Immunol*. 2021;11:621225. https://doi.org/10.3389/fimmu.2020.621225
22 Simon S, Bugos G, Salter AI, Riddell SR. Synthetic receptors for logic gated T cell recognition and function. *Curr Opin Immunol*. 2022;74:9–17. https://doi.org/10.1016/j.coi.2021.09.003
23 Umadevi K, Sundeep D, Kumar U, Pal D, Parashar D. Next-generation CARs and beyond. In: Kumar U, Kumar A, Parashar D, eds. *Advancement in CAR T-cell therapy in tumors*. Springer; 2026:257–295.
24 Nolan-Stevaux O, Smith R. Logic-gated control of immunotherapy for solid tumors. *Front Immunol*. 2024;15:1490911.
25 Liu S, Zhong Z, Tu Q, Jin M. Synthetic biology approaches to enhance cancer immune responses. *Front Immunol*. 2025;16:1698158. https://doi.org/10.3389/fimmu.2025.1698158
26 Joechner AH, Mach M, Li Z. The current landscape of modular CAR T cells. *Int J Mol Sci*. 2025;26(24):11898.
27 Liu D, Zhao J, Song Y. Engineering switchable and programmable universal CARs for CAR T therapy. *J Hematol Oncol*. 2019;12(1):69.
28 Streltsova MA, Boyko AA, Alekseeva NA, et al. Genetically modified NK cells with a switchable CAR for HER2-positive cancers. *Biochimie*. 2025;238:30–42. https://doi.org/10.1016/j.biochi.2025.07.021
29 Kang X, Li D, Sun R. Nanotechnology and NK cell immunotherapy in gastrointestinal tumors. *Front Med (Lausanne)*. 2025;12:1647737.
30 Maia A, Tarannum M, Romee R. Genetic manipulation to enhance NK cell-based immunotherapy. *Stem Cells Transl Med*. 2023;13:230.
31 Yu X, Chen X, Yang Y, Tian Y, Jia J, Tong X. Nanomaterial assisted natural killer cell therapy. *Front Immunol*. 2025;16:1558701. https://doi.org/10.3389/fimmu.2025.1558701
32 Yang P, Wang W, Liu Q, et al. Metabolic reprogramming of NK cells drives anti-tumor immunity. *Crit Rev Oncol Hematol*. 2025;215:104859. https://doi.org/10.1016/j.critrevonc.2025.104859
33 Moinuddin A, Poznanski SM, Portillo AL, Monteiro JK, Ashkar AA. Metabolic adaptations determine whether NK cells fail or thrive within the TME. *Immunol Rev*. 2024;323:19–39. https://doi.org/10.1111/imr.13316
34 Fox AC, Blazeck J. Applying metabolic control strategies to engineered T cell cancer therapies. *Metab Eng*. 2024;86:250–261. https://doi.org/10.1016/j.ymben.2024.10.009
35 Kennedy PR, Arvindam US, Phung SK, et al. Metabolic programs drive function of therapeutic NK cells in hypoxic tumor environments. *Sci Adv*. 2024;10:eadn1849. https://doi.org/10.1126/sciadv.adn1849
36 Yang Y, Nilsson MB, Yu X, et al. The activity of EGFR CAR NK and CAR T cells against EGFR inhibitor-resistant NSCLC and drug-tolerant persister cells. *Clin Cancer Res*. 2025;31(22):4745–4762. https://doi.org/10.1158/1078-0432.CCR-25-1693
37 Wang W, Jiang J, Wu C. CAR-NK for tumor immunotherapy: clinical transformation and future prospects. *Cancer Lett*. 2020;472:175–180. https://doi.org/10.1016/j.canlet.2019.11.033
38 Jørgensen LV, Christensen EB, Barnkob MB, Barington T. The clinical landscape of CAR NK cells. *Exp Hematol Oncol*. 2025;14(1):46. https://doi.org/10.1186/s40164-025-00633-8
39 Yao P, Liu YG, Huang G, Hao L, Wang R. The development and application of CAR-NK cells for cancer therapy. *Exp Hematol Oncol*. 2024;13:118. https://doi.org/10.1186/s40164-024-00583-7
40 Chu Y, Nayyar G, Tian M, et al. Targeting neuroblastoma with anti-ROR1 CAR NK cells and N-803. *Mol Ther Oncol*. 2024;32:200820. https://doi.org/10.1016/j.omton.2024.200820
41 Švubová V, Janstová L, Jedlička M, et al. TGF-β decreases NK cell mobility and cytotoxic efficacy in leukemia microenvironment models. *Immunotargets Ther*. 2025;14:589.
42 Melaiu O, Lucarini V, Cifaldi L, Fruci D. Influence of the TME on NK cell function in solid tumors. *Front Immunol*. 2020;10:3038. https://doi.org/10.3389/fimmu.2019.03038
43 Chen Z, Han F, Du Y, Shi H, Zhou W. Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions. *Signal Transduct Target Ther*. 2023;8(1):70. https://doi.org/10.1038/s41392-023-01332-8
44 Goldenson BH, Zhu H, Wang YZ, et al. Umbilical cord blood and iPSC-derived NK cells: key differences in cytotoxic activity and KIR profiles. *Front Immunol*. 2020;11:561553. https://doi.org/10.3389/fimmu.2020.561553
45 Chacim S, Monjardino T, Cunha JL, et al. Costs, effectiveness, and safety associated with CAR T-cell therapy. *PLoS One*. 2022;17:e0278950. https://doi.org/10.1371/journal.pone.0278950
46 Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J*. 2021;11(4):69. https://doi.org/10.1038/s41408-021-00459-7
47 Lin X, Sun Y, Dong X, Liu Z, Sugimura R, Xie G. IPSC-derived CAR-NK cells for cancer immunotherapy. *Biomed Pharmacother*. 2023;165:115123. https://doi.org/10.1016/j.biopha.2023.115123
48 Wei X, Su C, Liu Y, et al. IPSCderived NK cells for immunotherapy and therapeutic perspective (Review). *Mol Med Rep*. 2025;32(2):222. https://doi.org/10.3892/mmr.2025.13587