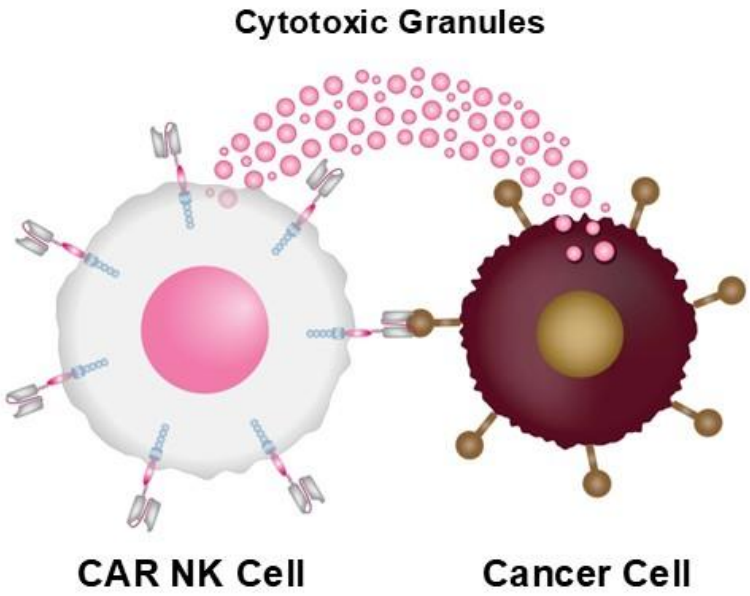
MarinBio BLOG

Making CAR NK Cell Therapy Accessible for Everybody



Introduction

The emergence of chimeric antigen receptor (CAR)-based immunotherapies has revolutionized the treatment of hematologic malignancies.

While CAR-T cell therapies dominate the clinical landscape, enthusiasm is growing for CAR-engineered natural killer (CAR-NK) cells, which leverage the cytotoxic capacity of NK cells with the specificity of CAR constructs. Natural killer (NK) cells are a type of innate lymphocyte that play a critical role in the early defense against viral infections and cancer. They can recognize and kill abnormal cells without prior sensitization, distinguishing them from adaptive immune cells like T lymphocytes. NK cells mediate their effects through direct cytotoxic activity. Unlike T cells, NK cells do not require antigen presentation via HLA molecules, making them capable of targeting tumor cells that escape immune detection through downregulation of MHC class I. CAR-NK therapies carry lower risks of severe toxicities such as cytokine release syndrome and graft-versus-host disease, providing a favorable safety profile for allogeneic, “off-the-shelf” applications.

A surge of high-impact research has advanced our understanding of CAR-NK therapies. These studies have dissected the molecular architecture of CARs optimized for NK biology, developed novel approaches for large-scale CAR-NK generation, expanded the antigenic repertoire for hematologic and solid tumors, and have begun to address the problem of rejection of allogenic 'off the shelf' NK cells by recipient immune cells. Together, they highlight a new era in cellular immunotherapy in which CAR-NK cells are positioned not as competitors but as complementary agents to CAR-T cells.

Cellular Sources for Generating CAR NK-Cells

CAR NK-cells can be generated from multiple cellular sources, each with unique biological and therapeutic attributes. The origin of NK cells affects their expansion, cytotoxicity, persistence, and suitability for large-scale manufacturing. The three most common and clinically relevant sources are peripheral blood NK cells, umbilical cord blood NK cells, and induced pluripotent stem cell (iPSC)-derived NK cells, which together form the foundation of current CAR NK-cell platforms.

The most common source of NK cells for CAR NK therapies is peripheral blood-derived NK cells (PB-NK). PB-NK cells have been preferable for 3 main reasons:

1. PB-NK cells are readily accessible from healthy donors, making them practical for both autologous and allogeneic applications.
2. They exhibit strong innate cytotoxicity and mature NK phenotypes, which translates to immediate anti-tumor activity.
3. Many early-phase clinical trials, including those targeting hematologic malignancies, have used PB-NK cells because of their established safety profile.

Manufacturing Process for CAR NK Cell Therapy

The main steps involved in developing a CAR NK cell therapeutic are listed below.

Peripheral Blood NK Cell Collection

Collect NK cells from the patient (autologous) or a healthy donor (allogeneic) via leukapheresis.

Isolate NK cells from the collected peripheral blood mononuclear cells (PBMCs).

NK Cell Activation and Expansion

Culture NK cells with cytokines such as IL-2, IL-15, or feeder cells to activate and expand them.

Monitor NK cell proliferation and viability.

Genetic Modification (CAR Transduction/Transfection)

Introduce the Chimeric Antigen Receptor (CAR) gene into NK cells using viral vectors (lentivirus, retrovirus) or non-viral methods (mRNA electroporation, transposons).

Verify CAR expression on NK cells using flow cytometry or other molecular assays.

Ex Vivo Expansion and Quality Control

Further expand CAR-expressing NK cells to reach therapeutic doses.

Perform sterility testing, phenotype characterization, and functional assays (cytotoxicity, cytokine production).

CAR NK Cell Infusion

Infuse CAR NK cells intravenously into the patient under clinical monitoring.

Monitor for safety, cytokine release, and early signs of efficacy.

Table 1. Cellular Sources for Generating CAR NK Cells

Cellular Source	Description	Advantages	Limitations
Peripheral Blood NK Cells (PB-NK)	Mature NK cells isolated from donor peripheral blood using density gradient or magnetic bead separation.	Readily available from healthy donors; strong cytotoxic potential; compatible with autologous or allogeneic use.	Donor variability; limited proliferation and transduction efficiency; short in vivo persistence.
Umbilical Cord Blood NK Cells (CB-NK)	Immature NK cells derived from cryopreserved umbilical cord blood units and expanded ex vivo.	High proliferative and expansion capacity; low risk of graft-versus-host disease (GvHD); suitable for off-the-shelf use.	Require differentiation and maturation to achieve full cytotoxicity; limited initial cell numbers.
iPSC-Derived NK Cells (iPSC-NK)	NK cells differentiated from induced pluripotent stem cells under defined culture conditions.	Uniform, renewable, and scalable source; genetically modifiable at the stem cell stage; ideal for standardized off-the-shelf products.	Complex manufacturing process; differentiation variability; potential regulatory and safety hurdles.

Chimeric Antigen Receptor (CAR) Design for NK cells

The CAR designed specifically for NK cells typically consists of an extracellular antigen-recognition domain, a transmembrane domain, and intracellular signaling domains that activate NK cell effector functions. The extracellular domain is usually a single-chain variable fragment (scFv) derived from an antibody that binds a tumor-associated antigen with high specificity. This is linked via a hinge region to a transmembrane domain, often derived from CD28, CD8α, or NK-specific molecules such as NKG2D, which anchors the receptor in the membrane. The intracellular region contains signaling motifs that drive NK cell activation, cytokine production, and cytotoxicity, commonly incorporating CD3ζ and costimulatory elements optimized for NK biology. Several molecular modifications have been introduced to improve CAR NK cell performance. For example, inclusion of NK-specific signaling domains such as DAP10 or DAP12 enhances activation through natural cytotoxicity pathways; and engineering the hinge and transmembrane regions to be derived from NK-associated molecules improves receptor stability and signaling efficiency.

Molecular Engineering Strategies to Enhance CAR NK Cell Antitumor Activity

Recent advances in genetic engineering have led to significant improvements in the efficacy of CAR NK cells against cancer. One major strategy involves cytokine engineering, in which CAR NK cells are modified to express cytokines such as IL-15, IL-21, or membrane-bound IL-2. These cytokines provide autocrine support that promotes survival, proliferation, and cytotoxic function of the CAR NK cells without the need for exogenous cytokine supplementation.

A second approach targets the inhibitory pathways that suppress NK cell function within the tumor microenvironment. By introducing checkpoint inhibition mechanisms, such as expressing dominant-negative receptors for TGF-βR or NKG2A, CAR NK cells can maintain cytolytic activity even under immunosuppressive conditions. Enhanced killing can also be achieved by optimizing intracellular signaling domains, such as combining CD3ζ with co-stimulatory or

adaptor molecules like 2B4, DNAM-1, or DAP12, which strengthen the immune synapse and amplify activation signals.

Another complementary strategy focuses on enabling CAR NK cells to resist the physical and metabolic barriers of the tumor microenvironment. This can be achieved by introducing genes into the CAR NK cells, such as heparanase (HPSE) to degrade the extracellular matrix or cytokines such as IL-12 and IL-18 to reprogram the local immune milieu toward a proinflammatory state.

Finally, the development of armored or multi-target CAR NK cells allows simultaneous recognition of multiple tumor-associated antigens or co-expression of NK-activating ligands such as CD16 or NKG2D ligands, reducing the likelihood of tumor antigen escape and promoting broader immune engagement. Collectively, these molecular strategies represent a multifaceted effort to enhance CAR NK cell persistence, tumor infiltration, and cytotoxicity, ultimately improving their therapeutic potential in both hematologic malignancies and solid tumors.

Table 2. Molecular Approaches to Enhance CAR NK-Cell Tumor Killing

Molecular Approach	Molecular Strategy	Primary Effect	Example
Cytokine Engineering	Expression of IL-15, IL-21, or membrane-bound IL-2	Enhances NK cell survival, proliferation, and cytotoxicity	IL-15-expressing CAR NK cells show improved persistence
Checkpoint Inhibition / Dominant-Negative Receptors	Blockade of PD-1/PD-L1, TGF-βR, or NKG2A pathways	Prevents tumor-induced NK cell suppression	CAR NK cells with dominant-negative TGF-βR retain function
Enhanced Synapse Formation and Signal Amplification	Use of 2B4, DNAM-1, DAP12, or optimized CD3ζ signaling motifs	Strengthens activation signaling and immune synapse formation	2B4-CD3ζ CAR constructs improve cytotoxic response
Resistance to Tumor Microenvironment	Expression of HPSE, IL-12, or IL-18	Improves infiltration and resistance to suppression	HPSE-expressing CAR NK cells penetrate solid tumors
Armored / Multi-Target CAR NK Cells	Dual or trispecific antigen targeting; co-expression of NK ligands	Reduces antigen escape and recruits host immunity	Dual CD19/CD22 CAR NK cells prevent B-cell relapse

Target Antigens for CAR-NK Cells

The therapeutic scope of CAR NK cells hinges on the antigenic repertoire. CD19 remains the most validated target, where CAR NK therapy produced durable responses in B-cell malignancies with limited toxicity. Expanding beyond these studies have shown EGFR mutants and CD70 as promising cancer targets. CD70 is an attractive target for CAR NK cell therapy because it is aberrantly and transiently expressed on a wide range of malignancies, such as renal cell carcinoma, glioblastoma, acute myeloid leukemia (AML), and certain lymphomas, while showing limited or no expression on most normal tissue. EGFR mutants are tumor targets for CAR NK cells because these mutations drive oncogenic signaling in many cancers and are often expressed selectively or at higher levels on tumor cells compared to normal tissues,

making them ideal tumor-associated antigens. Other targets include mesothelin, PSCA, GD2, and HER2. Notably, multi-target or dual-CAR constructs are emerging to counter antigen escape. This trend highlights the strategic advantage of NK cells in engaging diverse tumor ligands while minimizing off-target toxicity.

Table 3. Multi-Target CAR NK Constructs

Construct Name/Design	Targets	Tumor Type(s)	Purpose / Advantage
Dual CAR NK (HER2 + IL13Rα2)	HER2 and IL13Rα2	Glioblastoma	Broadens tumor recognition and minimizes antigen escape in heterogeneous glioblastoma expressing either or both antigens
Bispecific CAR NK (CD19 + CD22)	CD19 and CD22	B-cell acute lymphoblastic leukemia (B-ALL)	Prevents relapse due to loss or downregulation of CD19; ensures sustained killing of malignant B cells expressing either antigen
Tri-specific CAR NK (EGFR + EGFRvIII + HER2)	EGFR, EGFRvIII, and HER2	Solid tumors (glioblastoma, lung, and breast cancers)	Enhances recognition of multiple receptor variants and overexpressed forms, improving efficacy across tumor subtypes
Dual CAR NK (GD2 + B7-H3)	GD2 and B7-H3	Neuroblastoma, melanoma, osteosarcoma	Targets both a tumor-associated glycolipid (GD2) and an immune checkpoint ligand (B7-H3) to enhance killing and overcome immune evasion

Allogenic (Off the Shelf) CAR NK-Cell Therapy

Most approved CAR-cell products are autologous, meaning a patient's own immune cells are collected, genetically modified to express the CAR, expanded *ex vivo*, and reinfused. While effective, this approach is costly, time-consuming, and often unfeasible in patients whose health is rapidly deteriorating. Allogeneic CAR cells are derived from healthy unrelated donors and infused into patients. Advantages are rapid availability, standardized manufacturing, and reduced cost, offering a more scalable therapy model.

However, the introduction of CAR cells derived from an un-related donor into an immune competent patient provokes immune recognition and destruction of the graft through transplant rejection mechanisms. The challenge of transplant rejection is a key obstacle to effective allogeneic CAR therapies. Overcoming immune barriers while maintaining therapeutic efficacy has therefore become a central focus area of research, with the hope of developing "universal" allogenic CAR cells that are not rejected when infused into patients.

Classical HLA class I molecules (HLA-A, HLA-B, HLA-C) are expressed on nearly all nucleated cells and present endogenous peptides to $CD8^+$ cytotoxic T cells. In the context of transplantation or allogeneic cell therapy (including CAR T or CAR NK cells), these molecules are a major cause of immune rejection. Recipient T cells recognize non-self HLA molecules on donor cells as foreign, leading to activation of cytotoxic $CD8^+$ T cells.

Approaches to Decrease HLA Class I Molecules in CAR Cell Therapy

Several strategies are being explored to reduce immunogenicity of allogeneic CAR cells by decreasing classical HLA class I expression:

Gene editing (CRISPR/Cas9, TALENs, or ZFNs):

- Knocking out B2M (β2-microglobulin), which is essential for HLA class I surface expression, prevents presentation of peptides to $CD8^+$ T cells.

RNA interference or antisense oligonucleotides:

- Temporarily suppress HLA class I transcription to reduce immunogenicity.

HLA engineering / replacement:

- Knock out classical HLA molecules but retain non-classical HLA (HLA-E or HLA-G) to provide inhibitory signals to NK cells, preventing NK-mediated killing of "missing self" cells.

Conventional molecular approaches for decreasing rejection indiscriminately suppress all MHC class I molecules. While this effectively cloaks cells from killing by CD8+ T cells, it simultaneously removes the critical inhibitory signal HLA-E. HLA-E is an inhibitory cell surface ligand, and inhibits reactive host NK cells and CD8+ T cells by binding to NKG2A. Loss of HLA-E expression renders the modified CAR NK cells vulnerable to host NK cell and CD8+ T cell mediated rejection.

A recent study by Liu et al. (2025) (1) presents a solution to this problem, demonstrating that selective HLA knockdown in CAR NK-cells combined with induced PD-L1 or single-chain HLA-E expression not only prevents rejection of allogeneic CAR NK cells but also enhances their anti-tumor activity and safety profile. The core approach lies in the development of highly specific shRNA constructs that achieve potent knockdown of classical HLA class I molecules (HLA-A, -B, -C) while sparing the expression of the inhibitory non-classical HLA-E. The study evaluates immune checkpoint molecule PD-L1 expression on CAR NK cells as a potential shield. Exogenous PD-L1 expression on allogeneic CAR NK cells provides moderate inhibition of host T cell responses, reducing activation, proliferation, and cytotoxicity. More strikingly, the expression of an introduced single-chain HLA-E gene proves exceptionally potent at inhibiting host NK cell killing of HLA-ABC-reduced NK cells, almost completely abolishing NK cell activation and target lysis *in vitro*.

In vivo, In humanized mouse models, the data showed unmodified donor CAR NK cells are rapidly eliminated, while their modified counterparts persist and dominate the NK cell compartment over time, coinciding with transient activation host T cells. Overall the data points to a clear path forward for improving CAR NK cell therapy

Conclusion

Recent studies have transformed the CAR-NK field from an experimental concept to a translationally validated platform with clinical and mechanistic credibility. Structural innovations have tailored CARs to NK biology, ensuring more robust activation while minimizing exhaustion. Advances in scalable generation platforms, particularly iPSC-derived CAR-NKs, have positioned the therapy for global dissemination as an off-the-shelf product. Expansion of the antigenic repertoire now includes hematologic, solid tumor, and even autoimmune indications. Mechanistic studies reveal that CAR-NKs combine the specificity of engineered receptors with the resilience of innate immune responses, offering a distinctive therapeutic niche.
Looking forward, challenges remain, particularly in enhancing CAR-NK persistence in vivo.

The study by Liu et al. demonstrated engineered CAR-NK cells can evade host immune rejection while simultaneously gaining enhanced tumor-killing power, overcoming critical barriers to implementation of "off-the-shelf" CAR NK cell therapies.

As large-scale clinical trials mature, CAR-NKs may define the next wave of cellular immunotherapy, broadening the therapeutic horizon not only in oncology but also in immunology and regenerative medicine.

Reference

1. Liu, F., Tarannum, M., Zhao, Y. et al. Selective HLA knockdown and PD-L1 expression prevent allogeneic CAR-NK cell rejection and enhance safety and anti-tumor responses in xenograft mice. Nat Commun 16, 8809 (2025). https://doi.org/10.1038/s41467-025-63863-8