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CAR-NK Cells and the Tumor Microenvironment: Emerging Opportunities, Challenges, and the Road Beyond Controversies

Asieh Emami Nejad[1], Saham Shaverdi[1], Marjan Taherian[2], Azim Forouzan[3], Ali Sadoogh Abbasian[4], Mohammadreza Rohani[5], Mojtaba Ahmadlou[6], Sayed Mohammad Matin Ishaghi[7], Elnaz Sheydaee[8], Simin Najafgholian[9], and Mostafa Manian[10]

[1] Department of Biology, Payame Noor University, Tehran, Iran
[2] Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
[3] Department of Gastroenterology and Hepatology, Clinical Research Development Unit, Amiralmomnin Hospital, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[4] Department of Nephrology, Clinical Research Development Unit of Amiralmomnin Hospital, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[5] Department of Gastroenterology and Hepatology Internal Medicine, School of Medicine, Saveh University of Medical Sciences, Saveh, Iran
[6] Department of Biostatistics, Clinical Research Development Unit of Amiralmomnin Hospital, School of Medicine, Arak University of Medical Sciences, Arak, Iran
[7] Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran
[8] Department of Hematology, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran
[9] Department of Emergency Medicine, Clinical Research Development Unit, Valiasr Hospital, Arak University of Medical Science, Arak, Iran
[10] Department of Medical Laboratory Science, KerMS.C., Islamic Azad University, Kermanshah, Iran

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ABSTRACT

Chimeric antigen receptor–natural killer (CAR-NK) cell therapy holds significant promise for cancer immunotherapy due to its efficient recognition and lysis of malignant cells. Despite the potential of CAR-NK therapy as a safer and more effective immunotherapeutic strategy, researchers are actively focusing on addressing its limitations. These include enhancing persistence, optimizing genetic engineering methods, and standardizing the production process for wider clinical applicability. The development of novel generations of CAR-NK cells, combined with a deeper understanding of their behavior in solid tumors, could potentially revolutionize cancer cell therapy and improve patient outcomes in the near future. However, to improve clinical outcomes and facilitate the broader application of CAR-NK cell therapies, we must address challenges related to the optimization of CAR constructs, in vivo persistence, tumor penetration, safety, and regulatory considerations. Overall, the article presents an extensive review of the challenges and potential

Corresponding Authors: Simin Najafgholian, MD;
Department of Emergency Medicine, Clinical Research Development Unit, Valiasr Hospital, Arak University of Medical Science, Arak, Iran. Tel: (+98 86) 6565 2328, Email: S.najafgholian@arakmu.ac.ir

Mostafa Manian, PhD;
Department of Medical Laboratory Science, KerMS.C., Islamic Azad University, Kermanshah, Iran. Tel: (+98 990) 7393 978, Email: manian.m@iau.ir, mostafamanian@gmail.com,

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strategies for improving the long-term antitumor efficacy of CAR-NK cell therapy, emphasizing the importance of combination therapies, drug delivery methods, and immune checkpoint blockade in enhancing the effectiveness of NK cell–based immunotherapy. The paper provides valuable insights into the intricate mechanisms and potential future applications of these strategies in cancer immunotherapy.

Keywords: Cancer-associated fibroblasts; Cancer immunotherapy; CAR-NK cell therapy; CAR-T cells therapy; Chimeric antigen receptor; Natural killer cells; Tumor microenvironment

INTRODUCTION

Natural killer (NK) cells, a subset of innate lymphoid cells with diversified killing mechanisms, have recently become prominent in the application of immunotherapy. These cells originate from hematopoietic stem cells (HSCs) in the bone marrow and are characterized by their ability to directly lyse tumor cells in a non–MHC-restricted manner.[1] NK cells can rapidly detect and eliminate aberrant cells, virus-infected cells, and cancer cells through a combination of surface receptors that recognize various molecules on target cells without requiring prior sensitization to antigens. In fact, they serve as the first responders in the immune system's fight against cancer development.[2,3]

NK cells express a variety of activating and inhibitory receptors on their surface. The balance between activating and inhibitory receptor signaling determines whether an NK cell will kill a target cell.[1] However, NK cells control tumor growth by interacting directly with tumor cells or affecting the function of other populations of innate and adaptive immunity in the tumor microenvironment (TME).[4,5] Moreover, clinical data indicate that a higher number of NK cells in the TME is associated with improved outcomes in patients with various types of cancer, such as hepatocellular carcinoma, melanoma, breast cancer, non-small-cell lung cancer, squamous cell carcinoma of the lungs, pulmonary adenocarcinoma, renal cell carcinoma, and gastric cancer.[6]

Genetic modification techniques have demonstrated the capacity to customize NK cells by including chimeric antigen receptors (CARs) and blocking inhibitory genes. These methods enable patients with hematological malignancies to efficiently eliminate their own tumor cells that were previously resistant to destruction by the same NK cells lacking CARs.[2] Preclinical research with CAR-NK cells has demonstrated identical in vivo effectiveness to CAR-T cells in xenograft mouse models, with reduced cytokine production and improved survival rates.[7] The successful adoptive transfer of allogeneic NK cells into patients further establishes NK cells as a promising platform for CAR engineering and the development of off-the-shelf products for broad clinical application.[8] To date, CAR-NK cells have shown impressive efficacy in the treatment of hematological malignancies and have been widely studied in the treatment of solid tumors, such as glioblastoma, breast cancer, and ovarian cancer, with numerous breakthroughs.[9,10] In this review, we present CAR-NK cell therapy for solid tumors as a potential platform to revolutionize cancer treatment and improve patient outcomes due to its advantages over CAR-T cell therapy, such as reduced risk of graft-vs-host disease (GvHD) and cytokine release syndrome (CRS).

Additionally, we discuss the challenges and effects of immunosuppressive TME mechanisms for successful progression in clinical trials. Moreover, we provide data on recently published preclinical and clinical studies of CAR-NK therapy. Finally, we evaluate the progression in CAR-NK clinical trial challenges and describe existing strategies that optimize the persistence, function, safety, and efficacy of CAR-NK cells and expand the solid tumor penetration of infused cells, which can assist in developing a therapeutic approach.

CAR-NK Cell Therapy for Solid Tumors

The cellular cytotoxic activity, MHC-unrestricted recognition, and tumor-infiltrating ability allow NK cells to be exploited as a promising therapeutic option for the treatment of solid tumors.[11,12] Accordingly, NK cells have been examined to produce CAR-NK cells against various tumor antigens in multiple preclinical studies and clinical trials (Supplementary tables 1 and 2).

Activation Signal for CAR-NK Cells

CAR-NK cells can be activated against tumor cells through CAR-independent mechanisms that are exploited by NK cells. CAR-NK cells exert cytolytic activity for killing tumor cells that do not express the

target antigen in a CAR-independent manner.[13] Similarly, CAR-NK cells retain innate natural cytotoxicity when engaged by varioius activating receptors. For instance, the activation of natural cytotoxicity receptors (eg, NKG2D, NKp30, NKp44, NKp46), and activating KIRs (eg, KIR2DS1, KIR2DS4, and KIR2DL4), as well as costimulatory receptors (e.g., DNAM-1), can induce caspase-mediated apoptosis of targeted tumor cells.[14,15] Target cell apoptosis can also be induced through the Fas ligand/TNF-related apoptosis-inducing ligand (FasL/TRAIL) pathway, as well as interferon-γ (IFN-γ) and tumor necrosis factor (TNF) produced by NK cells.[16,17] Furthermore, CD16 on the NK cells can trigger antibody-dependent cell-mediated cytotoxicity (ADCC) to eliminate tumor cells.[18] Activated CAR-NK cells can also induce target cell lysis by releasing granzyme and perforin (Figure 1).

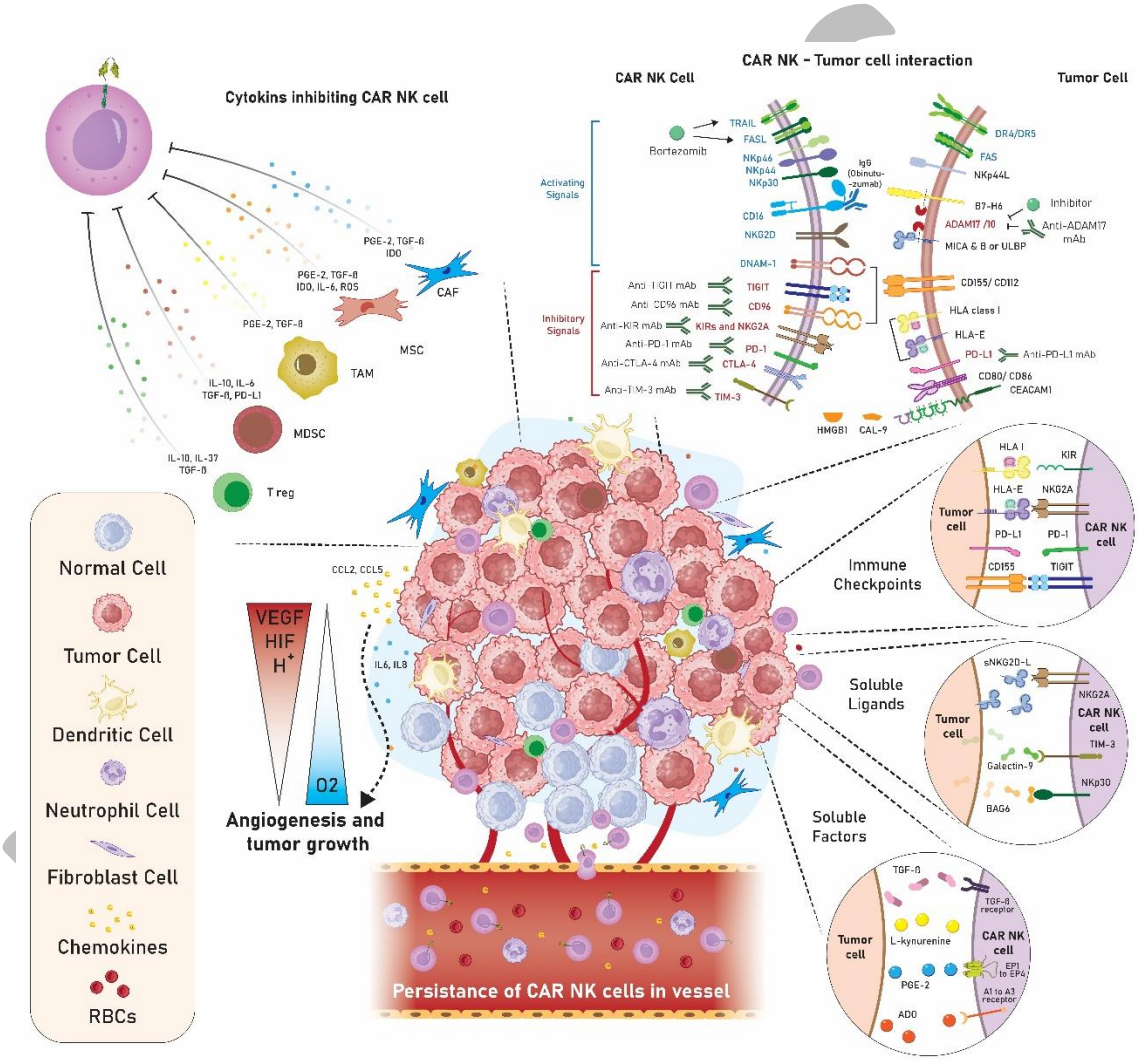

Figure 1. The possible involvement of immunosuppressive variables in the tumor microenvironment (TME) that could lead to the ineffectiveness of CAR-NKs. It also provides a description of treatment strategies aimed at enhancing the detection and activation of natural killer (NK) cells in the solid tumor microenvironment. TME inhibits the identification of tumor cells by natural killer (NK) cells through multiple mechanisms. In response to cytokine signaling, the expression of internal checkpoints such as CBLB, SOCS3, and CIS regulates the activation of natural killer (NK) cells and the development of immunological synapses with tumor cells. The inhibitory tumor microenvironment also contributes to the suppression of CAR-NKs by releasing suppressive molecules such as TGF-β. Within this particular structure, the potency of CAR-NK is diminished, resulting in reduced efficacy against tumor cells. The homing of CAR-NKs is also controlled by chemokine receptors, such as CCR5, which directs the cells to the liver in response to CCL3. This reduces the effectiveness of CAR-NKs against malignancies located in the bone-marrow. The function and

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cytotoxicity of CAR-NKs are regulated by intrinsic mechanisms within natural killer (NK) cells. For instance, when tumor ligands interact with NK immunomodulatory checkpoint receptors including TIGIT, PD-1, NKG2A, TIM-3, and Siglec-7, it hinders the response of CAR-NKs to target cells. ADAM17 inhibits the NK cell ADCC response by releasing the CD16 receptor from the surface of the NK cell. The primary agents involved in these processes are potential targets for therapy to reinstate NK cell recognition and activation. (1) The proteasome inhibitor bortezomib enhances the ability of natural killer (NK) cells to kill cancer cells by increasing the cytotoxicity of TRAIL and FasL. (2) The ability of natural killer (NK) cells to identify tumor antigens is greatly hindered by the action of proteases ADAM10 and ADAM17, which cause the shedding of CD16, B7-H6, and NKG2DL (MICA, MICB, and ULBP). Therefore, the administration of anti-ADAM17 monoclonal antibodies and ADAM inhibitors can enhance the identification and activation of natural killer (NK) cells through the engagement of CD16, NKp30, and NKG2D receptors. (3) Furthermore, the genetically modified "off-the-shelf" NK cells, known as FT516, have a high affinity for non-cleavable CD16 and are resistant to protease-mediated cleavage. Currently, clinical trials are underway to evaluate the therapeutic efficacy of FT516. (4) The activating receptor DNAM engages in competition with the inhibitory receptors TIGIT and CD96 for ligands CD155 and CD112. Monoclonal antibody treatments that specifically target TIGIT and CD96 have been shown to significantly hinder the advancement of cancer and the spread of cancer cells to other parts of the body by inhibiting the depletion of natural killer (NK) cells generated by TIGIT and CD96. (5) Killer cell immunoglobulin-like receptors (KIRs) and NKG2A have essential functions in the "missing self" process of natural killer (NK) cells. Nevertheless, tumor cells exploit this process to elude the immune monitoring carried out by natural killer (NK) cells. By using monoclonal antibodies, the inhibitory signals can be blocked, which in turn restores the ability of natural killer cells to destroy tumor cells. (6) The immune checkpoint molecules PD-1, PD-L1, CTLA-4, and TIM-3 cause NK cell fatigue by binding to specific ligands on tumor cells. ICIs primarily increase the identification and cytotoxicity of natural killer (NK) cells against tumor cells by targeting these molecules. This illustration was generated using biorender.com. Cancer cells express immunological checkpoint ligands on their cell surface, which provide a suppressive interaction with NK cells. In addition, tumor cells can inhibit the activity of NK cells by releasing soluble ligands into the surrounding environment, such as BAG-6, galectin-9, and soluble NKG2D-L (sNKG2D-L), as well as other soluble substances, including cytokines like transforming growth factor-β (TGF-β), enzymes, and metabolites. Several soluble factors are also generated by immune cells found in the tumor microenvironment (TME), including Tregs, tumor-associated macrophages (TAM), and myeloid-derived suppressor cells (MDSC). Platelets release the metalloproteinases ADAM-10 and ADAM-17, which cause the shedding of NKG2D-L. Additional non-immune cells, such as derived-mesenchymal stromal cells (MSC) and cancer-associated fibroblasts (CAF), also generate indoleamine 2, 3 dioxygenase (IDO) or reactive oxygen species (ROS) that diminish the function of NK cells. In addition, hypoxia, elevated levels of fatty acids, food deprivation, and acidity, along with other metabolic variables, collectively create a complex immunosuppressive tumor microenvironment that impairs the efficiency of natural killer (NK) cells against hematologic malignancies. Various techniques can counteract the immunosuppressive process of the tumor microenvironment (TME). (A) Antibodies that block immunological checkpoints prevent the suppression of NK cell cytotoxicity. When the adenosine A2A receptor (A2AR) binds to extracellular adenosine (ADO), it also inhibits the function of NK cells. Inhibiting the CD73 ectoenzyme, responsible for producing ADO, decreases the concentration of this metabolite in the tumor microenvironment (TME), consequently enhancing the cytotoxic activity of NK cells. Moreover, the inhibitory impact of NK cells is hindered by anti-TGF-β neutralizing antibodies, which disrupt the connection between this cytokine and its receptor (TGF-βR). (B) The expression of dominant-negative receptor (DNR) obstructs the inhibitory signaling that is activated by PD-1 and TGF-βR when PD-L1/L2 or TGF-β is present. (C) Small molecule inhibitors targeting GSK-3β affect the metabolism of NK cells and enhance their ability to kill target cells. Additional inhibitors are specifically designed to impede the kinase activity of TGF-βR. ; PD-1, Programmed Death 1; TIGIT, T cell immunoglobulin and ITIM domain; TIM-3, T cell immunoglobulin and mucin-domain containing-3; NKG2A, natural killer group 2A; PD-L1/2, Programmed Death ligand-1/2; HLA-E, HLA class I histocompatibility antigen, alpha chain E; ADAM-17, A disintegrin and metalloprotease 17; TGF- β, Transforming growth factor beta; TGFβR-2, Transforming growth factor beta receptor type 2; CCL2 Chemokine (C-C motif) ligand 2; CAR, chimeric antigen receptor; CIS, cytokine-inducible SH2-containing protein; SOCS3, suppressors of cytokine signaling; LAT, linker for activation of T cell; CBLB, Casitas B-lineage lymphoma protooncogene B ; ADCC, Antibody-dependent cellular cytotoxicity;HLA-I, HLA class I histocompatibility antigen; KIR, Killer-cell immunoglobulin-like receptor; HLA-E, HLA class I histocompatibility antigen, alpha chain E; PD-L1, Programmed Death ligand-1; PD-1, Programmed Death 1; TIGIT, T cell immunoglobulin and ITIM domain; BCL2-associated Athanogene 6 (BAG-6); sNKG2D-L, soluble natural killer group 2D ligands; TIM-3, T cell immunoglobulin and mucin-domain containing-3; PGE-2, prostaglandin E2; NO, nitric oxide; A Disintegrin And Metalloproteinase (ADAM).

The CAR is designed to recognize a specific antigen expressed in certain tumors, which can serve as a target for tumor therapy (Figure 2). Installing a CAR on immune cells, such as NK cells, allows them to recognize the specific antigen and subsequently attack the tumor expressing it.[19] Utilizing the NK-specific intracellular signaling domains in CAR constructs that enhance NK cell functions can improve their persistence in vivo as well.[20,21] Most activating receptors on NK cells, triggering their cytotoxic and secretory functions, signal through cytosolic Tyr-based motifs, like immunoreceptor tyrosine-based activation motif (ITAM) and immunoreceptor Tyr-based switch motif (ITSM). DAP10 and DAP12 are adaptor molecules containing the ITAM and contribute to activation signaling in NK cells. FcRγ and CD3ζ are other ITAM-containing chains. The activating receptor 2B4, belonging to the signaling lymphocytic activation molecule (SLAM) family, exploits ITSM to transmit signals.[22]

Recently, Eitler et al[23] indicated that downregulation of the adhesion molecule ICAM-1 on breast cancer cells is a critical escape mechanism from ErbB2 (HER2)–specific monoclonal antibody trastuzumab-mediated NK cell cytotoxicity, while engineered NK cells expressing high-affinity FcγRIIIa (CD16) in combination with an ErbB2-specific CAR can overcome this resistance through bypassing the LFA-1 signaling (Figure 3).

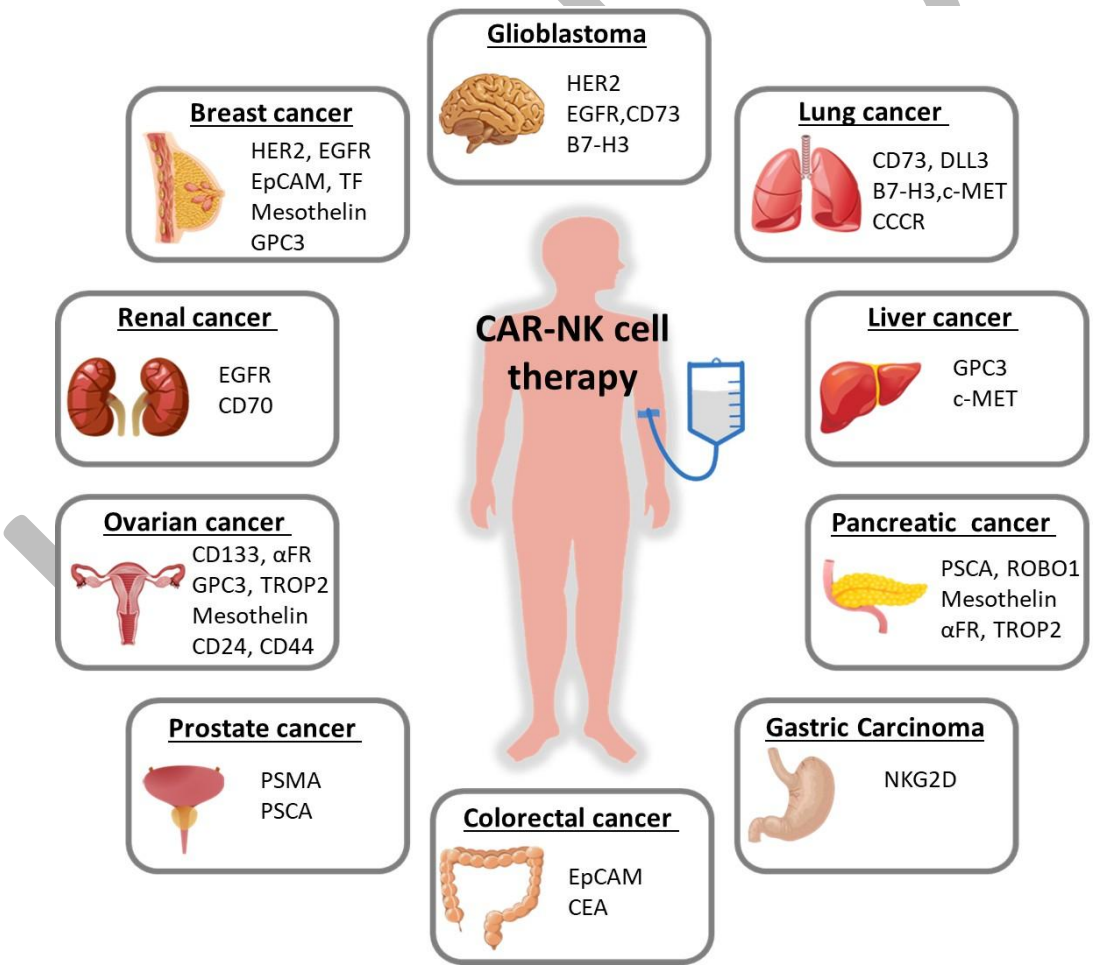

Figure 2. Targets of CAR NK therapy in different cancers.

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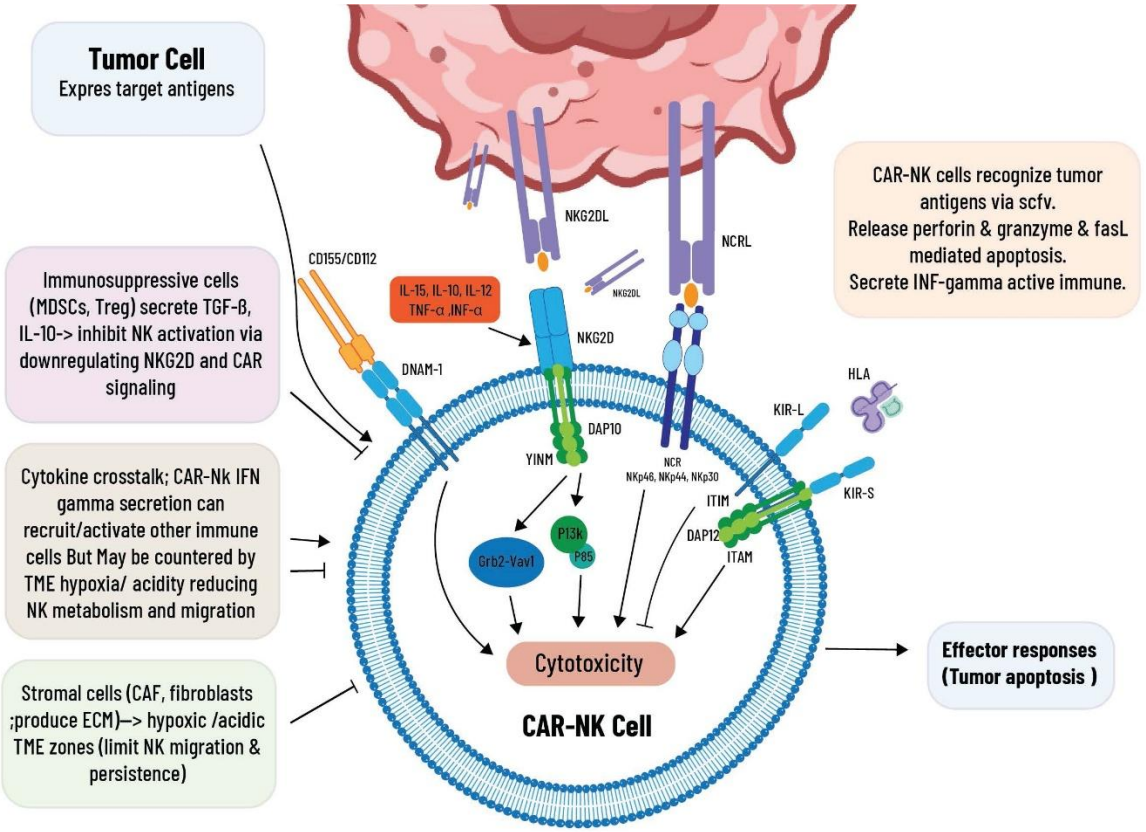

Figure 3. This diagram illustrates the mechanism by which natural killer (NK) cell surface receptors modulate the function of chimeric antigen receptor (CAR)-NK cells. The NKG2D receptor, expressed on the surface of CAR-NKs, interacts with its ligands (NKG2DL) present on target cells. Upon binding, NKG2D forms a complex with the adaptor protein DAP10. This association triggers downstream signaling pathways, including the activation of the p85 subunit of phosphoinositide 3-kinase (PI3K) and the Grb2-Vav1 pathway, which collectively enhance the cytotoxic activity of CAR-NKs.The tumor microenvironment (TME) plays a crucial role in regulating NKG2D receptor activity. Cytokines such as IL-15, IL-10, IL-12, TNF-α, and IFN-α present in the TME can upregulate or activate NKG2D receptors, thereby promoting CAR-NK cytotoxicity. Conversely, soluble forms of NKG2DL (sNKG2DL) found in the TME can bind to NKG2D receptors and inhibit their function, potentially dampening the anti-tumor response.Killer immunoglobulin-like receptors (KIRs) are another important class of NK cell surface receptors. These receptors are categorized based on the signaling motifs in their cytoplasmic domains: activating KIRs (KIR S) contain immunoreceptor tyrosine-based activation motifs (ITAMs), while inhibitory KIRs (KIR L) contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The balance between activating and inhibitory KIR signals influences the overall responsiveness of CAR-NKs.Additionally, DNAM-1 (CD226) is a co-stimulatory receptor that, upon engagement with its ligands CD155 and CD112 on target cells, further augments the cytotoxic potential of CAR-NKs. Natural cytotoxicity receptors (NCRs), including NKp46, NKp44, and NKp30, are a group of activating receptors expressed on CAR-NKs. Their interaction with specific ligands on target cells contributes to the activation and effector functions of CAR-NKs, reinforcing their anti-tumor activity.

CAR-NK Cell Expansion and Persistence

In the absence of cellular contacts, ex vivo expansion of NK cells is inefficient, and the resulted number of NK cells is not sufficient for a clinical approach.[24] K562 cells were transduced with mIL-15 or 4-1BB ligand (4-1BBL), and both of them showed improved efficiency for stimulation of NK cell proliferation.[25] However, studies have shown that K562/mb15/41BBL-activated NK cells become unresponsive to stimulation and senesce earlier than T lymphocytes, possibly due to telomere shortening.[26]

"Memory-like NK" cells that are generated by a brief

preactivation of NK cells with IL-12, IL-15, and IL-18 exert enhanced responses to cytokines or activating receptor restimulation for weeks to months after preactivation and respond more potently to tumor cells.[27,28] "Armored CARs" are designed to secrete cytokines or other molecules, counteracting the immunosuppressive nature of the TME and promoting CAR-cell persistence and efficacy.[29] These CAR constructs can be armored with stimulatory cytokine-transgenes encoding, for example, IL-2, IL-7, IL-15, and IL-21, which trigger NK cell expansion and survival.[11] However, preclinical research shows a link between toxicity and systemic IL-15 release.[30] Although systemic administration of IL-2 has been associated with serious complications, partly due to the uncontrolled stimulation of effector cells and partly because of the potential to induce the growth of regulatory cells, including regulatory T (Treg) cells.[30]

However, these systemic cytokines can be associated with a high risk of capillary leak syndrome and further accelerate the exhaustion of infused NK cells upon overstimulation.[31,32] The researchers genetically engineered MHC class I negative (missing self as "signal 1") K562 cells to enforce the expression of CD48, 4-1BBL, and membrane-bound IL-21.[33]

Understanding the Tumor Microenvironment

The TME represents a complex ecosystem with altered extracellular matrices, aberrant vascularization, and hypoxic zones that surround tumor cells and various nonmalignant cell types, such as stromal cells and immune cells.[34,35] A variety of innate and adaptive immune cells, including macrophages, NK cells, dendritic cells, and T cells, contribute to the antitumor immune response.[36] However, various regulatory cells in the TME can adversely affect these immune cells, exerting a protumorigenic function that supports cancer aggressiveness, distant metastasis, and resistance to therapy.[37] Furthermore, solid tumors recruit myeloid-derived suppressor cells (MDSCs), which inhibit cytotoxic lymphocytes and reinforce the suppressive TME.[38,39]

NK cell function is impaired by MDSCs through metabolic disturbances and inhibitory cytokines. Certain signaling pathways, including arginase activity and reactive oxygen species generation, are implicated in MDSC-mediated repression.[40] Major suppressor cells in the context of antitumor immunity have been identified as MDSCs. MDSCs promote the development of protumor macrophages (M2) and Tregs by producing immunosuppressive cytokines such as IL-10 and transforming growth factor β (TGF-β). Additionally, these inhibitory mediators reduce the expression of the activating receptor NKG2D on NK cells, thereby diminishing their cytotoxic activity. Additionally, tumor-associated hypoxia promotes an increase in programmed cell death ligand 1 (PD-L1) expression on the surface of MDSCs.[41,42] However, metabolic reprogramming of the TME is the most commonly used mechanism by MDSCs to suppress tumor-fighting cells. MDSCs significantly influence the antitumor immune response by creating local tolerance within the tumor through a complex network of cellular components, including enzymes, transporters, and transcription factors involved in glucose, lipid, and amino acid metabolism.[41] MDSCs can be associated with changes in the TME, such as downregulation of genes encoding glucose transporters and glycolysis-related enzymes, disruption of lactate and pH regulation, elevated levels of CD39 and CD73, and increased adenosine accumulation.[41]

Moreover, the abnormal vasculature of solid tumors lacks hierarchical organization and expresses lower adhesion molecules on the endothelium, impairing the infiltration of immune cells.[43,44] The immunosuppressive cytokines and metabolites within the TME (TGF-β, IL-10, IL-37, indoleamine 2,3-dioxygenase [IDO], adenosine, and prostaglandin E2), can further limit the NK cell antitumor functions.[45–47] TGF-β reduces the recruitment of functional NK cells, downregulates their activating receptors, and suppresses the release of perforin.[48] Furthermore, the immunosuppressive TME contains upregulated immune checkpoint ligand expression that interacts with immune checkpoint receptors on tumor-infiltrating immune cells and suppresses their effector function. NK cells express the immune checkpoint receptor programmed cell death 1 (PD-1), which can inhibit their cytotoxic function upon interaction with PD-L1 on tumor cells.[49,50]

Furthermore, tumor-associated neutrophils (TANs) can express PD-L1 under the immunosuppressive condition of TME.[51] The upregulation of PD-L1 on TANs has been associated with a decrease the responsiveness of NK-activating receptors, NKp46 and NKG2D, through the PD-L1/PD-1 interplay.[52] In addition, tumor-associated macrophages, particularly M2-like tumor-associated macrophages, and cancer-associated fibroblasts (CAFs), exhibiting protumor

phenotypes, contribute to suppressing NK cell cytotoxicity, in particular by secreting anti-inflammatory cytokines and immunosuppressive mediators in the TME.[53,54] This is worsened by CAFs, which attract Tregs and M2 macrophages and produce extracellular matrix components that inhibit NK cell infiltration.[40] The interaction between CAFs and tumor cells through the IL-6 produced by CAFs increases the Treg:$CD8^+$ T cell ratio.[42] Using co-culture systems, the interaction between fibroblasts and breast cancer cells increased levels of cytokines (TGF-β, IL-6, and IL-8) as well as chemokines (CXCL1 and CXCL3). These molecules are involved in cell migration and the development of epithelial-mesenchymal transition.[55] Overall, NK cells' function in the TME resulted from a sophisticated interplay of activating and inhibiting receptors with corresponding ligands or cytokines. Through these receptors, NK cells identify biomarkers presented on the tumor cells, providing the stage for either tumor surveillance or tumor immune escape[56] (Figure 1).

Overcoming the Immunosuppressive TME

One of the major challenges facing CAR cell therapy in solid tumors is overcoming the immunosuppressive TME (Figure 1).

TGF-β

The cytotoxicity of NK cells is significantly reduced once TGF-β binds to TGF-βR on NK cells, leading to phosphorylation and activation of SMAD2/3 followed by SMAD4. Additionally, it has been shown that TGF-β decreases NK cell ADCC, IFN-γ secretion, and granzyme A and B production by activating SMAD3. Silencing TGF-β's downstream mediator, SMAD3, is another strategy to reduce the detrimental effects of TGF-β on NK cells and enhance NK cell cytotoxicity.[57] Downregulating NK cell–activating receptors (NKG2D, DNAM1, and NKp30, TGF-β) reduces the cytotoxic function of NK cells by decreasing their capacity to degranulate, produce cytokines, and engage in metabolic and mTOR signaling pathways. Moreover, TGF-β increases the expression of CXCR3 and CXCR4 on NK cells, which prevents their development into functional immune cells and interferes with their egress from the bone marrow. Notably, unfavorable prognoses are associated with elevated TGF-β expression in several solid tumors, including pancreatic, gastric, lung, breast, and liver cancers.[58]

Researchers have engineered NK cells to make them resistant to the suppressive effect of TGF-β, for example, by the TGF-βR2 knockout using CRISPR-Cas9 gene editing,[59] or the expression of a dominant negative TGF-βR2 using retroviral transduction.[60] The engineered NK-92 cells expressing a CAR with the extracellular TGF-βR2 domain and intracellular NKG2D domain were found to be resistant to TGF-β–induced suppressive effects and exhibit enhanced IFN-γ production and cytotoxicity, as well as more cytotoxicity and a better chemoattractant in the presence of TGF-β.[61] Thus, converter CARs can be a promising strategy for developing novel generations of CAR-NK cells to reverse the immunosuppressive TME, improving outcomes in the treatment of solid tumors.

According to Oh et al,[62] CAR-NK cells were engineered to release UP01a, a TGF-β receptor inhibitory peptide that disrupts the TGF-β signaling cascade in the TME and increases NK cell infiltration in the TME. Another study uses a peptide called P6, which targets mesothelin in pancreatic tumors, to deliver self-activating CAR-NK cells that block TGF-β1 signaling in the TME and restore NK cell activity, metabolism, and cytotoxicity.[63] In the presence of exogenous TGF-β, Chaudry et al demonstrated that cotransducing NK cells with a B7H3 CAR and a TGF-β dominant negative receptor can maintain cytolytic function. However, since this was only evaluated in vitro, further preclinical studies are needed to determine whether this is a viable strategy.[58,64]

Adenosine

Cancer cells release large amounts of adenosine triphosphate in the hypoxic environment, which is then broken down into adenosine by the ectoenzymes CD39 and CD73. Adenosine is a potent purinergic mediator that can instruct NK cells to suppress their antitumor activities.[58] Both NK cells and T cells are broadly immunosuppressed by this buildup, and the antitumor function of NK cells can be inhibited by CD39-expressing Tregs.[40]

Adenosine interacts with the A2A adenosine receptor (A2AR) on NK cells to limit their activity. Therefore, researchers have exploited NK cell–specific conditional deletion of A2AR to preserve the proliferative capacity and antitumor function of NK cells.[65] The possibility of adapting CAR-NK cells to hypoxia within the TME of solid tumors has been the subject of only a few investigations to date. Nonetheless,

several studies have already explored how NK cell–based therapies might be modified, and it is possible that CAR-NK cells could use the same tactics.[58] To restore oxygen levels in the TME, Duan et al used biodegradable manganese dioxide nanoparticles, called MnOX nanoenzymes. These MnOX nanoenzymes enhance infiltration and antitumor activity when combined with CAR-NK cells in mice.[66]

Solocinski et al[67] used high-affinity NK (haNK) cells in a different approach. These cells contain internal IL-2 and a high-affinity CD16 receptor, which enhances ADCC and activation. They demonstrated that haNKs retain their cytotoxicity in hypoxic environments. By integrating an anti-CD73 scFv CAR into genetically engineered NK cells, Chambers et al[68] successfully eradicated lung cancer cells with high CD73 expression, even in hypoxic environments. Even with these encouraging methods, much more research is still needed to successfully and safely implement such strategies in a clinical setting.

By focusing on the intricate interactions among immune cells, stromal components, and metabolic factors that promote tumor growth and therapy resistance, modulating the TME has emerged as a rapidly advancing field in cancer therapy aimed at overcoming resistance and improving patient outcomes. These strategies have demonstrated improved NK cell infiltration, persistence, and cytotoxicity in preclinical in vitro and animal models, providing proof of concept for their therapeutic potential. However, translating these findings into human applications faces challenges, including the complex and heterogeneous nature of the human TME, variability among patients, and the requirement to manufacture CAR-NK cells products under strict Good Manufacturing Practice (GMP) conditions to ensure safety and reproducibility. TME heterogeneity, the lack of predictive biomarkers, and adaptive resistance mechanisms often limit clinical efficacy, despite encouraging outcomes from early-phase trials of TME modulation in certain cancer types. Real-time TME profiling is driving the development of combination therapies and personalized approaches to overcome resistance and maximize benefit. Despite this progress, limitations remain, including potential toxicities, regulatory hurdles, and the need for long-term efficacy data. Continued multidisciplinary research and well-designed clinical studies are essential to fully realize the potential of TME modulation strategies in CAR-NK cancer immunotherapy (Figure 4).

Infiltration Capabilities of CAR-NK Cells

Various immunosuppressive TME-related factors counteract the infiltration capabilities of CAR-NK cells, preventing NK cell homing into solid tumors.[69] The limited tumor infiltration by NK cells prevents a sustained antitumor immune response and the successful application of CAR-NK cell therapy. Research demonstrates that overexpressing the chemokine receptor CXCR3 on human NK cells during ex vivo expansion enhances their ability to migrate toward solid melanoma tumors that express CXCL10.[70] Similarly, enforcing a CXCL16 gradient in the pancreatic tumor environment increases the tumor tissue infiltration of NK cells, improves the antitumor response, and reduces the tumor burden in mice.[71] Therefore, the enhanced engagement of chemokines with cognate receptors on the CAR-NK cells has emerged as one approach to improving their migration toward the tumor site. For instance, the genetic engineering of CAR-NK cells to express the chemokine receptor CXCR4 conferred a specific chemotaxis to CXCL12/SDF-1α–secreting glioblastoma cells, intensified tumor infiltration,[72] and retained the functional and cytotoxic activity of CAR-NK cells against target cells.[73]

Clinical Trials and Research Progress

Presently, multiple clinical trials are proceeding to investigate the therapeutic utility of CAR-NK cells in various solid and hematological malignancies, like neuroblastoma, prostatic cancer, and glioblastoma. These trials aim to validate the clinical efficacy and safety profile of CAR-modified NK therapy in a real-world setting.[74] Numerous ongoing clinical trials investigating CAR-NK cell therapies are focused on pancreatic cancer, which remains one of the most challenging diseases to treat.[30]

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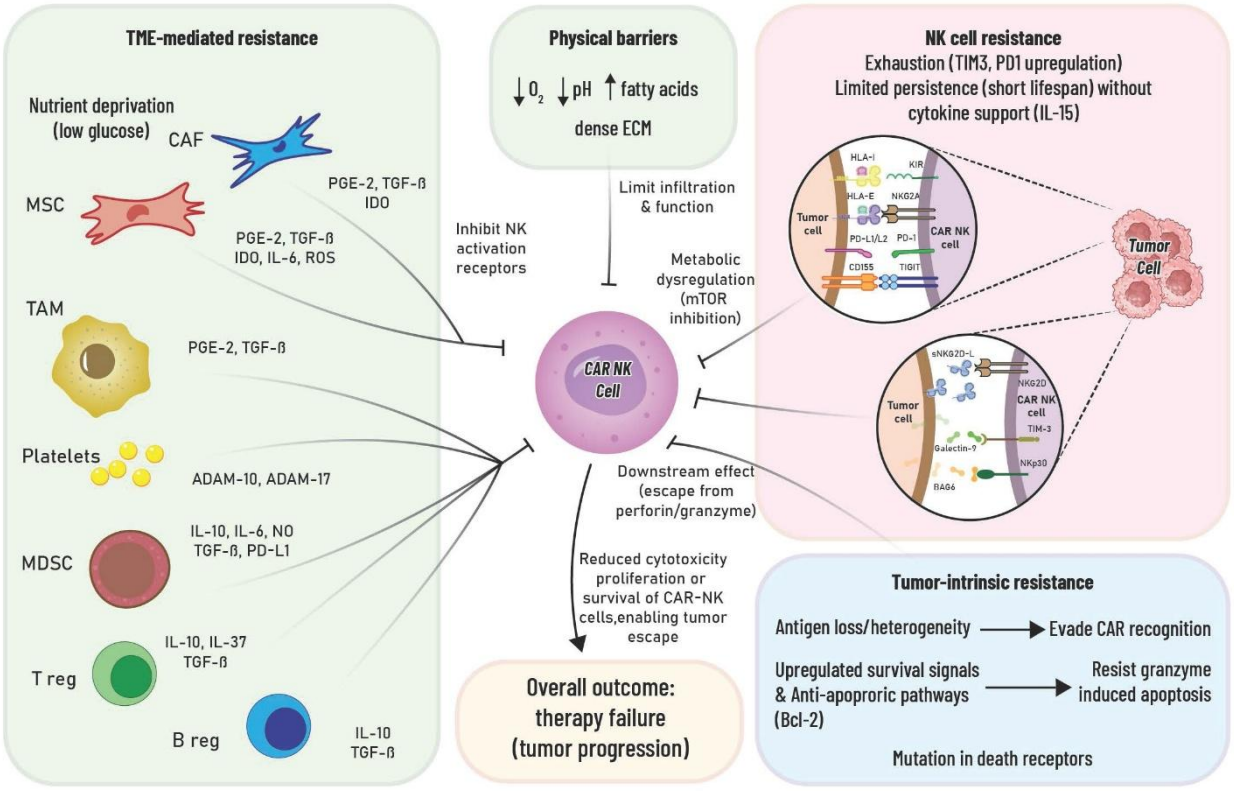

Figure 4. The immune-suppressive tumor microenvironment (TME) plays a critical role in impairing the function of chimeric antigen receptor natural killer (CAR-NK) cells. Cancer cells actively contribute to this suppression by expressing immune checkpoint ligands on their surface, which engage inhibitory receptors on NK cells and dampen their cytotoxic activity. In addition to membrane-bound ligands, tumor cells release a variety of soluble factors into the surrounding milieu that further inhibit NK cell function. These include proteins such as BAG-6, galectin-9, and soluble NKG2D ligands (sNKG2D-L), as well as cytokines like transforming growth factor-beta (TGF-β), enzymes, and metabolic byproducts.Many of these immunosuppressive soluble factors are not only produced by tumor cells but also by immune cells infiltrating the TME. Regulatory T cells (Tregs), regulatory B cells (Bregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) all contribute to the suppression of NK cell activity by secreting inhibitory molecules and cytokines. Platelets also play a role by releasing metalloproteinases ADAM-10 and ADAM-17, which promote the shedding of NKG2D ligands from the tumor cell surface, thereby reducing NK cell recognition and activation.Non-immune stromal cells within the TME, such as mesenchymal stromal cells (MSCs) and cancer-associated fibroblasts (CAFs), further exacerbate immunosuppression by producing indoleamine 2,3-dioxygenase (IDO) and reactive oxygen species (ROS), both of which negatively impact NK cell function. Moreover, the metabolic landscape of the TME—characterized by hypoxia, elevated levels of fatty acids, nutrient deprivation, and acidic pH—creates additional barriers that limit the effectiveness of NK cells, particularly in the context of hematologic malignancies.Collectively, these diverse mechanisms generate a highly immunosuppressive environment that significantly hinders the anti-tumor activity of CAR-NKs. However, several therapeutic strategies are being explored to counteract these immunosuppressive pathways and restore NK cell function within the TME.

Early clinical research, however, remains limited and heterogeneous. Furthermore, CAR-NK cells face several challenges, including inefficient transduction of CAR gene constructs, difficulties in maintaining cell potency after cryopreservation, variable persistence following infusion, and inconsistent durability of clinical responses. These obstacles have hindered the widespread adoption of CAR-NK cell therapy in mainstream clinical practice.[75] However, information about the manufacturing process of CAR-NK cells was

not consistently documented, and significant variations existed in both published and ongoing research regarding CAR-NK cell dosage, administration schedules, and routes of administration.[75] Some studies have reported using freshly collected products immediately after ex vivo manufacturing, whereas only 2 studies have described infusing cryopreserved CAR-NK cell products. Even though most trial registration data do not specifically mention this, it remains difficult to determine the feasibility of using cryopreserved materials until the results are published.[75] Characterization of cell products was not consistently described. The majority of studies used cell surface markers and CAR transgene persistence to assess the potency and viability of CAR-NK cells, whereas cell viability and potency assays were infrequently reported.[75] Cellular senescence often limits the ability of NK cells to proliferate in ex vivo cultures. According to some preclinical studies, generating NK cells from expanded HSCs or induced pluripotent stem cells (iPSCs) may help prevent cellular senescence.[75] Furthermore, data on dose responsiveness, the relationship between CAR-NK cell persistence and efficacy, and new information on potential biomarkers associated with efficacy may be obtained from future research.[75] To prevent the unchecked activation and proliferation of CAR-NK cells in vivo, it is essential to address the risks of insertional mutagenesis and potential neoplastic transformation resulting from the random integration of CAR-carrying vectors.[30] A significant obstacle is the host immune system's ability to recognize and eliminate grafted cells, especially since most ongoing clinical trials use an off-the-shelf allogeneic approach for CAR-NK cells.[30] Fever and exhaustion are the most common adverse effects of CAR-NK therapy and can result from elevated serum levels of IL-6 and C-reactive protein.[76]

Treatment for solid tumors is now undergoing early-stage clinical trials with considerable variation in design, end points, and reporting. The majority of published CAR-NK cell therapy clinical trials, including those targeting solid tumors, have very small sample sizes. Most active registered trials (over 50 trials with a total of 2102 participants) plan to recruit more subjects[30]; however, the majority focus on hematologic malignancies rather than solid tumors. In solid tumor trials, these small sample sizes limit statistical power and generalizability. Current trials have inconsistent and often nonstandardized end points. Safety (including adverse event rates and dose-limiting toxicities), feasibility, and overall response rate are typical primary end points. Progression-free survival, overall survival, and CAR-NK cell persistence serve as common secondary end points. While objective response rates and response duration are frequently reported for solid tumors, biomarker analysis and surrogate outcomes are not consistently incorporated.

Most trials have short durations, while some last up to a year to assess response durability. Frequently, median follow-up times are either not reported or not reached. There is a scarcity of long-term data on durability and late toxicities. Clinical trials are currently testing a wide variety of dosing regimens, reflecting the uncertainty about the dose required to maximize efficacy while minimizing potential side effects in patients. The dosage schedules in the selected clinical trials demonstrate a variety of approaches, ranging from single to multiple doses. Because CAR-NK cells have lower postinfusion durability and expansion than CAR-T cells, dosage may also be more important. Optimizing the source and manufacturing processes of CAR-NK cells could be significant step toward improving their clinical efficacy. Potential biases include heterogeneity in CAR-NK cell sources, manufacturing techniques, which complicate cross-study comparisons. Other potential biases include patient selection (heavily pretreated or refractory patients) and uncontrolled study designs, as the majority of published trials are single-arm and lack control groups. Most importantly, while negative or inconclusive studies may remain unpublished, positive outcomes are more likely to be publicized.

Clinical Trials for CAR-NK Cell Therapy

BCMA-CAR-NKs modified with CXCR4 significantly reduced the tumor burden and extended the survival of tumor-bearing mice.[77,78] Preclinical studies targeting specific antigens, including EGFRvIII, HER2, HLA-G, and cancer stem cell markers, have shown enhanced cytotoxicity against solid tumors (ovarian cancer, breast cancer, and glioblastoma) in vitro and in vivo, indicating the potential of CAR-NK cell therapies in the treatment of malignancies.[77,79,80] Murakami et al developed CARKHYG-1 that specifically suppresses the growth of GBM cells by inducing apoptosis through the expression of EGFRvIII.[81] Also, Liu et al[82] demonstrated that EGFR-CAR NK cell types inhibited breast cancer cell line–derived xenograft and patient-

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derived xenograft tumors in mice, which holds potential as a viable technique for managing triple-negative breast cancer in patients.

According to Wang et al,[83] the creation of multifunctional genetically modified human NK cells, namely CD73 mCAR pNK cells, has the potential to produce potent antiglioblastoma (GBM) effects. This is attributed to the diverse nature of tumors and the various immunosuppressive properties present in the TME of GBM. In vitro and in orthotopic GBM xenograft models, Zhang et al provided evidence of the powerful and selective anticancer activity of second-generation CAR NK-92/5.28.z, which specifically targets the growth factor receptor tyrosine kinase ErbB2 (HER2).[84] In addition, preclinical studies have confirmed the efficacy of CAR-NK cells in targeting glioblastoma and EpCAM-positive colorectal cancer cells in xenograft models. However, limited clinical data exist on their potential in solid tumor treatment. In China, 3 phase I/II clinical studies demonstrated the feasibility of nonhematological tumor treatment with CAR-NK cells in pancreatic ductal adenocarcinoma and relative solid tumors with ROBO-1 expression.[85–88]

EpCAM-specific second-generation CAR cells have been established to specifically target and destroy EpCAM-positive colorectal cancer cells, exhibiting focused cytotoxicity. The research showed that using both regorafenib and CAR-NK-92 cells together was more effective at suppressing the growth of established EpCAM-positive tumor xenografts than using either CAR-NK-92 cells or regorafenib alone.[89] The ongoing clinical trials aim to evaluate the safety and feasibility of CAR-NK therapy, as well as its capacity to induce tumor regression and enhance patient outcomes. These trials are crucial for evaluating the persistence and expansion of CAR-NKs in vivo, and identifying any potential adverse effects associated with this novel immunotherapy approach.[90] In terms of trial targets, some CAR-NKs target metastatic solid tumors that express tumor-associated antigens (HER2, PSMA, mesothelin, ROBO1, or MUC1). A clinical trial is now assessing the effectiveness of CAR therapy that targets NKG2DL in treating solid tumors. This approach is based on the discovery that stress molecules identified by NKG2D are frequently increased in metastatic solid tumors.[74,91]

Predictive and Prognostic Biomarkers in CAR-NK Cell Therapy for Solid Tumors

Tumor antigen density, NK cell–intrinsic factors, TME modulators, and donor cell quality are emerging as significant predictors of CAR-NK cell response in solid tumors, although no single biomarker can accurately predict this response. Functional assays and multiplexed biomarker panels can further improve patient selection and treatment optimization. The variation in therapy effectiveness based on the TME is noteworthy.[92,93] The respond of tumor cells to CAR-NK cells appears to be significantly influenced by the presence of CD44 in the extracellular matrix. Both in vitro and in vivo studies have shown that CAR-NK cells significantly suppressed tumors and induced cytotoxicity in CD44-negative N87 tumor-bearing mice. Conversely, CAR-NK cells exhibited reduced efficacy in CD44-positive JIMT-1 tumor-bearing mice, as they effectively penetrate the extracellular matrix and reach tumor antigens.[92] Therefore, CD44 expression in the TME may be assayed to predict CAR-NK cell responsiveness.

The translationally relevant biomarkers of cellular immunotherapy associated with the effectiveness, durability, and safety of CAR therapy were assessed by Layman et al.[93] Specifically, they evaluated the presence and concentration of several candidate biomarker proteins related to (1) immune activation and cytotoxicity (IFN-γ, granzyme B, IL-2, IL-12p70, perforin), (2) cellular immunotherapy persistence (IL-7, IL-15, IL-18, CD40L), and (3) adverse events (CRS and immune effector cell-associated neurotoxicity syndrome [ICANS] [IL-1β, IL-10, IL-6, MCP-1]). Elevated levels of IFN-γ, IL-12p70, and perforin have been associated with robust immune activation and cytotoxicity, serving as positive predictive biomarkers for CAR-NK cell efficacy. Conversely, excessive levels of IL-6, IL-10, or MCP-1 are correlated with CRS and immune-related toxicities, acting as prognostic indicators of treatment safety rather than efficacy. Together, these findings suggest that integrating tumor phenotyping (eg, CD44 expression, antigen density) with systemic immune signatures (eg, cytokine profiles) could guide patient stratification and enable biomarker-driven optimization of CAR-NK therapies for solid tumors.

Challenges of the NK Cell Sources in Clinical Trials

Peripheral blood NK cells: The effectiveness of autologous NK cells is often lower; therefore, allogeneic peripheral blood (PB)–NK cells are typically preferred for treatment. However, because NK cells are scarce in peripheral blood, it is difficult to collect a sufficient number, necessitating extensive expansion.[76] Despite

improvements in stimulatory biological combinations, PB-NK cells still face low transduction efficiency and potential exhaustion from prolonged culture, despite being mature cells that do not require extensive differentiation.[76] Interdonor variability and the lack of standardized protocols for NK cell activation and expansion can lead to heterogeneity in CAR-NK cell products. Moreover, applying established CAR-T manufacturing techniques to genetically modify PB-NK cells using lentiviral vectors is difficult, likely due to their intrinsic antiviral defenses. One approach under investigation to enhance NK cell transduction efficiency involves using alternative lentiviral pseudotypes to alter the tropism of the lentiviral delivery system.[30]

Cord blood NK cells: Cord blood's high NK cell yield makes it a convenient alternative. Although cord blood (CB)–NK cells may express fewer cytotoxicity receptors, they have shown promise in clinical trials, with current research demonstrating their efficacy and durability in vivo.[76] Moreover, prolonged ex vivo NK cell expansion is required to reach clinically relevant dosages due to the limited volume obtainable from a cord blood sample.[30]

NK cell lines: Because they are simple to expand and maintain, NK cell lines, such as NK-92 facilitate the production of large quantities of NK cells. NK-92 cells have potent antitumor activity and can be genetically modified.[76] Their poor in vivo persistence has been a significant drawback in NK-92 cell trials, impeding the development of long-lasting remissions.[76] The high expression of activating receptors and low expression of inhibitory receptors indicate that NK-92 cells are highly cytotoxic. However, the potential for tumor engraftment raises several safety concerns regarding the infusion of immortal NK cell lines.[30] Residual K562 cells may be present in the final product, necessitating thorough purification, which complicates and increases the cost of manufacturing. Tumor-derived feeder cells, such as K562 are difficult to use in clinical protocols because of the strict standards for cell-based therapeutics enforced by European regulatory agencies, including the European Medicines Agency.[76]

Stem cell–derived NK cells: iPSCs or human embryonic stem cells (hESCs) can be used to generate NK cells, providing a standardized, commercially available product that reduces donor variability. Although it takes 3 to 5 weeks to produce NK cells from these stem cells, the final product represents the most consistent and reliable approach.[76] Complex mechanisms required for pluripotent stem cell differentiation limit the capacity to cost-effectively scale up the production of clinical-grade CAR-NK cells under GMP. Producing large quantities of CAR-NK cells is time-consuming, and during the expansion phase, cultured cells may acquire oncogenic mutations, which could pose safety risks.[30] Concerns about genomic instability, the potential for malignant transformation, informed consent for donor tissue use, genetic manipulation, and data privacy are among the key ethical challenges associated with the use of iPSC-derived NK cells.[94] Regarding hESCs, the primary safety concern in iPSC-based therapies is the risk of teratoma formation, which may occur if patients receive iPSC-derived cells containing residual undifferentiated iPSCs. To evaluate the clinical potential of iPSCs and their differentiated derivatives, further in vitro and in vivo animal studies are needed to develop optimized growth and differentiation protocols, along with reliable safety assessments.[95]

In addition to eliminating unnecessary barriers to iPSC research and therapy, laws and standards must be established to ensure the ethical integrity of iPSC production and application. GMP conditions should be maintained at every stage of iPSC-based cell therapy, including donor somatic cell isolation, iPSC generation and differentiation, and the transplantation of iPSC-derived products. Rigorous quality control procedures are also required, particularly when genetic modification of cells is involved.[96]

Manufacturing and Scale-up Challenges for Clinical Translation of CAR-NK Cell Therapy

The complexity and cost of customized manufacturing present challenges. Innovative manufacturing techniques have recently been the focus of research aimed at improving the accessibility of CAR-NK therapy. Off-the-shelf CAR-NK products can be manufactured in large quantities and stored for immediate use, significantly reducing production time and costs compared to autologous CAR-NKs, which require customized manufacturing.[97] It is difficult to produce a sufficient quantity of high-quality CAR-NK cells for clinical use, and strict GMP-compliant procedures are required. The methods used to produce CAR-NK cell therapies vary depending on the platforms and proprietary technologies employed, as well as the starting materials.[30] Bioreactor systems enable precise control of critical parameters (temperature, dissolved oxygen, pH, and nutrient levels), which are vital for

maintaining cell integrity, ensuring reproducibility, and optimizing productivity.[98] Every step of the production process—from storing donor cells and genetic modification and differentiation to formulating the finished product—incorporates rigorous quality control procedures. These methods include sterility testing, immunophenotyping, cytotoxicity assays, genetic stability evaluations, and assessments of transgene expression levels.[98]

GMP-Compliant Manufacturing and Cost Consideration for Off-the-Shelf CAR-NK Products

Scalable, off-the-shelf therapeutic production is enabled by the ability to obtain CAR-NK cells from various sources, including PB, CB, iPSC, or NK-92 cell lines, NK101 cell lines, and hematopoietic progenitor cells.[169] However, manufacturing clinical-grade CAR-NK cells is challenging, and variability in the cell product's properties may impact outcomes. Further optimization of donor or cell source selection, cell culture conditions, cryopreservation protocols, and dosing methods is necessary to ensure consistent quality. To produce high-quality, cost-effective infusion products at scale, automated and standardized manufacturing systems, along with robust quality control procedures, are essential.[99] To meet regulatory requirements for safety and efficacy, GMP practices require stringent in-process controls, validated quality control testing (including flow cytometry for purity, identification, and potency), and comprehensive documentation.[100] Feeder-free and serum-free media, along with GMP-grade reagents and vectors, are increasingly used in GMP-compliant protocols to minimize contamination and batch-to-batch variability. GMP processes also include controlled shipping to preserve product integrity for "off-the-shelf" use, as well as validated cryopreservation techniques.[100]

Liu et al[101] employed a cryopreservation technique specifically designed for CAR-iNK cells to replicate actual clinical conditions. After 6 months of cryopreservation in clinical-grade CS10 freezing medium, the cytotoxicity of the cryopreserved CAR-iNK cells remained almost unchanged. This technique has enabled the creation of an off-the-shelf CAR-iNK cell product, allowing us to overcome the challenges associated with NK cell cryopreservation. Furthermore, since secondary T-cell malignancies induced by CAR-T therapy have been reported following treatment, cryopreserved NK cells provide sufficient time to evaluate the potential risks of CAR-iNK tumorigenesis. Given the properties of cryopreserved iNK cells in cellular therapy applications, a 20% to 30% increase in the initial infusion dose is necessary to achieve the appropriate effective dose.[101]

By reducing the potential for contamination and operator error—thereby lowering the failure rate—automated closed manufacturing systems minimize manufacturing costs, improve product quality, and increase patient access to cell therapies. To establish appropriate facility and equipment requirements along with monitoring plans, the US Food and Drug Administration (FDA) encourages the use of closed manufacturing processes. Automated closed systems offer significant benefits but involve a trade-off between high upfront capital costs and long-term operational cost savings. Although the long-term advantages outweigh the initial investment, these costs often pose substantial challenges for academic laboratories and small biotech companies.[102] Even though the existing workflow is reliable, there is always room for process optimization, particularly by reducing manual tasks and simplifying paperwork. The efficiency of batch review and deviation management could be improved by switching from paper-based to electronic batch records. Furthermore, this transition would decrease manual labor and enhance scalability for commercial and clinical applications. Large-scale production is enabled by automated, closed-system manufacturing, which lowers per-dose costs and allows the processing of batches for multiple patients in a single run.[102]

Many expansion techniques are used to produce the necessary number of NK cells for clinical use. These include cytokine combinations, membrane-bound cytokines in K562 cell–based systems, and advanced automated expansion technologies. Despite advancements in expansion methods, it can still take several weeks to reach clinically meaningful cell numbers, and maximizing transduction efficiency while preserving cell viability remains challenging.[40]

Donor Variability and Reproducibility in CAR-NK Expansion

For NK cell research and their effective therapeutic application, an essential prerequisite is the development of robust ex vivo expansion techniques that produce functional NK cells in adequate numbers. While many studies concentrate on comparing activating agents or optimizing cytokine cocktails, basic culture parameters

such as the initial seeding density have received very little attention. The initial seeding density significantly influences both the phenotypic profile of NK cells and their overall expansion potential.[103]

Additionally, intrinsic donor variability may introduce significant heterogeneity in experimental results, jeopardizing both reproducibility and translational applicability. Since such interindividual variations may affect both the functional output and therapeutic potential of expanded NK cells, understanding them is crucial for both basic research and clinical applications.[103] It is widely known that there are significant differences in CD16a expression among healthy individuals. In disease contexts such as gastric cancer and non-small-cell lung cancer, reduced NKp46 expression has been previously reported and often correlates with decreased NK cell function and disease progression. Although the fundamental mechanisms remain unclear, interindividual differences in NK cell responsiveness may result from genetic polymorphisms, epigenetic regulation, and prior immunological activation.[103] In the long run, integrating single-nucleotide polymorphism analyses into donor selection processes may help predict the quality of cell products, guide treatment approaches, and reduce interindividual variation in clinical outcomes. To assess how phenotypic and genotypic variations translate into effector functions, future research should build on these findings by including larger donor cohorts and functional assays such as cytotoxicity testing, cytokine release assays, and especially ADCC. Extending genetic analysis beyond receptor gene variants—ideally through whole—genome or exome sequencing-may reveal additional metabolic or regulatory pathways involved in NK cell proliferation.[103] Reproducible generation of clinical-grade CAR-NKs to effectively treat solid tumors requires overcoming interdonor variability through careful donor selection and improved expansion techniques.

Safety Switches in CAR-NK Design

Treatment with CAR-NKs is not entirely risk-free, although these cells are generally safer than CAR-Ts. CRS, neurotoxicity, on-target/off-tumor toxicities, and leukemic transformation of CAR-NKs are some of the potential side effects. These concerns emphasize the need to develop strategies for the selective elimination of injected CAR-NKs in cases of severe adverse effects.[57] However, these adverse events still occur and need to be carefully managed clinically.

When administering CAR-NK therapy, managing CRS-related toxicity is essential. First, it is important to monitor biomarkers predictive of CRS risk, such as cytokine levels, and track their changes during treatment. Second, clinicians should aim to reduce tumor burden prior to CAR-NK infusion.[104] Anti–IL-6 therapy, intravenous fluid boluses, low-dose vasopressors, tocilizumab or other human-murine chimeric monoclonal antibodies against IL-6, and corticosteroids are all components of a systematic treatment approach for CRS and neurotoxicity.[105,106]

Suicide switches enable the selective elimination of CAR-expressing cells, thereby deactivating the therapy. The obvious consequences of this are the inability to continue the therapy and the need to either cease treatment or develop new CAR constructs.[107] Small molecule–dependent homodimerization (AP1903/rimiducid or AP20187) forms the basis of the iCas9 switch's mechanism of action. In the presence of rimiducid, the modified human caspase-9 (which lacks the native dimerization domain) is linked to the FKBP molecule, allowing dimerization and, consequently, activation of caspase-9 via chemically induced dimerization. These switches are among the best inactivating switches because of their high efficiency, exceeding 85% to 90% with a single injection of AP1903.[107] The iRC9 system, a variant of the iCas9 system, utilizes the heterodimerization of the FRB molecule (FKBP-rapamycin binding) and FKBP12. This modification enables the creation of an orthogonal switch, in which rimiducid activates the MyD88 costimulator, and rapamycin triggers caspase-9 dimerization.[108]

One study reported increased apoptosis and introduced a rapamycin-induced caspase-9–based safety switch (iRC9) alongside iCO. It has been demonstrated that these switches can coexist within the cell without interfering with its biochemical processes. This approach was successfully tested in CAR-NKs.[109] As is also being investigated in T cells, conditional safety switches incorporated into the cells provide a solution by allowing the selective removal of CAR-NK cells in patients, reversing any negative effects. According to certain research, the CAR design of CAR-NK cells incorporates an inducible caspase-9–based suicide gene (iCasp9). When undesirable responses are detected, the iCasp9 component acts as a safety switch, allowing the CAR-NK cells to undergo pharmacologically induced cell death. As demonstrated with CAR-T cells, this

method adds an extra layer of safety by enabling the rapid and targeted elimination of CAR-NK cells when necessary.[99] ADCC against NK cells modified with this EGFR variant can be mediated by cetuximab, a clinically approved monoclonal antibody. As a result, it enables the specific elimination of CAR-NK cells.[99]

The herpes simplex virus type 1 thymidine kinase (HSV-TK) must be introduced into CAR cells for ganciclovir to become activated. Native human cells do not express this enzyme. Ganciclovir (as well as acyclovir and penciclovir) is converted into a toxic metabolite by HSV-TK, leading to cell death through a mechanism that remains to be fully elucidated.[110] Numerous clinical trials have evaluated this technology, demonstrating its efficacy and safety.[107] With 5 altered amino acids and a 14-fold reduction in Michaelis constants, an artificial version of HSV-sr39TK is currently in use, ensuring greater efficiency and safety.[111] HSV-TK cell membrane markers (truncated CD34 or NGFR) enable magnetic selection, which in turn enriches CAR-T populations expressing the desired switch. Although switches that are expressed on the membrane, such as those relying on the ADCC effect, do not require the vector to carry additional markers like intracellular switches do, similar techniques for confirming switch expression could also be applied to other switches.[107]

Similar outcomes are obtained when using HSV-TK compared to iCas9; however, this system's drawbacks include the need to maintain ganciclovir at a highly alkaline pH[11] and the immunogenicity of HSV-TK.[107] Nonetheless, the ganciclovir-based method offers the advantage of enabling in vivo positron emission tomography (PET) imaging studies. The ^{18}F-labeled compound can be phosphorylated and retained within the cell by using the *HSV-TK* gene as a reporter gene (the mutant HSV-srTK is more sensitive to ^{18}F). Since the intensity of the PET signal correlates with HSV-srTK activity, this allows us to track the location of CARs in the body and evaluate the function of the switch upon activation (a decrease in signal intensity indicates a reduction in cell number).[112,113]

Challenges of the Clinical Application of Car-Nk Therapy

A primary challenge is the sensitivity of NK cells to the freeze-thaw procedure, which greatly reduces their viability and cytotoxic activity. Nevertheless, some studies have demonstrated that the inclusion of IL-2 can partially restore the functionality of cryopreserved NK cells. Hence, approaches for optimal cryopreservation need to be explored to make CAR-NK therapy feasible as an off-the-shelf product.[3]

Furthermore, the heterogeneity of NK cells from different sources presents a challenge in standardizing the production of CAR-NKs. Each source exhibits variations in potency and persistence, making it necessary to optimize protocols for the generation and expansion of CAR-NKs from different sources to achieve consistent and reproducible results in clinical applications.[114]

The application of CAR-NKs in clinical trials for cancer therapy is still in the early stages, with limited data on their efficacy and safety in treating solid tumors. More extensive clinical studies are needed to fully evaluate their potential in various cancer types.[115] One of the key challenges in the clinical application of CAR-NK therapy for solid tumors is overcoming the limitations that decrease their efficacy in this setting. These limitations may include the heterogeneity of antigen expression, barriers limiting the trafficking of CAR-NK cells to the solid tumor site, TME-mediated suppression, and secretion of factors that disrupt the immune response.[115,116] Furthermore, the heterogeneity of tumor cells can lead to the loss of target antigens, posing a challenge for CAR therapy. In this regard, the innate cytotoxic capacity of NK cells allows CAR-NKs to recognize and eliminate tumor cells through CAR-dependent mechanisms as well as through their natural cytotoxic functions. Additionally, their ability to target diverse antigens provides an advantage in overcoming potential antigen loss in solid tumors.[117,118]

As mentioned, one of the challenges in treating solid tumors with CAR therapy is the limited intratumoral penetration and trafficking of CAR-modified immune cells. However, CAR-NKs offer potential solutions to this issue. NK cells have an inherent ability to migrate into tissues, including tumor sites, which may enhance their intratumoral infiltration compared to CAR-Ts. Moreover, NK cells possess diverse cytotoxic mechanisms, both CAR-dependent and CAR-independent, that can effectively target tumor cells.[114] Additionally, NK cells are capable of targeting cancer stem cells, known for their resistance to conventional therapies. This is facilitated by the decreased MHC-I expression on the surface of cancer stem cells and the presence of ligands stimulating activating receptors on NK cells.[117,119]

Immunosuppressive Barrier

CAR-NKs, with their inherent antitumor activity, hold potential for overcoming the immunosuppressive TME. In a preclinical study, the upregulation of chemokine receptors CXCR4 or CXCR1 in CAR-NKs has been shown to substantially enhance their ability to infiltrate glioblastoma[120] and ovarian cancer,[121] respectively. The unique biological features of NK cells, such as their lack of HLA-matching restriction, contribute to their superior safety and potent antitumor activity, making them a promising platform for CAR-based therapies in the context of solid tumors.[117,118] In addition, the inherent characteristics of NK cells, including their diverse cytotoxic mechanisms and their ability to target cancer stem cells, position CAR-NK therapy as a promising avenue for developing effective treatments for solid tumors.[118]

Challenges That Affect Cytotoxicity and Persistence in Solid Tumors

Cytotoxicity and persistence of CAR-NK cells are essential for their effectiveness in targeting solid tumors. However, there are several challenges that affect these aspects when treating solid tumors. One key challenge is the dense stroma formed by CAFs that inhibits CAR-NK cell infiltration into the tumor site, thereby limiting their cytotoxic effects on solid tumors. Furthermore, intratumoral CAR cells can become exhausted due to continuous stimulation with target antigens and the immunosuppressive TME.[122] This can lead to a decrease in the ability of these cells to proliferate and persist as they are discharged to the lymph nodes due to a combination of internal and external forces.

Strategies to enhance NK cell homing to solid tumors include modifying NK cells to express chemokine receptors like CCR7, which can facilitate their infiltration into tumor tissues. These strategies are being explored in preclinical studies with a planned evaluation of efficacy in upcoming clinical trials.[72,123]

One such strategy involves using electroporation to introduce mRNA that encodes the chemokine receptor CCR7 into NK cells. This enhances their ability to migrate toward lymph nodes that express the chemokine CCL19. Nevertheless, the efficacy of these methods necessitates additional validation through clinical trials.[124]

Furthermore, ensuring the controlled in vivo persistence of CAR-NK cells is crucial for maintaining their antitumor efficacy. It is essential to strike the balance between promoting expansion and persistence of CAR-NK cells while preventing uncontrolled proliferation.[125] NK cells normally survive for only approximately 2 weeks after infusion, in contrast to T cells, which can develop into long-lived memory cells.[76]

To improve the longevity of NK cells, externally administered cytokines have been employed to augment the proliferation and persistence of adoptive NK cells.[126] However, this approach may result in undesirable side effects, such as the proliferation of suppressive immune subsets like Tregs. Moreover, there is a potential for host T cells to reject allogeneic NK cells, which is a crucial factor to consider in cell therapy involving allogeneic NK cells. Additional investigation is required to examine the role of transmembrane-bound IL-15 in enhancing the long-term presence of NK cells.[127]

Tumor escape is another challenge that affects the cytotoxicity and persistence of CAR-T and CAR-NKs in solid tumors. Additionally, the immune evasion mechanisms of tumors, such as the secretion of prostaglandin E2, which blocks $CD8^+$ cytotoxicity and reduces NK cell survival and activity, can hinder the persistence and effectiveness of CAR-T and CAR-NKs.[122,128]

An effective approach to addressing this challenge is the engineering of CAR-NKs that can mitigate certain immunosuppressive effects. Employing CRISPR-Cas9 technology to disable genes associated with NK cells can achieve this. Furthermore, employing genome editing to eliminate checkpoint components as a means of enhancing NK cell function is an alternative strategy to address the issue of NK cell exhaustion in the TME.[1]

Indeed, tumor-associated mechanisms that contribute to NK cell exhaustion involve the prolonged stimulation of NK cells by tumor cells, resulting in diminished effector functions and reduced NK cell infiltration into tissues.[129,130]

Moreover, the susceptibility of expanded NK cells to freeze-thaw cycles, which affects their cytotoxic activity and post-transfer viability, presents a significant challenge. Nevertheless, certain studies have demonstrated that the inclusion of IL-2 can partially restore the functionality of cryopreserved NK cells.[3] Cryopreservation protocols need to be optimized to preserve the integrity, viability, function, and potency of CAR-T and CAR-NK cells after thawing, particularly for future off-the-shelf production and cryopreservation.[122]

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Isolating, purifying, and expanding primary NK cells and achieving efficient transduction of CAR constructs are technical challenges in the manufacturing process of CAR-NKs. There is a need for optimized large-scale production methods under GMP conditions.[131]

Transduction of CAR-NK Cells and Delivery

In recent trials, the most commonly utilized vectors include lentivirus, retrovirus, and adeno-associated virus vectors. Engineering NK cells is more challenging than engineering T cells, due to their inherent antiviral capacity, higher sensitivity to apoptosis, and limited expansion potential.[132,133] While retroviral transduction is the most commonly used method for gene delivery into NK cells, it often results in a modest percentage of NK cells being successfully transduced. Alternative nonviral methods (electroporation, lipofection) provide transient expression of the CAR construct, which may not offer long-term effectiveness.[114] Furthermore, research has demonstrated the high efficiency of such techniques, including the adeno-associated virus for delivery of the CAR construct and its precise integration into the NK cell genome utilizing CRISPR-Cas9 technology.[114,134]

Viral transduction: Retroviruses are frequently used as viral vectors to introduce genes into NK cells. Retroviruses (lentiviruses, gammaretroviruses, and alpharetroviruses) cause permanent gene expression.[57] However, retroviral vectors have the ability to integrate into the genome, which can lead to risks such as cancer development and cellular transformation.[58] The effectiveness of lentiviral vectors in primary NK cells is limited because cell viability is often adversely affected.[135] Although lentiviral transduction has lower genotoxicity, its low efficiency requires multiple rounds of transduction, making the procedure costly and labor-intensive. Because of their better safety profiles, nonviral methods are becoming increasingly popular.[58]

Polybrene, RetroNectin, and Vectofusin-1 are transduction enhancers that increase the efficiency of lentiviral transduction.[57] Polybrene promotes viral entry, is frequently used to increase the transduction efficiency of mostly virally resistant NK cells. Retronectin functions by colocalizing with the virus on the cell surface. Since its initial discovery to significantly enhance lentiviral transduction of HSCs, vectofusin-1 has been utilized in the field of NK cells.[135] A baboon envelope-pseudotyped lentivirus (BaEV-LV) has demonstrated superior transduction efficiency. Additionally, stimulating NK cells with cytokines before transduction increases efficiency, facilitating viral uptake.[135] Müller et al[136] showed that a combination of Vectofusin-1 and RD114-TR–pseudotyped lentiviral vectors is an efficient method to transduce PB-derived NK cells to produce highly cytotoxic CD19-CAR NK cells.

Nonviral transduction: DNA or mRNA plasmid electroporation is a nonviral method for genetically modifying NK cells.[135] Although DNA electroporation has limited effectiveness, primary NK cells have demonstrated electroporation efficiencies ranging from 80% to 90% when mRNA-based plasmids are used in conjunction with prior NK cell activation.[137,138] These developments open the door to more accessible and individualized cancer treatments, potentially improving therapeutic outcomes in challenging cases such as solid tumors.[139] mRNA electroporation is a crucial approach to generate CAR-modified NK cells for adoptive immunotherapy, enabling effective CAR expression and targeted tumor recognition. This technique avoids genome integration and reduces the risk of insertional mutagenesis by temporarily permeabilizing cell membranes with an electric field to deliver CAR-encoding mRNA, providing a safer, nonviral alternative to viral vectors.[40]

The temporary expression that results from using electroporation to produce CAR expression is a disadvantage because stable integration of the construct into the DNA is not possible. The therapeutic window is significantly shortened since CAR constructs on primary NK cells can be expressed on the surface for only 3 to 5 days before this expression is lost. Therefore, it is not possible to produce a stable CAR-NK product using electroporation.[135] Since mRNA transfection does not require cell division, it is more effective than DNA transfection.[140] Unlike DNA transfection, mRNA transfection appears to be a faster, more efficient, and temporary method for producing CAR-NKs. Furthermore, it is anticipated that CAR mRNA-engineered NK cells will become a valuable therapeutic strategy for treating solid tumors. To enhance the cytolytic activity of NK cells, some researchers developed a CAR by fusing the extracellular domain of NKG2D to DAP12, demonstrating promising therapeutic potential for metastatic colorectal cancer.[139] Using the Current Good Manufacturing Practice–compliant mRNA electroporation approach, Carlsten and colleagues[141] effectively transfected NK cells,

causing rapid and reproducible transgenic expression in CAR-NKs without adversely affecting cytotoxic function, viability, or phenotype. Ionizable lipid nanoparticles are constantly used to transfer RNA into NK cells.[139]

However, compared to DNA, mRNA has a shorter half-life, which enhances safety but may also make nonviral gene editing more challenging.[140]

CRISPR-Cas9: Compared to other techniques such as transcription activator-like effector nucleases or zinc-finger nucleases, the discovery of the CRISPR-Cas9 system made targeted gene integration possible with high efficiency.[142] A novel method for precise genome editing has been made possible using CRISPR-associated nuclease 9 (CRISPR-Cas9) technology.[143] Two primary domains are utilized by the CRISPR-Cas9 system to create DNA alterations: the Cas9 enzyme and the guide RNA.[144] Targeted CAR insertion combined with gene knockout in NK cells creates new opportunities to enhance primary CAR-NK products. Finding the optimal CAR-NK product may be facilitated by focusing on key transcription factors involved in NK cell exhaustion, terminal differentiation, and clonal expansion, as observed in adaptive NK cells, as well as regulators of cell cycle progression and apoptosis.[135] Recent reports on successful CRISPR editing of CAR-NKs highlight them as a highly promising tool to address current challenges such as tumor escape mechanisms, therapy resistance, and difficult-to-treat solid tumors.[140]

Daher et al[145] used CRISPR-Cas9 technology to delete the *CISH* gene in CAR-NKs, enhancing their metabolic potential and antitumor efficacy. Additionally, exhausted NK cells in the TME have been treated using CRISPR-Cas9.[139] It has been shown that upregulating the expression of the NKG2D ligand using an engineered CRISPR-Cas9 system effectively enhances the immune response against pathogenic cells.[139] Furthermore, using CRISPR-Cas9 technology to disrupt inhibitory pathways and overcome immune checkpoint obstacles offers a novel approach to enhance NK cell effector functions. Blocking NK group 2A (NKG2A) may improve the effectiveness of NK cells in adoptive cell therapy.[146] Researchers effectively eliminated inhibitory signaling genes in NK cells, including *ADAM17* and *PD-1*, using CRISPR-Cas9. The findings demonstrated that these genetically modified NK cells exhibited increased cytotoxicity, cytokine production, and overall NK cell activity.[139] Knockout of the IL-15 cytokine-inducible Src homology 2 (SH2)–containing protein (CISH) enhanced the in vivo persistence and cytotoxic function of IL-15–secreting CAR-NKs, according to one of the first studies on further modified, virally transduced CAR-NKs using RNP-based CRISPR-Cas9 complexes.[140] In a recent study, Choi et al[147] employed CD70-directed CAR-NKs in conjunction with the CRISPR-Cas9 method to knock down *CD70* in NK cells. In addition to producing important cytokines, the CD70-eliminated CAR-NKs with enhanced IL-15 expression demonstrated strong cytotoxicity against several CD70-positive solid tumor cell lines. Shankar et al[148] disrupted the *KLRC1* gene, which encodes the HLA-E–binding NKG2A receptor, using CRISPR-Cas9 technology. Notably, they also employed CRISPR to introduce a GD2 (disialoganglioside)–targeting CAR into human blood-derived NK cells via a virus-free genome editing method, enhancing the safety of CAR-NKs. The modified KLRC1-GD2-CAR-NKs demonstrated strong viability, proliferation, and precise targeting of $GD2^{+}$ tumors. Additionally, they overcame HLA-E–mediated immunosuppression.

However, it has been documented that CRISPR-Cas9 genome editing can induce p53-mediated DNA damage responses and cause cell cycle arrest in human cells. Furthermore, it is important to consider the potential for preexisting humoral and cell-mediated adaptive immune responses to the CRISPR-Cas9 system in humans when using it for in vivo applications in clinical trials.[139]

Nevertheless, the drawbacks of increased immunogenicity, potential insertional mutagenesis, restricted insert size, elevated cost for GMP-grade viral production, and related regulatory complications have prompted the investigation of nonviral delivery routes as cost-effective alternatives.[132,149] The nonviral transposon platforms Sleeping Beauty and PiggyBac offer a safer method for integrating CAR designs, although their efficiency is lower.[122,132] The delivery of mRNA-based CAR designs, using either electroporation or lipid nanoparticles, results in high CAR expression efficiency. However, this expression is temporary and not long-lasting.[133,150] Scientists have also created nonviral gene editing technologies, such as CRISPR-Cas9, that allow for targeted integration of CAR and other beneficial genes, as well as the removal of negative regulators of immune cells, T-cell receptor (TCR), or MHC-related genes, respectively.[122,151]

In conclusion, although viral vectors are the most popular technique for introducing genes into human cells, they have several disadvantages, including challenging and costly production, batch-to-batch variability, high carcinogenic risk, immunogenicity, and low DNA packaging capacity. As a result, scientists are paying more attention to nonviral gene delivery methods. Overall, the challenges affecting the cytotoxicity and persistence of CAR-NK cells in solid tumors highlight the need for continued research and development to overcome these barriers and increase the efficacy of cell-based immunotherapies against solid tumors.

Comparative Analysis of CAR-NK and CAR-T Therapies

Advantages of NK Cells as a CAR Platform

CAR-NKs are capable of efficiently recognizing and targeting aberrant cells without the need for antigen-specific priming. Additionally, NK cells exhibit a lack of HLA-matching restriction, which is often a limitation in T cell–based therapies. This feature makes them a more universal therapeutic platform for CAR-based strategies.[114]

Furthermore, the safety profile of CAR-NK therapy is an advantage. CAR-NKs have been reported to have encephalopathy syndrome, minimal GVHD, and ICANS and fewer incidences of CRS, a common side effect associated with CAR-T therapy. CAR-NKs can also be produced from allogeneic sources, like healthy donor blood, and can be used as an "off-the-shelf" therapeutic product. This feature allows for more accessible and cost-effective production.[114]

CAR-NKs can discriminate between healthy and tumor cells. In contrast, CAR-Ts may target both malignant and nonmalignant cells with the targeted antigen, potentially leading to on-target, off-tumor toxicity, which is a concern in CAR-T therapy.[152]

In addition, the ability to generate CAR-NKs from diverse sources, particularly the possibility of creating a homogeneous and clinically scalable product from genetically modified human iPSCs, offers the advantage of providing an "off-the-shelf" CAR-NK therapy. This eliminates the need for personalized and patient-specific products, addressing a significant challenge in current CAR-T therapies.[19]

Previous studies have demonstrated the limitations and challenges of CAR-T cell therapy, such as difficulties in collecting normal T cells from patients, time-consuming manufacturing processes, and increased attention to gene editing. This has led to a shift in focus toward exploring CAR-NK therapy as a promising alternative. Umbilical cord blood is identified as a better source of NK cells due to its gene expression advantages, convenience of collection, and lower risk of GVHD. Additionally, NK cell lines, particularly NK-92 and KHYG-1 cell lines, are highlighted as potential sources for rapid and abundant NK cells for immunotherapy. Notably, NK-92 cells have been applied in clinical trials and have shown promising results in the treatment of solid tumors.[77,153]

In addition, another benefit of CAR-NK therapy is its potential to reduce antigen loss relapse. Antigen loss is a recognized mechanism of cancer relapse and immune escape following CAR-T therapy. In contrast, CAR-NKs can recognize cancer cells through multiple mechanisms, reducing reliance on a single antigen.[152,154,155]

The predominant adverse effects associated with CAR-NK therapy encompass fever, fatigue, and anorexia. To effectively implement CAR-NK therapy in clinical settings, additional research is required to validate the fundamental experimental findings across various solid tumors and provide tailored recommendations for distinct TMEs within the human body.[153]

The activity of CAR-NKs against solid tumors has been the subject of an increasing number of in vitro and in vivo studies. The most extensively investigated solid tumors to evaluate their therapeutic potential are glioblastoma, breast cancer, and ovarian cancer. Although these cells offer unique advantages, there have been biological and technical challenges in translating CAR-NK therapy from hematologic malignancies to solid tumors. These challenges include, among others, issues with cell persistence, overcoming TME-mediated suppression, and maximizing transduction efficiency.[156] The mechanisms underlying CAR-NK efficacy in solid tumors, particularly in liver cancer, cholangiocarcinoma, and urological malignancies, remain poorly understood despite ongoing clinical trials.[156]

Compared to CAR-NKs, CAR-Ts have the following advantages: treatment centers have more expertise in the production and distribution processes of CAR-Ts, strong cytotoxicity, high circulating T-lymphocyte counts, and ease of freezing and storage. However, CAR-NKs have the following drawbacks: low circulating NK cell frequencies, high sensitivity to freezing and thawing,

dependence on cytokine support essential for their persistence, limited global manufacturing experience, and fatigue and exhaustion caused by suppressive cytokines and Tregs.[157] Therefore, when administered to immunocompetent patients, allogeneic TCRα chain CAR-T and CAR-NKs remain susceptible to a host-vs-graft response.[158]

NK cell expansion in vitro is more challenging because NK cells require cytokines and exhibit slower rates of proliferation. Robust expansion protocols have been developed for CAR-Ts; however, they are also time-consuming and require specific cytokines. Although CAR-NK therapy is still in the early stages of clinical application, CAR-T therapy has 6 FDA-approved treatments.[159] The design of CAR-NKs still has flaws compared to the relatively well-developed CAR-T therapy, particularly in the design of the activation domain, which is critical.[160]

CAR-NK vs CAR-T: Persistence, Transduction Efficiency, and Safety

Long-lasting responses are linked to the greater in vivo persistence of CAR-Ts, which often lasts for months or even years. Although this persistence can sometimes cause adverse effects, it is a key factor in their long-term effectiveness. In vivo persistence of CAR-NKs is usually shorter, lasting days to weeks. This problem is exacerbated by immunological rejection, limited expansion, and replicative senescence. Nonetheless, in preclinical and clinical research, engineering techniques have enhanced CAR-NK persistence. In contrast to CAR-T, persistence is still a major drawback for CAR-NK despite these developments. Reprogramming CAR-NKs to acquire memory or memory-like characteristics is actively being investigated with the goal of achieving long-term tumor control. This approach may help overcome the limitations related to NK cell persistence in CAR-NK therapy.[161]

In terms of safety profiles, CAR-T cells may present issues with off-tumor toxicity, which could limit their use in solid tumors. CAR-NKs, on the other hand, may offer a safer treatment alternative because they are designed to retain high cytotoxic capability even in the presence of immunosuppressive substances commonly found in solid tumors. Crucially, CAR-NKs are less likely to induce neurotoxicity compared to CAR-T cells.[162]

Strong CAR expression and product consistency are achieved through the high transduction efficiency of CAR-Ts using common viral vectors (lentiviral or retroviral). The low viral transduction efficiency and subsequent apoptosis of NK cells are likely caused by their inherent immune characteristics.[163] CAR-NKs are inherently more difficult to transduce, showing lower efficiency and greater variability depending on the transduction method and source. Although CAR-NK transduction rates have recently improved due to the introduction of specific pseudotyped viral vectors and enhancers such as Vectofusin-1, they remain lower and less consistent than those in CAR-Ts. This technical challenge affects the scalability and consistency of CAR-NK products.[161,164,165] These gaps are being narrowed by ongoing advances, but each platform has unique advantages and limitations for clinical use.

CAR-NK Therapy in Solid Tumors: Glioblastoma, Ovarian, and Pancreatic Cancers at the Forefront

Glioblastoma: There are currently no effective treatments for glioblastoma (GB).[166] To effectively control GBM with immunotherapy, it is important to identify tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) as targets. The epidermal growth factor receptor variant III (EGFRvIII), CD133, interleukin-13 receptor α subunit 2 (IL-13Rα2), and melanoma antigen-encoding genes (MAGE)-1 and -3 are among the TAAs and TSAs associated with GBM.[162] The safety profile, off-the-shelf availability, and ease of production of CAR-engineered NK cells are advantages in treating GBM. Improved outcomes in GBM tumor treatment may result from further research and advancements in engineering CAR-NKs with memory-like characteristics and enhanced durability.[162]

Since the antitumor activity of NK cells is not solely dependent on CAR function, their diverse oncolytic mechanisms present a highly promising approach for treating GBM, which can exhibit highly heterogeneous cellular composition even within a single tumor.[162] CAR-NKs offer a safe treatment alternative because they are designed to retain high cytotoxic activity even in the presence of immunosuppressive substances commonly found in solid tumors. Since CAR-NK cells do not face the obstacle of GVHD, they offer a potentially much more practical method for treating GBM.[162]

Clinical trials have shown that CAR-NKs can effectively target residual tumor cells, which may undergo phenotypic changes over time—a challenge faced by CAR-T cells.[162] To improve binding to gliomas

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during adoptive transfer, EGFRvIII-targeted CAR-NKs overexpressing CXCR4 receptors were created. These EGFRvIII-targeted CAR-NKs successfully eliminated EGFRvIII-positive human glioblastoma cell lines. In a mouse model of GBM, significant inhibition of tumor growth and extended survival were observed.[167]

In 9 patients with recurrent GBM, a clinical trial demonstrates the safety and feasibility of intracranial injections of HER2-CAR NK cells. The CAR construct also targets ROBO-1, MUC1, PD-1, FRα, and DR4/5. At doses up to 1×10^8, the CAR2BRAIN study reports very few side effects. None of the patients developed ICANS or CRS, and the therapy did not show dose-dependent toxicity. Notably, the degree of $CD8^+$ T-cell infiltration in the tumor tissue before treatment correlates with patient outcomes. The median overall survival for all patients is 31 weeks, while the median progression-free survival is 7 weeks.[168] The goal of Ma et al's study on the glioma mouse model is to examine the impact of combination therapy using an IL-15/IL-15R–producing oncolytic virus and second-generation EGFR-CAR NK cells with a CD28 costimulatory domain. According to previous research with endogenous NK cells in animal models, the oncolytic virus can enhance the intracranial infiltration of EGFR-CAR NK cells when administered intravenously. Compared to the saline group, OV-IL15C combined with EGFR-CAR NK cells demonstrated the greatest reduction in tumor volume, along with increased intracranial infiltration and activation of NK and $CD8^+$ T cells.[169,170]

The feasibility and safety of HER2-targeted CAR-NKs have been demonstrated in early trials, but treatment durability remains a challenge, as evidenced by inconsistent results in patients with recurrent GBM. Combinatorial strategies will likely be necessary to overcome these obstacles. These developments are crucial to making CAR-NK therapy a practical treatment option for GBM, especially given the poor prognosis of this aggressive cancer.[167]

Pancreatic cancer: Treatment of advanced pancreatic cancer (PC) is significantly hindered by the unique TME of PC, characterized by dense connective tissue formation and high levels of immunosuppressive cell infiltration.[167] Allogeneic FRα-targeted CAR-NKs were engineered by researchers to express ligands that induce apoptosis. These modified CAR-NKs trigger significant tumor cell apoptosis by initiating DR4/5-mediated selective cell death in tumors positive for both FRα and DR4/5.[167] In pancreatic cancer models, adding CXCR2 to CAR-NKs enhanced tumor cell destruction and increased CAR-NK infiltration into tumor areas.[171]

PD-L1 and ROBO1 are important targets currently being studied in clinical trials for pancreatic tumors. Other targets include Claudin 18.2, TROP2, and MUC1—2 glycoproteins that are commonly overexpressed in solid tumors—as well as the stress-induced NKG2DL group ligands (MICA/B and ULBP1-6), which are natural NK cell ligands frequently overexpressed in a variety of malignancies.[99]

A CAR-NK therapy targeting PSCA was created using primary human NK cells obtained from umbilical cord blood. The PSCA-positive human pancreatic cancer cell line Capan-1 and the PSCA-negative human pancreatic cancer cell line PANC-1 were independently cocultured in vitro with these CAR-NKs and with unmodified NK cells. The results showed that the coculture of PSCA-CAR-NKs with Capan-1 cells led to significantly higher secretion of IFN-γ and a markedly lower survival rate of Capan-1 cells. Furthermore, in a pancreatic cancer mouse model implanted with Capan-1 cells, PSCA-CAR-NKs demonstrated exceptional therapeutic efficacy, resulting in a notable reduction in tumor growth without serious adverse side effects.[172] In the AsPC-1 cell line, MSLN-positive human pancreatic cancer cells were effectively eradicated by MSLN-CAR-NKs combined with a stimulator of interferon genes (STING) agonist. Further in vivo research demonstrated that, in the AsPC-1 mouse transplant model, MSLN-CAR-NKs dramatically inhibited tumor growth, resulting in prolonged survival.[173] An innovative method to boost anticancer immunity is the synergistic effect of STING agonists combined with MSLN-CAR-NKs; however, further research is needed to precisely modulate immune responses to avoid potential side effects.[167]

A study showed that in both 2D and 3D models, CD44v6-CAR-NK92 cells demonstrated significant cytotoxicity against glioblastoma cell lines. The findings indicated that CD44v6 is a promising target for immunotherapy in pancreatic cancer and exhibited notable efficacy without creating systemic toxicity across all models used. These results support earlier research suggesting that CAR-NKs offer a viable strategy to combat pancreatic cancer by enhancing the immune response and overcoming immunological barriers within the TME. The effectiveness of CD44v6-

CAR-NK92 cells in vivo was demonstrated by a decrease in tumor burden.[174]

Ovarian cancer: The process of producing CAR-expressing NK/ILC cells from CAR-transduced iPSCs was described in a recent study, which also demonstrated stable expression of CD45, CD7, and CAR. When these cells were exposed to tumor cells expressing GPC3, they produced IFN-γ and exhibited effective cytotoxicity. The survival of immunocompromised mice with GPC3-positive ovarian tumors was significantly increased by the CAR-NK/ILC cells, with no adverse effects observed.[175]

Zhang's group created MSLN-targeted CAR-NKs, which demonstrated strong and specific cytotoxicity against OVCAR-3 and SKOV3, 2 MSLN-positive human ovarian cancer cell lines. In animal models, these cells effectively halted the growth of intraperitoneal and subcutaneous ovarian tumors.[176] Additionally, CD24-positive ovarian cancer cell lines OVCAR-3 and SKOV3 were efficiently and specifically eliminated by engineered CD24-targeted CAR-NKs both in vitro and in vivo.[177] The presence of CD24 in normal epithelial tissues and hematopoietic cells raises concerns about potential side effects, necessitating further investigation in future preclinical and clinical trials, despite the limited knowledge of the on-target, off-tumor effects associated with the CD24 marker.[171] Additionally, FRα-targeted CAR-NKs demonstrated significant cytotoxicity against ovarian cancer cells. However, maintaining persistence and overcoming immunosuppressive microenvironments continue to be difficult tasks.[167] Researchers investigated the potential of using CAR-NK immunotherapy to target CLDN6 in ovarian cancer. The results showed that CAR-NKs targeting CLDN6 exhibit low cross-reactivity with normal cells and high selectivity for tumor cells.[178]

A natural killer–like T (NKT) cell line, known as universal NKT (UNKT), was used to develop a novel treatment strategy for solid tumors. Compared to both wild-type UNKT and MSLN-CAR-Ts, the CAR-modified UNKT cells demonstrated superior cytotoxicity against MSLN-positive ovarian cancer (OC) when cocultured with tumor cells expressing MSLN. Notably, in mouse models, CAR-UNKT cells showed strong efficacy against OC without inducing immunological memory responses.[179] In vitro, Jan discovered that HLA-G CAR-transduced NK cells exhibited strong cytolytic activity against ovarian, breast, brain, and pancreatic cancer cells. Additionally, in an orthotopic mouse model, these cells significantly reduced xenograft tumor growth, resulting in longer median survival. This strategy could benefit future CAR-NK treatment applications for various solid tumors.[80,180]

Deployment of CAR-NKs in Tumor Combination Therapy

The application strategy of CAR-NKs in tumor combination therapy entails combining CAR-NK therapy with other immunotherapy methods, radiation, and chemotherapy. This combination technique seeks to boost both the antitumor activity of CAR-NKs and the sensitivity of tumor cells, achieving a more effective therapeutic outcome[181] (Figure 5).

One approach is the combination of CAR-NK immunotherapy with lymphodepleting chemotherapy, which has been found to reduce tumor size by decreasing MDSCs and Tregs in the TME.[91,182] A study has developed modified CAR NK-92 cells that include nanoparticles coated with paclitaxel to transport the chemotherapeutic drug to the tumor and demonstrated encouraging antitumor outcomes.[183]

Immune checkpoint blockade therapies targeting inhibitory receptors (PD-1, TIM-3, LAG-3, and TIGIT) in combination with CAR modification of NK cells represent a potential strategy to activate the antitumor immune response of NK cells and correct the exhausted state of NK cells in the TME.[181,184] The great abundance of PD-1$^+$ NK cells in the ascites of ovarian cancer patients indicates that they may be stimulated or expanded inside the TME.[185] In recent years, studies have shown that NK cells, including the NK-92 cell line, can express PD-1, and this expression may be upregulated in response to the TME or cytokine stimulation. Moreover, blocking the PD-1/PD-L1 axis is shown to enhance the sensitivity of NK cells to tumor cells and increase their antitumor activity.[181,186–188] Research has indicated that incorporating an anti–PD-L1 antibody into the combination of macrophages and NK cells can effectively reduce the suppressive impact of macrophages on NK cell proliferation and ADCC.[189]

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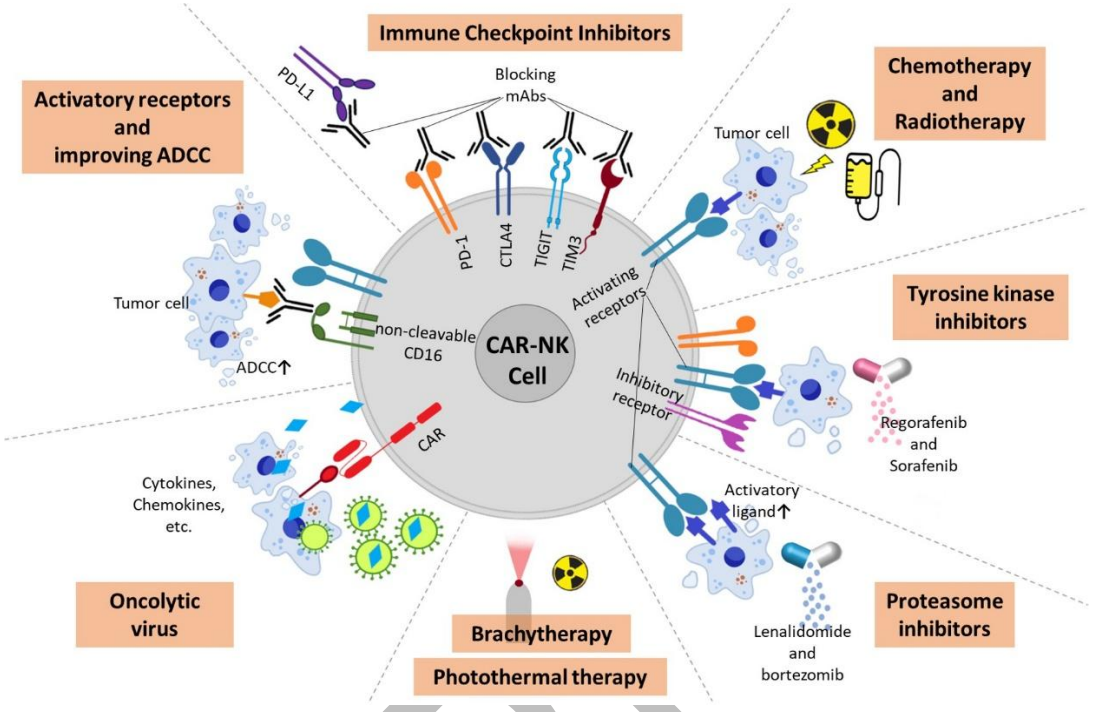

Figure 5. Utilization of CAR-NKs in the context of tumor combination therapy. The tumor microenvironment (TME) represents a significant obstacle to CAR-NK therapy, mostly because of many variables, such as the secretion of immunosuppressive components by inhibitory cells inside the TME. The primary focus in CAR-NK therapy is to address the immunosuppressive role of the tumor microenvironment (TME). This is crucial when considering combination therapies, including anti-PD-1/PD-L1, chemotherapy, kinase inhibitors, proteasome inhibitors, oncolytic virus, and photothermal therapy. These techniques can induce the release of tumor antigens and DAMPs, hence facilitating the activation, proliferation, and elimination of CAR-NKs. There are many molecules that can be targeted and classified as TSA (tumor-specific antigens), TAA (tumor-associated antigens).

In a mouse glioblastoma model, a preclinical study combining CAR-NKs targeting HER2 and anti–PD-1 checkpoint inhibitors demonstrated a synergistic antitumor effect, successfully treating advanced tumors resistant to NK-92/5.28.z monotherapy. Additionally, the combined treatment stimulated the immune system and generated a cytotoxic, rather than immunosuppressive, TME.[190] In patients with recurrent HER2-positive glioblastoma, the CAR2BRAIN study combines intravenous ezabenlimab with NK-92/5.28.z cells. No dose-limiting toxicities, CRS, or neurotoxicity were observed in this clinical trial. Additionally, higher levels of $CD8^+$ T-cell infiltration in tumors were associated with delayed disease progression.[168] The Liu et al group focused on a slightly different strategy, despite the promise of this therapy. They demonstrated that in a mouse model of nasopharyngeal carcinoma, CAR-NKs expressing anti–PD-L1 combined with the checkpoint inhibitor nivolumab induced tumor regression.[191] Additionally, pembrolizumab, which inhibits the PD-1 receptor, irradiated PD-L1 CAR-NKs, and N-803 are being used in a clinical trial targeting refractory gastric and head and neck cancers. This trial is currently in the recruitment phase.[192]

Combining CAR-NKs with radiotherapy and chemotherapy may enhance NK-cell trafficking, infiltration, and tumor recognition.[58] Notably, recent research has shown that the combination of CD44-CAR-NKs and cisplatin exhibits higher antitumor activity than sequential therapy.[193] Similarly, the combination of CAR-NK92 cells targeting CD133 and cisplatin has been found to have the strongest antitumor effect on ovarian cancer without affecting the cytotoxicity and viability of CAR-NKs.[194] Moreover, the synergistic effect of CAR-T/NK cells and radiotherapy in glioblastoma and pancreatic cancer has been established, underscoring the potential of combining radiotherapy, chemotherapy, and immunotherapy for a synergistic

antitumor effect.[181] Lin et al demonstrated that irradiation could effectively enhance the antitumor activity of CAR-NKs targeting glypican-3 (GPC3) on hepatocellular carcinoma cells in vivo. Notably, this effect was observed only with high-dose radiation (8 Gy).[195] However, further research is needed to determine the ideal dosage, duration, and sequence of this treatment combination, as well as to evaluate the potential of these strategies in other types of solid tumors and in preventing radiation-induced immunosuppression.[58]

Future Perspectives

The development of novel generations of CAR-NK cells, together with a deeper understanding of their behavior in solid tumors, could potentially revolutionize cancer cell therapy and improve patient outcomes in the future. Currently, MD Anderson clinical trials are utilizing clinical-grade Universal Antigen Presenting Cells to generate NK and CAR-NKs. This innovative method facilitates the expansion of highly pure GMP-grade CAR-NK cells in quantities suitable for adoptive CAR-NK-based cancer immunotherapy.[196]

In future investigations, it may be feasible to utilize SNAP-CAR NK cells as a potent adapter method for precisely directing modified cells to various antigens by covalent chemistry. Additionally, the introduction of synNotch CAR could serve as an innovative method to reduce off-target adverse effects and enhance the safety of CAR-NK therapy, making it suitable for future clinical and preclinical investigations.[74]

Improving the proliferation and persistence of NK cells in vivo is a crucial area of current research necessary to enhance the therapeutic efficacy of CAR-NKs.[76] To address their short lifespan, adoptive cell therapy cells have been engineered to produce cytokines that enhance their proliferation and persistence in vivo. Another approach to improving the durability of immune cell–based therapies is to infuse memory cells. However, since memory NK populations have only recently been identified, incorporating their characteristics represents a novel strategy for enhancing the durability of CAR-NK treatments.[159]

The versatility of g-NK cells suggests potential applications beyond hematological malignancies. Their compatibility with various therapeutic antibodies also opens up treatment possibilities for solid tumors.[159]

NK cell receptor engineering holds great promise for reprogramming immune responses to cancer cells. A strategy to boost the efficacy of NK cell therapy is to combine engineered targets. In addition to multiple engineering methods, artificial intelligence can help develop combination treatments with conventional treatments or other immunotherapies by predicting patient responses. Importantly, safety issues remain intrinsically related to engineered NK cell therapies, such as off-target activation.[197] To prevent harmful effects on healthy tissue and minimize potential side effects, it is crucial to ensure the specificity of NK cell therapy. In addition, to refine NK cell activation and selectively target cancer cells, tightly tuned logic-gated circuits should be developed.[197] Moreover, further research should be done to investigate the computational models to predict and dynamically adjust cell responses. The frequent use of integrative vectors to modify NK cells also comes with the risk of insertional mutagenesis, which may lead to NK cell lymphomas. The current FDA recommendation is to have a vector copy number less than 5.[197,198] Quality control standards must be defined to check the engineering efficacy, purity, phenotype and tumorigenicity of NK cells. Indeed, depending on the donor, the source and the culture conditions, NK cell subsets can vary.[199] For these latter points, new techniques to expand, modify and cryopreserve primary NK cells ex vivo would be extremely useful for scaling up production for clinical application, as high doses and multiple injections are required.[197,200] The creation of “off-the-shelf” CAR-NK products, along with advancements in cryopreservation methods, could significantly reduce treatment costs and improve patient accessibility.[139] The infrastructures developed for the production of cell and viral vectors, as well as the tools available to engineer cells (among which in vivo editing strategies), are expected to synergistically improve NK cell therapy.

The commitment to achieving the therapeutic potential of CAR-NKs is demonstrated by ongoing clinical trials targeting different antigens. This stage is crucial for transforming the landscape of cancer treatment.[201] In navigating the future of cancer immunotherapy, the complex processes involved in activating NK cells and the ability of CAR-NKs to recognize various tumor antigens shed light on the extensive research needed to effectively incorporate them into mainstream oncology. These cellular treatments have the potential to revolutionize precision immunotherapy, offering patients with difficult cancers renewed hope and improved outcomes as they transition from scientific promise to clinical reality. Furthermore,

integrating immune checkpoint inhibition and targeting the TME may offer a novel approach to CAR-NK–based immunotherapy. Amidst this changing environment, NK cells and CAR-NKs emerge as highly promising entities, leading us toward a future where cancer treatment transcends mere combat and becomes a tailored, precise, and efficacious therapeutic approach.

Further research is still needed to determine the long-term durability, viability, and potency of these off-the-shelf products after freeze-thaw cycles. These studies will help in understanding the long-term effects of CAR-NK therapy, including any potential adverse effects.[76]

The successful transition of CAR-NK therapy from preclinical development to large-scale clinical use relies on overcoming key barriers in translation and manufacturing. Standardizing large-scale expansion protocols, ensuring batch-to-batch consistency, and maintaining GMP compliance are essential for achieving consistent clinical results. Automating NK cell culture systems, integrating closed bioreactors, and developing cryostable, off-the-shelf products will be vital for industrial scaling. Creating harmonized regulatory frameworks and cost-effective production pipelines will further support clinical translation and global access. Future progress depends on multidisciplinary collaborations among academia, industry, and regulatory agencies to ensure CAR-NK therapies move from promising experimental tools to widely accessible, effective cancer treatments.

DISCUSSION

CARs have been introduced into NK cells, making them selective and effective in targeting and cytotoxicity. The absence of observed adverse effects such as GVHD and CRS, commonly associated with CAR-T therapy, further underscores the therapeutic potential of CAR-NK therapy. However, there are challenges associated with the development and implementation of CAR-NK therapy. These include the difficulty in transducing NK cells, the need for efficient in vitro expansion and sustaining shorter in vivo persistence compared to T cells, and the potential biohazard arising from the use of animal-derived products. Efforts are being directed toward addressing these challenges and developing safe, effective, and scalable protocols for obtaining CAR-NKs. Current research and clinical trials are focused on evaluating the biological properties of CAR-NKs, optimizing CAR compositions, and developing methodologies for the preparation of CAR-NKs. Importantly, safety considerations related to genetic engineering are also being thoroughly investigated. The aim is to ensure that CAR-NK therapy can be applied effectively in clinical settings with minimal risk to patients. Notably, various clinical trials are underway to assess the efficacy of CAR-NK therapy in treating different types of cancer, including hematological malignancies and solid tumors. Additionally, the use of CAR-NKs in combination with other immunotherapies is being investigated to further enhance their therapeutic potential. This includes efforts to optimize the recovery of CAR-NKs after freezing, simplify the manufacturing process, and enhance the potency and persistence of CAR-NKs. Strategies to reprogram CAR-NKs to overcome TME-mediated suppression and escape, as well as developing recombinant CAR-NKs with memory properties, are also being explored.[202]

The ability of NK cells to undergo ADCC, a process in which they selectively attack cells that are covered with antibodies, thereby enhancing their effectiveness, has led researchers to investigate advanced techniques for gene editing to enhance their durability, ability to kill cells, movement within the body, and capacity to combat the immune-suppressing environment seen in tumors. Current clinical trials are assessing the effectiveness of quadruple-engineered NK cells that are produced from iPSCs.[203,204]

In summary, the research progress in clinical trials and innovative strategies for reprogramming NK cells reflects the substantial advancements in the field of cellular immunotherapy. In conclusion, the ongoing investigations and clinical trials demonstrate the potential of NK cells as a promising alternative for cancer therapy, which continues to expand the scope of treatment options for a broad range of malignancies. The potential of NK cell–based therapies, especially in combination with innovative strategies and advanced gene editing technologies, signifies the evolving landscape of precision cancer treatment.[205]

There are currently no FDA-approved medications for CAR-NK treatments, but several are being tested in clinical trials. Their importance in cancer immunotherapy is increasing as the number of clinical trials grows. Nevertheless, more clinical trials are needed to optimize CAR-NK treatment for clinical use, despite encouraging results—particularly since phase 3 and 4 trials are not yet available.[58]

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

No new data were created. Data sharing is not applicable to this article.

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REFERENCES

1. Zhang B, Yang M, Zhang W, Liu N, Wang D, Jing L, et al. Chimeric antigen receptor-based natural killer cell immunotherapy in cancer: from bench to bedside. Cell death & disease. 2024;15(1):50.
2. Stoiber S, Cadilha BL, Benmebarek MR, Lesch S, Endres S, Kobold S. Limitations in the Design of Chimeric Antigen Receptors for Cancer Therapy. Cells. 2019;8(5).
3. Wang W, Jiang J, Wu C. CAR-NK for tumor immunotherapy: Clinical transformation and future prospects. Cancer letters. 2020;472:175-80.
4. Cózar B, Greppi M, Carpentier S, Narni-Mancinelli E, Chiossone L, Vivier E. Tumor-Infiltrating Natural Killer Cells. Cancer discovery. 2021;11(1):34-44.
5. Gauthier L, Morel A, Anceriz N, Rossi B, Blanchard-Alvarez A, Grondin G, et al. Multifunctional Natural Killer Cell Engagers Targeting NKp46 Trigger Protective Tumor Immunity. Cell. 2019;177(7):1701-13.e16.
6. Ran GH, Lin YQ, Tian L, Zhang T, Yan DM, Yu JH, et al. Natural killer cell homing and trafficking in tissues and tumors: from biology to application. Signal transduction and targeted therapy. 2022;7(1):205.
7. Gong Y, Klein Wolterink RGJ, Wang J, Bos GMJ, Germeraad WTV. Chimeric antigen receptor natural killer (CAR-NK) cell design and engineering for cancer therapy. Journal of hematology & oncology. 2021;14(1):73.
8. Zhang Y, Zhou W, Yang J, Yang J, Wang W. Chimeric antigen receptor engineered natural killer cells for cancer therapy. Experimental Hematology & Oncology. 2023;12(1):70.
9. Zhang Y, Zhou W, Yang J, Yang J, Wang W. Chimeric antigen receptor engineered natural killer cells for cancer therapy. Exp Hematol Oncol. 2023;12(1):70.
10. Khawar MB, Sun H. CAR-NK Cells: From Natural Basis to Design for Kill. Frontiers in immunology. 2021;12:707542.
11. Maalej KM, Merhi M, Inchakalody VP, Mestiri S, Alam M, Maccalli C, et al. CAR-cell therapy in the era of solid tumor treatment: current challenges and emerging therapeutic advances. Molecular Cancer. 2023;22(1):20.
12. Lin M, Luo H, Liang S, Chen J, Liu A, Niu L, et al. Pembrolizumab plus allogeneic NK cells in advanced non-small cell lung cancer patients. J Clin Invest. 2020;130(5):2560-9.
13. Khawar MB, Sun H. CAR-NK cells: from natural basis to design for kill. Frontiers in immunology. 2021;12:707542.
14. Sun C, Sun H-y, Xiao W-h, Zhang C, Tian Z-g. Natural killer cell dysfunction in hepatocellular carcinoma and NK cell-based immunotherapy. Acta Pharmacologica Sinica. 2015;36(10):1191-9.
15. Sun C, Sun H, Zhang C, Tian Z. NK cell receptor imbalance and NK cell dysfunction in HBV infection and hepatocellular carcinoma. Cellular & molecular immunology. 2015;12(3):292-302.
16. Lanier LL. NK cell recognition. Annu Rev Immunol. 2005;23:225-74.
17. Zitti B, Bryceson YT. Natural killer cells in inflammation and autoimmunity. Cytokine & Growth Factor Reviews. 2018;42:37-46.
18. Wu J, Mishra HK, Walcheck B. Role of ADAM17 as a regulatory checkpoint of CD16A in NK cells and as a potential target for cancer immunotherapy. Journal of leukocyte biology. 2019;105(6):1297-303.
19. Xie G, Dong H, Liang Y, Ham JD, Rizwan R, Chen J. CAR-NK cells: A promising cellular immunotherapy for cancer. EBioMedicine. 2020;59:102975.
20. Lanier LL, Corliss BC, Wu J, Leong C, Phillips JH. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. Nature. 1998;391(6668):703-7.
21. Billadeau DD, Upshaw JL, Schoon RA, Dick CJ, Leibson

PJ. NKG2D-DAP10 triggers human NK cell-mediated killing via a Syk-independent regulatory pathway. Nature immunology. 2003;4(6):557-64.
22. Kumar S. Natural killer cell cytotoxicity and its regulation by inhibitory receptors. Immunology. 2018;154(3):383-93.
23. Eitler J, Rackwitz W, Wotschel N, Gudipati V, Murali Shankar N, Sidorenkova A, et al. CAR-mediated targeting of NK cells overcomes tumor immune escape caused by ICAM-1 downregulation. J Immunother Cancer. 2024;12(2).
24. Imai C, Iwamoto S, Campana D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. Blood. 2005;106(1):376-83.
25. Fujisaki H, Kakuda H, Shimasaki N, Imai C, Ma J, Lockey T, et al. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer research. 2009;69(9):4010-7.
26. Fujisaki H, Kakuda H, Imai C, Mullighan CG, Campana D. Replicative potential of human natural killer cells. Br J Haematol. 2009;145(5):606-13.
27. Romee R, Schneider SE, Leong JW, Chase JM, Keppel CR, Sullivan RP, et al. Cytokine activation induces human memory-like NK cells. Blood, The Journal of the American Society of Hematology. 2012;120(24):4751-60.
28. Romee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. Science translational medicine. 2016;8(357):357ra123-357ra123.
29. Han J, Zhang B, Zheng S, Jiang Y, Zhang X, Mao K. The Progress and Prospects of Immune Cell Therapy for the Treatment of Cancer. Cell Transplantation. 2024;33:09636897241231892.
30. Jørgensen LV, Christensen EB, Barnkob MB, Barington T. The clinical landscape of CAR NK cells. Exp Hematol Oncol. 2025;14(1).
31. Raggi G, Roldan N, Micallef V, Rapet A, De Maddalena L, Imler T, et al. Interleukin-2–induced vascular leak syndrome: clinically relevant in vitro recapitulation with a patient-derived lung-on-chip. Eur Respiratory Soc; 2020.
32. Sivori S, Meazza R, Quintarelli C, Carlomagno S, Della Chiesa M, Falco M, et al. NK cell-based immunotherapy for hematological malignancies. Journal of Clinical Medicine. 2019;8(10):1702.
33. Liu E, Ang SO, Kerbauy L, Basar R, Li L, Tong Y, et al. GMP-compliant universal antigen presenting cells (uAPC) promote the metabolic fitness and antitumor activity of armored cord blood CAR-NK cells. Frontiers in immunology. 2021;12:626098.
34. Farc O, Cristea V. An overview of the tumor microenvironment, from cells to complex networks (Review). Exp Ther Med. 2021;21(1):96.
35. Trédan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. Journal of the National Cancer Institute. 2007;99(19):1441-54.
36. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. Signal transduction and targeted therapy. 2021;6(1):263.
37. de Visser KE, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. Cancer Cell. 2023;41(3):374-403.
38. Burga RA, Thorn M, Point GR, Guha P, Nguyen CT, Licata LA, et al. Liver myeloid-derived suppressor cells expand in response to liver metastases in mice and inhibit the anti-tumor efficacy of anti-CEA CAR-T. Cancer Immunology, Immunotherapy. 2015;64:817-29.
39. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. The Journal of clinical investigation. 2015;125(9):3356-64.
40. Balkhi S, Zuccolotto G, Di Spirito A, Rosato A, Mortara L. CAR-NK cell therapy: promise and challenges in solid tumors. Front Immunol. 2025;16:1574742.
41. Hofer F, Di Sario G, Musiu C, Sartoris S, De Sanctis F, Ugel S. A Complex Metabolic Network Confers Immunosuppressive Functions to Myeloid-Derived Suppressor Cells (MDSCs) within the Tumour Microenvironment. Cells. 2021;10(10).
42. Hou A, Hou K, Huang Q, Lei Y, Chen W. Targeting Myeloid-Derived Suppressor Cell, a Promising Strategy to Overcome Resistance to Immune Checkpoint Inhibitors. Front Immunol. 2020;11:783.
43. Schmidt J, Ryschich E, Maksan S-M, Werner J, Gebhard MM, Herfarth C, et al. Reduced basal and stimulated leukocyte adherence in tumor endothelium of experimental pancreatic cancer. International journal of pancreatology. 1999;26:173-9.
44. Schmidt J, Ryschich E, Daniel V, Herzog L, Werner J, Herfarth C, et al. Vascular structure and microcirculation of experimental pancreatic carcinoma in rats. European Journal of Surgery. 2000;166(4):328-35.
45. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. Nature Reviews Cancer. 2016;16(1):7-19.

46. La Fleur L, Botling J, He F, Pelicano C, Zhou C, He C, et al. Targeting MARCO and IL37R on immunosuppressive macrophages in lung cancer blocks regulatory T cells and supports cytotoxic lymphocyte function. Cancer Research. 2021;81(4):956-67.
47. Dasgupta S, Bhattacharya-Chatterjee M, O'Malley BW, Chatterjee SK. Inhibition of NK cell activity through TGF-β1 by down-regulation of NKG2D in a murine model of head and neck cancer. The Journal of Immunology. 2005;175(8):5541-50.
48. Lazarova M, Steinle A. Impairment of NKG2D-mediated tumor immunity by TGF-β. Frontiers in immunology. 2019;10:487971.
49. Kim MJ, Ha S-J. Differential role of PD-1 expressed by various immune and tumor cells in the tumor immune microenvironment: expression, function, therapeutic efficacy, and resistance to cancer immunotherapy. Frontiers in Cell and Developmental Biology. 2021;9:767466.
50. Sun H, Sun C. The rise of NK cell checkpoints as promising therapeutic targets in cancer immunotherapy. Frontiers in immunology. 2019;10:484823.
51. Kwantwi LB, Wang S, Zhang W, Peng W, Cai Z, Sheng Y, et al. Tumor-associated neutrophils activated by tumor-derived CCL20 (C-C motif chemokine ligand 20) promote T cell immunosuppression via programmed death-ligand 1 (PD-L1) in breast cancer. Bioengineered. 2021;12(1):6996-7006.
52. Sun R, Xiong Y, Liu H, Gao C, Su L, Weng J, et al. Tumor-associated neutrophils suppress antitumor immunity of NK cells through the PD-L1/PD-1 axis. Transl Oncol. 2020;13(10):100825.
53. Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. Nature reviews Drug discovery. 2022;21(11):799-820.
54. Monteran L, Erez N. The dark side of fibroblasts: cancer-associated fibroblasts as mediators of immunosuppression in the tumor microenvironment. Frontiers in immunology. 2019;10:475160.
55. Camp JT, Elloumi F, Roman-Perez E, Rein J, Stewart DA, Harrell JC, et al. Interactions with fibroblasts are distinct in Basal-like and luminal breast cancers. Mol Cancer Res. 2011;9(1):3-13.
56. Li T, Niu M, Zhang W, Zhou J, Yi M. CAR-NK cells for cancer immunotherapy: recent advances and future directions. Frontiers in Immunology. 2024;15:1361194.
57. Dehghan F, Metanat Y, Askarizadeh M, Ahmadi E, Moradi V. Novel gene manipulation approaches to unlock the existing bottlenecks of CAR-NK cell therapy. Front Cell Dev Biol. 2024;12:1511931.
58. Doeppner CA, Binder AK, Bremm F, Feuchter N, Dörrie J, Schaft N. Rendering NK Cells Antigen-Specific for the Therapy of Solid Tumours. Int J Mol Sci. 2025;26(13).
59. Daher M, Basar R, Shaim H, Gokdemir E, Uprety N, Kontoyiannis A, et al. The TGF-β/SMAD signaling pathway as a mediator of NK cell dysfunction and immune evasion in myelodysplastic syndrome. Blood. 2017;130:53.
60. Yvon ES, Burga R, Powell A, Cruz CR, Fernandes R, Barese C, et al. Cord blood natural killer cells expressing a dominant negative TGF-β receptor: Implications for adoptive immunotherapy for glioblastoma. Cytotherapy. 2017;19(3):408-18.
61. Wang Z, Guo L, Song Y, Zhang Y, Lin D, Hu B, et al. Augmented anti-tumor activity of NK-92 cells expressing chimeric receptors of TGF-βR II and NKG2D. Cancer Immunol Immunother. 2017;66(4):537-48.
62. Oh M-S, Seo M, Yoon S, Jang M. 292 Unleashing CAR-NK cells against solid tumors by disrupting TGF-β signaling in the tumor microenvironment. Regular and Young Investigator Award Abstracts. 2023.
63. Shin SH, Lee YE, Yoon HN, Yuk CM, An JY, Seo M, et al. An innovative strategy harnessing self-activating CAR-NK cells to mitigate TGF-β1-driven immune suppression. Biomaterials. 2025;314:122888.
64. Chaudhry K, Geiger A, Dowlati E, Lang H, Sohai DK, Hwang EI, et al. Co-transducing B7H3 CAR-NK cells with the DNR preserves their cytolytic function against GBM in the presence of exogenous TGF-β. Molecular therapy Methods & clinical development. 2022;27:415-30.
65. Young A, Ngiow SF, Gao Y, Patch AM, Barkauskas DS, Messaoudene M, et al. A2AR Adenosine Signaling Suppresses Natural Killer Cell Maturation in the Tumor Microenvironment. Cancer Res. 2018;78(4):1003-16.
66. Duan J, Zhao S, Duan Y, Sun D, Zhang G, Yu D, et al. Mno(x) Nanoenzyme Armed CAR-NK Cells Enhance Solid Tumor Immunotherapy by Alleviating the Immunosuppressive Microenvironment. Adv Healthc Mater. 2024;13(11):e2303963.
67. Solocinski K, Padget MR, Fabian KP, Wolfson B, Cecchi F, Hembrough T, et al. Overcoming hypoxia-induced functional suppression of NK cells. J Immunother Cancer. 2020;8(1).
68. Chambers AM, Lupo KB, Wang J, Cao J, Utturkar S, Lanman N, et al. Engineered natural killer cells impede the immunometabolic CD73-adenosine axis in solid tumors. Elife. 2022;11.

69. Cózar B, Greppi M, Carpentier S, Narni-Mancinelli E, Chiossone L, Vivier E. Tumor-infiltrating natural killer cells. Cancer discovery. 2021;11(1):34-44.
70. Wennerberg E, Kremer V, Childs R, Lundqvist A. CXCL10-induced migration of adoptively transferred human natural killer cells toward solid tumors causes regression of tumor growth in vivo. Cancer Immunol Immunother. 2015;64(2):225-35.
71. Lee J, Kang TH, Yoo W, Choi H, Jo S, Kong K, et al. An Antibody Designed to Improve Adoptive NK-Cell Therapy Inhibits Pancreatic Cancer Progression in a Murine Model. Cancer Immunol Res. 2019;7(2):219-29.
72. Müller N, Michen S, Tietze S, Töpfer K, Schulte A, Lamszus K, et al. Engineering NK Cells Modified With an EGFRvIII-specific Chimeric Antigen Receptor to Overexpress CXCR4 Improves Immunotherapy of CXCL12/SDF-1α-secreting Glioblastoma. Journal of immunotherapy (Hagerstown, Md : 1997). 2015;38(5):197-210.
73. Jamali A, Hadjati J, Madjd Z, Mirzaei HR, Thalheimer FB, Agarwal S, et al. Highly Efficient Generation of Transgenically Augmented CAR NK Cells Overexpressing CXCR4. Front Immunol. 2020;11:2028.
74. Elahi R, Heidary AH, Hadiloo K, Esmaeilzadeh A. Chimeric Antigen Receptor-Engineered Natural Killer (CAR NK) Cells in Cancer Treatment; Recent Advances and Future Prospects. Stem cell reviews and reports. 2021;17(6):2081-106.
75. Rana S, Thomas L, Kirkham AM, Shorr R, Visram A, Maganti H, et al. CAR-NK cells to treat patients with cancer: a systematic scoping review of published studies and registered clinical trials. Cytotherapy. 2025.
76. Balkhi S, Zuccolotto G, Di Spirito A, Rosato A, Mortara L. CAR-NK cell therapy: promise and challenges in solid tumors. Front Immunol. 2025;16.
77. Li H, Song W, Li Z, Zhang M. Preclinical and clinical studies of CAR-NK-cell therapies for malignancies. Frontiers in immunology. 2022;13:992232.
78. Ng YY, Du Z, Zhang X, Chng WJ, Wang S. CXCR4 and anti-BCMA CAR co-modified natural killer cells suppress multiple myeloma progression in a xenograft mouse model. Cancer gene therapy. 2022;29(5):475-83.
79. Abbott RC, Verdon DJ, Gracey FM, Hughes-Parry HE, Iliopoulos M, Watson KA, et al. Novel high-affinity EGFRvIII-specific chimeric antigen receptor T cells effectively eliminate human glioblastoma. Clin Transl Immunology. 2021;10(5):e1283.
80. Jan CI, Huang SW, Canoll P, Bruce JN, Lin YC, Pan CM, et al. Targeting human leukocyte antigen G with chimeric antigen receptors of natural killer cells convert immunosuppression to ablate solid tumors. J Immunother Cancer. 2021;9(10).
81. Murakami T, Nakazawa T, Natsume A, Nishimura F, Nakamura M, Matsuda R, et al. Novel Human NK Cell Line Carrying CAR Targeting EGFRvIII Induces Antitumor Effects in Glioblastoma Cells. Anticancer research. 2018;38(9):5049-56.
82. Liu Y, Zhou Y, Huang KH, Fang X, Li Y, Wang F, et al. Targeting epidermal growth factor-overexpressing triple-negative breast cancer by natural killer cells expressing a specific chimeric antigen receptor. Cell proliferation. 2020;53(8):e12858.
83. Xu M, Wang Y, Xia R, Wei Y, Wei X. Role of the CCL2-CCR2 signalling axis in cancer: Mechanisms and therapeutic targeting. Cell proliferation. 2021;54(10):e13115.
84. Zhang C, Burger MC, Jennewein L, Genßler S, Schönfeld K, Zeiner P, et al. ErbB2/HER2-Specific NK Cells for Targeted Therapy of Glioblastoma. Journal of the National Cancer Institute. 2016;108(5).
85. Wrona E, Borowiec M, Potemski P. CAR-NK Cells in the Treatment of Solid Tumors. International journal of molecular sciences. 2021;22(11).
86. Geller MA, Cooley S, Judson PL, Ghebre R, Carson LF, Argenta PA, et al. A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer. Cytotherapy. 2011;13(1):98-107.
87. Yang Y, Lim O, Kim TM, Ahn YO, Choi H, Chung H, et al. Phase I Study of Random Healthy Donor-Derived Allogeneic Natural Killer Cell Therapy in Patients with Malignant Lymphoma or Advanced Solid Tumors. Cancer immunology research. 2016;4(3):215-24.
88. Zhang L, Meng Y, Feng X, Han Z. CAR-NK cells for cancer immunotherapy: from bench to bedside. Biomarker research. 2022;10(1):12.
89. Zhang Q, Zhang H, Ding J, Liu H, Li H, Li H, et al. Combination Therapy with EpCAM-CAR-NK-92 Cells and Regorafenib against Human Colorectal Cancer Models. Journal of immunology research. 2018;2018:4263520.
90. Marin D, Li Y, Basar R, Rafei H, Daher M, Dou J, et al. Safety, efficacy and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19(+) B cell tumors: a phase 1/2 trial. Nature medicine. 2024;30(3):772-84.
91. Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105(8):3051-7.

92. Gergely B, Vereb MA, Rebenku I, Vereb G, Szöőr Á. Targeting HER2-Positive Solid Tumors with CAR NK Cells: CD44 Expression Is a Critical Modulator of HER2-Specific CAR NK Cell Efficacy. Cancers (Basel). 2025;17(5).
93. Layman H, Jackson B, Belkina N, Ranganathan S, Wohlstadter JN. Multiplexed Cytokine Assessment of T- and NK-Cell Responses to Tumor Cells: Parallel Assessment of Biomarkers Associated with Efficacy, Safety, and Persistence. Blood. 2024;144:4854.
94. Chen Q, Lv J, Xie X, Zhu H, Xiao Z, Peng Y. Ethical and Regulatory Considerations in the Clinical Translation of Pluripotent Stem Cell-Derived NK Cell Therapies. Cell Prolif. 2025:e70129.
95. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, et al. Ethical and Safety Issues of Stem Cell-Based Therapy. Int J Med Sci. 2018;15(1):36-45.
96. Moradi S, Mahdizadeh H, Šarić T, Kim J, Harati J, Shahsavarani H, et al. Research and therapy with induced pluripotent stem cells (iPSCs): social, legal, and ethical considerations. Stem Cell Res Ther. 2019;10(1):341.
97. Zhao T, You J, Wang C, Li B, Liu Y, Shao M, et al. Cell-based immunotherapies for solid tumors: advances, challenges, and future directions. Front Oncol. 2025;15.
98. Huang JJ. Ramping Up GMP-Compliant CAR-NK Manufacturing At Scale With iPSCs.
99. Jørgensen LV, Christensen EB, Barnkob MB, Barington T. The clinical landscape of CAR NK cells. Experimental Hematology & Oncology. 2025;14(1):46.
100. Oberschmidt O, Morgan M, Huppert V, Kessler J, Gardlowski T, Matthies N, et al. Development of Automated Separation, Expansion, and Quality Control Protocols for Clinical-Scale Manufacturing of Primary Human NK Cells and Alpharetroviral Chimeric Antigen Receptor Engineering. Hum Gene Ther Methods. 2019;30(3):102-20.
101. Liu Y, Zhang M, Shen X, Xia C, Hu F, Huang D, et al. Mesothelin CAR-engineered NK cells derived from human embryonic stem cells suppress the progression of human ovarian cancer in animals. Cell Prolif. 2024;57(12):e13727.
102. Kok N, Dekkers Z, Loos A, Bos T, van den Broek P, Post I, et al. Closed-system manufacturing of therapeutic NK cells using automated cell enrichment and concentration processes enables scalable, robust and cost-effective solutions. Front Bioeng Biotechnol. 2025;13:1586912.
103. Kusch N, Storm J, Macioszek A, Knabbe C, Kaltschmidt B, Kaltschmidt C. Donor Variability and Seeding Density Shape NK-Cell Proliferation and Surface Receptor Expression: Insights from an Integrated Phenotypic and Genetic Analysis. Cells. 2025;14(16).
104. Zhang X, Guo Y, Ji Y, Gao Y, Zhang M, Liu Y, et al. Cytokine Release Syndrome After Modified CAR-NK Therapy in an Advanced Non-small Cell Lung Cancer Patient: A Case Report. Cell Transplant. 2022;31:9636897221094244.
105. García Roche A, Díaz Lagares C, Élez E, Ferrer Roca R. Cytokine release syndrome. Reviewing a new entity in the intensive care unit. Med Intensiva (Engl Ed). 2019;43(8):480-8.
106. Zhong Y, Liu J. Emerging roles of CAR-NK cell therapies in tumor immunotherapy: current status and future directions. Cell Death Discovery. 2024;10(1):318.
107. Głowacki P, Rieske P. Application and Design of Switches Used in CAR. Cells. 2022;11(12).
108. Duong MT, Collinson-Pautz MR, Morschl E, Lu A, Szymanski SP, Zhang M, et al. Two-Dimensional Regulation of CAR-T Cell Therapy with Orthogonal Switches. Mol Ther Oncolytics. 2019;12:124-37.
109. Wang X, Jasinski DL, Medina JL, Spencer DM, Foster AE, Bayle JH. Inducible MyD88/CD40 synergizes with IL-15 to enhance antitumor efficacy of CAR-NK cells. Blood advances. 2020;4(9):1950-64.
110. Fillat C, Carrió M, Cascante A, Sangro B. Suicide gene therapy mediated by the Herpes Simplex virus thymidine kinase gene/Ganciclovir system: fifteen years of application. Curr Gene Ther. 2003;3(1):13-26.
111. Li LQ, Shen F, Xu XY, Zhang H, Yang XF, Liu WG. Gene therapy with HSV1-sr39TK/GCV exhibits a stronger therapeutic efficacy than HSV1-TK/GCV in rat C6 glioma cells. ScientificWorldJournal. 2013;2013:951343.
112. Murty S, Labanieh L, Murty T, Gowrishankar G, Haywood T, Alam IS, et al. PET Reporter Gene Imaging and Ganciclovir-Mediated Ablation of Chimeric Antigen Receptor T Cells in Solid Tumors. Cancer Res. 2020;80(21):4731-40.
113. Najjar AM, Manuri PR, Olivares S, Flores L, 2nd, Mi T, Huls H, et al. Imaging of Sleeping Beauty-Modified CD19-Specific T Cells Expressing HSV1-Thymidine Kinase by Positron Emission Tomography. Mol Imaging Biol. 2016;18(6):838-48.
114. Włodarczyk M, Pyrzynska B. CAR-NK as a Rapidly Developed and Efficient Immunotherapeutic Strategy against Cancer. 2023;15(1):117.
115. Ebrahimiyan H, Tamimi A, Shokoohian B, Minaei N, Memarnejadian A, Hossein-Khannazer N, et al. Novel insights in CAR-NK cells beyond CAR-T cell technology; promising advantages. International Immunopharmacology.

2022;106:108587.
116.Newick K, Moon E, Albelda SM. Chimeric antigen receptor T-cell therapy for solid tumors. Molecular therapy oncolytics. 2016;3:16006.
117.Yin T, Wang G, He S, Liu Q, Sun J, Wang YJCi. Human cancer cells with stem cell-like phenotype exhibit enhanced sensitivity to the cytotoxicity of IL-2 and IL-15 activated natural killer cells. 2016;300:41-5.
118.Holmes L, LaHurd A, Wasson E, McClarin L, Dabney K. Racial and Ethnic Heterogeneity in the Association Between Total Cholesterol and Pediatric Obesity. 2016;13(1):19.
119.Ferrari de Andrade L, Kumar S, Luoma AM, Ito Y, Alves da Silva PH, Pan D, et al. Inhibition of MICA and MICB shedding elicits NK-cell–mediated immunity against tumors resistant to cytotoxic T cells. 2020;8(6):769-80.
120.Müller N, Michen S, Tietze S, Töpfer K, Schulte A, Lamszus K, et al. Engineering NK cells modified with an EGFRvIII-specific chimeric antigen receptor to overexpress CXCR4 improves immunotherapy of CXCL12/SDF-1α-secreting glioblastoma. 2015;38(5): 197-210.
121.Ng YY, Tay JC, Wang SJMT-O. CXCR1 expression to improve anti-cancer efficacy of intravenously injected CAR-NK cells in mice with peritoneal xenografts. 2020;16:75-85.
122.Dagher OK, Posey AD. Forks in the road for CAR T and CAR NK cell cancer therapies. Nature immunology. 2023;24(12):1994-2007.
123.Wu AA, Drake V, Huang HS, Chiu S, Zheng L. Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells. Oncoimmunology. 2015;4(7):e1016700.
124.Lamers-Kok N, Panella D, Georgoudaki AM, Liu H, Özkazanc D, Kučerová L, et al. Natural killer cells in clinical development as non-engineered, engineered, and combination therapies. Journal of hematology & oncology. 2022;15(1):164.
125.Thistlethwaite FC, Gilham DE, Guest RD, Rothwell DG, Pillai M, Burt DJ, et al. The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. Cancer immunology, immunotherapy: CII. 2017;66(11):1425-36.
126.Pedroza-Pacheco I, Madrigal A, Saudemont A. Interaction between natural killer cells and regulatory T cells: perspectives for immunotherapy. Cellular & molecular immunology. 2013;10(3):222-9.
127.Stravokefalou V, Stellas D, Karaliota S, Nagy BA, Valentin A, Bergamaschi C, et al. Heterodimeric IL-15 (hetIL-15) reduces circulating tumor cells and metastasis formation improving chemotherapy and surgery in 4T1 mouse model of TNBC. Frontiers in immunology. 2022;13:1014802.
128.Majzner RG, Mackall CL. Tumor Antigen Escape from CAR T-cell Therapy. Cancer discovery. 2018;8(10):1219-26.
129.Coyle KM, Hawke LG, Ormiston ML. Addressing Natural Killer Cell Dysfunction and Plasticity in Cell-Based Cancer Therapeutics. Cancers. 2023;15(6).
130.Judge SJ, Murphy WJ, Canter RJ. Characterizing the Dysfunctional NK Cell: Assessing the Clinical Relevance of Exhaustion, Anergy, and Senescence. Frontiers in cellular and infection microbiology. 2020;10:49.
131.Blaeschke F, Stenger D, Kaeuferle T, Willier S, Lotfi R, Kaiser AD, et al. Induction of a central memory and stem cell memory phenotype in functionally active CD4(+) and CD8(+) CAR T cells produced in an automated good manufacturing practice system for the treatment of CD19(+) acute lymphoblastic leukemia. Cancer immunology, immunotherapy : CII. 2018;67(7):1053-66.
132.Schmidt P, Raftery MJ, Pecher G. Engineering NK Cells for CAR Therapy—Recent Advances in Gene Transfer Methodology. 2021;11.
133.El-Mayta R, Zhang Z, Hamilton AG, Mitchell MJ. Delivery technologies to engineer natural killer cells for cancer immunotherapy. Cancer gene therapy. 2021;28(9):947-59.
134.Naeimi Kararoudi M, Likhite S, Elmas E, Yamamoto K, Schwartz M, Sorathia K, et al. Optimization and validation of CAR transduction into human primary NK cells using CRISPR and AAV. Cell reports methods. 2022;2(6):100236.
135.Pfefferle A, Huntington ND. You Have Got a Fast CAR: Chimeric Antigen Receptor NK Cells in Cancer Therapy. Cancers (Basel). 2020;12(3).
136.Müller S, Bexte T, Gebel V, Kalensee F, Stolzenberg E, Hartmann J, et al. High Cytotoxic Efficiency of Lentivirally and Alpharetrovirally Engineered CD19-Specific Chimeric Antigen Receptor Natural Killer Cells Against Acute Lymphoblastic Leukemia. Frontiers in immunology [Internet]. 2019 2019; 10:[3123 p.]. Available from: https://doi.org/10.3389/fimmu.2019.03123
137.Carlsten M, Childs RW. Genetic Manipulation of NK Cells for Cancer Immunotherapy: Techniques and Clinical Implications. Front Immunol. 2015;6:266.
138.Xiao L, Cen D, Gan H, Sun Y, Huang N, Xiong H, et al.

Adoptive Transfer of NKG2D CAR mRNA-Engineered Natural Killer Cells in Colorectal Cancer Patients. Molecular Therapy. 2019;27(6):1114-25.

139.Zhou Z, Chen Y, Ba Y, Xu H, Zuo A, Liu S, et al. Revolutionising Cancer Immunotherapy: Advancements and Prospects in Non-Viral CAR-NK Cell Engineering. Cell Prolif. 2025;58(4):e13791.

140.Bexte T, Reindl LM, Ullrich E. Nonviral technologies can pave the way for CAR-NK cell therapy. Journal of Leukocyte Biology. 2023;114(5):475-86.

141.Carlsten M, Levy E, Karambelkar A, Li L, Reger R, Berg M, et al. Efficient mRNA-Based Genetic Engineering of Human NK Cells with High-Affinity CD16 and CCR7 Augments Rituximab-Induced ADCC against Lymphoma and Targets NK Cell Migration toward the Lymph Node-Associated Chemokine CCL19. Front Immunol. 2016;7:105.

142.Osborn MJ, Webber BR, Knipping F, Lonetree C-l, Tennis N, DeFeo AP, et al. Evaluation of TCR Gene Editing Achieved by TALENs, CRISPR/Cas9, and megaTAL Nucleases. Molecular Therapy. 2016;24(3):570-81.

143.Tian J, Bai T, Zhang Z, Zhai X, Wang K, Gao X, et al. Progress and prospects for use of cellular immunotherapy in pancreatic cancer. Journal of Cancer Research and Therapeutics. 2022;18(7).

144.Khoshandam M, Soltaninejad H, Hamidieh AA, Hosseinkhani S. CRISPR, CAR-T, and NK: Current applications and future perspectives. Genes & Diseases. 2024;11(4):101121.

145.Daher M, Basar R, Gokdemir E, Baran N, Uprety N, Nunez Cortes AK, et al. Targeting a cytokine checkpoint enhances the fitness of armored cord blood CAR-NK cells. Blood. 2021;137(5):624-36.

146.Bexte T, Alzubi J, Reindl LM, Wendel P, Schubert R, Salzmann-Manrique E, et al. CRISPR-Cas9 based gene editing of the immune checkpoint NKG2A enhances NK cell mediated cytotoxicity against multiple myeloma. Oncoimmunology. 2022;11(1):2081415.

147.Choi E, Chang J-W, Krueger J, Lahr WS, Pomeroy E, Walsh M, et al. Engineering CD70-Directed CAR-NK Cells for the Treatment of Hematological and Solid Malignancies. Blood. 2021;138(Supplement 1):1691-.

148.Shankar K, Zingler-Hoslet I, Tabima DM, Zima S, Shi L, Gimse K, et al. Virus-free CRISPR knockin of a chimeric antigen receptor into KLRC1 generates potent GD2-specific natural killer cells. Mol Ther. 2025;33(3):1014-30.

149.Balke-Want H, Keerthi V, Cadinanos-Garai A, Fowler C, Gkitsas N, Brown AK, et al. Non-viral chimeric antigen receptor (CAR) T cells going viral. Immuno-oncology technology. 2023;18:100375.

150.Moretti A, Ponzo M, Nicolette CA, Tcherepanova IY, Biondi A, Magnani CF. The Past, Present, and Future of Non-Viral CAR T Cells. Frontiers in immunology. 2022;13:867013.

151.Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science (New York, NY). 2020;367(6481).

152.Ebrahimiyan H, Tamimi A, Shokoohian B, Minaei N, Memarnejadian A, Hossein-Khannazer N, et al. Novel insights in CAR-NK cells beyond CAR-T cell technology; promising advantages. Int Immunopharmacol. 2022;106:108587.

153.Wang W, Liu Y, He Z, Li L, Liu S, Jiang M, et al. Breakthrough of solid tumor treatment: CAR-NK immunotherapy. Cell Death Discovery. 2024;10(1):40.

154.Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. The New England journal of medicine. 2014;371(16):1507-17.

155.Fischer J, Paret C, El Malki K, Alt F, Wingerter A, Neu MA, et al. CD19 Isoforms Enabling Resistance to CART-19 Immunotherapy Are Expressed in B-ALL Patients at Initial Diagnosis. Journal of immunotherapy (Hagerstown, Md : 1997). 2017;40(5):187-95.

156.Morcillo-Martín-Romo P, Valverde-Pozo J, Ortiz-Bueno M, Arnone M, Espinar-Barranco L, Espinar-Barranco C, et al. The Role of NK Cells in Cancer Immunotherapy: Mechanisms, Evasion Strategies, and Therapeutic Advances. Biomedicines. 2025;13(4).

157.Sadowski K, Olejarz W, Basak G. Modern Advances in CARs Therapy and Creating a New Approach to Future Treatment. Int J Mol Sci. 2022;23(23).

158.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):1089-108.

159.Andrea AE, Chiron A, Sarrabayrouse G, Bessoles S, Hacein-Bey-Abina S. A structural, genetic and clinical comparison of CAR-T cells and CAR-NK cells: companions or competitors? Front Immunol. 2024;15:1459818.

160.Xian Y, Wen L. CAR Beyond αβ T Cells: Unleashing NK Cells, Macrophages, and γδ T Lymphocytes Against Solid Tumors. Vaccines (Basel). 2025;13(6).

161.Peng L, Sferruzza G, Yang L, Zhou L, Chen S. CAR-T and CAR-NK as cellular cancer immunotherapy for solid

tumors. Cellular & Molecular Immunology. 2024;21(10):1089-108.
162.Sabahi M, Fathi Jouzdani A, Sadeghian Z, Dabbagh Ohadi MA, Sultan H, Salehipour A, et al. CAR-engineered NK cells versus CAR T cells in treatment of glioblastoma; strength and flaws. Journal of Neuro-Oncology. 2025;171(3):495-530.
163.Andrea AE, Chiron A, Sarrabayrouse G, Bessoles S, Hacein-Bey-Abina S. A structural, genetic and clinical comparison of CAR-T cells and CAR-NK cells: companions or competitors? Frontiers in Immunology. 2024;15:1459818.
164.Xue Y. Limitations and overcomes of CAR-NK for cancer immunotherapy. Highl Sci Eng Technol. 2023;36:944-8.
165.Soldierer M, Bister A, Haist C, Thivakaran A, Cengiz SC, Sendker S, et al. Genetic engineering and enrichment of human NK cells for CAR-enhanced immunotherapy of hematological malignancies. Frontiers in Immunology. 2022;13:847008.
166.Burger MC, Zhang C, Harter PN, Romanski A, Strassheimer F, Senft C, et al. CAR-engineered NK cells for the treatment of glioblastoma: turning innate effectors into precision tools for cancer immunotherapy. Frontiers in immunology. 2019;10:2683.
167.Li S, Jing J, Chen Y, Chi E, Wang B, Xie Z, et al. Precision sniper for solid tumors: CAR-NK cell therapy. Cancer Immunology, Immunotherapy. 2025;74(9):275.
168.Burger MC, Forster MT, Romanski A, Straßheimer F, Macas J, Zeiner PS, et al. Intracranial injection of natural killer cells engineered with a HER2-targeted chimeric antigen receptor in patients with recurrent glioblastoma. Neuro Oncol. 2023;25(11):2058-71.
169.Karamivandishi A, Hatami A, Eslami MM, Soleimani M, Izadi N. Chimeric antigen receptor natural killer cell therapy: A systematic review of preclinical studies for hematologic and solid malignancies. Human Immunology. 2025;86(1):111207.
170.Ma R, Lu T, Li Z, Teng KY, Mansour AG, Yu M, et al. An Oncolytic Virus Expressing IL15/IL15Rα Combined with Off-the-Shelf EGFR-CAR NK Cells Targets Glioblastoma. Cancer research. 2021;81(13):3635-48.
171.Ahmad GV, Nouri S, Mohammad gholian A, Abdollahi E, Ghorbaninezhad F, Tahmasebi S, et al. Breaking barriers: CAR-NK cell therapy breakthroughs in female-related cancers. Biomedicine & Pharmacotherapy. 2025;187:118071.
172.Teng KY, Mansour AG, Zhu Z, Li Z, Tian L, Ma S, et al. Off-the-Shelf Prostate Stem Cell Antigen-Directed Chimeric Antigen Receptor Natural Killer Cell Therapy to Treat Pancreatic Cancer. Gastroenterology. 2022;162(4):1319-33.
173.Da Y, Liu Y, Hu Y, Liu W, Ma J, Lu N, et al. STING agonist cGAMP enhances anti-tumor activity of CAR-NK cells against pancreatic cancer. Oncoimmunology. 2022;11(1):2054105.
174.Boulifa A, Franzén AS, Raftery MJ, Radecke C, Pecher G. Chimeric Antigen Receptor (CAR)-NK92 cells effective against glioblastoma, breast- and pancreatic cancer in vitro and in a murine xenograft model of ovarian cancer. Cancer Cell International. 2025;25(1):260.
175.Ueda T, Kumagai A, Iriguchi S, Yasui Y, Miyasaka T, Nakagoshi K, et al. Non-clinical efficacy, safety and stable clinical cell processing of induced pluripotent stem cell-derived anti-glypican-3 chimeric antigen receptor-expressing natural killer/innate lymphoid cells. Cancer Sci. 2020;111(5):1478-90.
176.Cao B, Liu M, Wang L, Liang B, Feng Y, Chen X, et al. Use of chimeric antigen receptor NK-92 cells to target mesothelin in ovarian cancer. Biochem Biophys Res Commun. 2020;524(1):96-102.
177.Klapdor R, Wang S, Morgan M, Dörk T, Hacker U, Hillemanns P, et al. Characterization of a Novel Third-Generation Anti-CD24-CAR against Ovarian Cancer. International journal of molecular sciences. 2019;20(3).
178.Anvar MT, Rashidan K, Arsam N, Rasouli-Saravani A, Yadegari H, Ahmadi A, et al. Th17 cell function in cancers: immunosuppressive agents or anti-tumor allies? Cancer Cell Int. 2024;24(1):355.
179.Jiang W, Gu G, Zhang Y, Song Y, Shi M, Wang G, et al. Novel mesothelin-targeted chimeric antigen receptor-modified UNKT cells are highly effective in inhibiting tumor progression. Pharmacol Res. 2023;197:106942.
180.Wang W, Liu Y, He Z, Li L, Liu S, Jiang M, et al. Breakthrough of solid tumor treatment: CAR-NK immunotherapy. Cell Death Discov. 2024;10(1):40.
181.Li J, Hu H, Lian K, Zhang D, Hu P, He Z, et al. CAR-NK cells in combination therapy against cancer: A potential paradigm. Heliyon. 2024;10(5):e27196.
182.Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nature reviews Immunology. 2012;12(4):269-81.
183.Siegler EL, Kim YJ, Chen X, Siriwon N, Mac J, Rohrs JA, et al. Combination Cancer Therapy Using Chimeric Antigen Receptor-Engineered Natural Killer Cells as Drug Carriers. Molecular therapy : the journal of the American Society of Gene Therapy. 2017;25(12):2607-19.
184.Zheng W, Ling S, Cao Y, Shao C, Sun X. Combined use of NK cells and radiotherapy in the treatment of solid

tumors. 2024;14.
185.Pesce S, Greppi M, Tabellini G, Rampinelli F, Parolini S, Olive D, et al. Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. The Journal of allergy and clinical immunology. 2017;139(1):335-46.e3.
186.Benson DM, Bakan CE, Mishra A, Hofmeister CC, Efebera Y, Becknell B, et al. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti–PD-1 antibody. Blood. 2010;116(13):2286-94.
187.Sivori S, Pende D, Quatrini L, Pietra G, Della Chiesa M, Vacca P, et al. NK cells and ILCs in tumor immunotherapy. Molecular Aspects of Medicine. 2021;80:100870.
188.Abdolahi S, Ghazvinian Z, Muhammadnejad S, Ahmadvand M, Aghdaei HA, Ebrahimi-Barough S, et al. Adaptive NK Cell Therapy Modulated by Anti-PD-1 Antibody in Gastric Cancer Model. Frontiers in pharmacology. 2021;12:733075.
189.Su S, Zhao J, Xing Y, Zhang X, Liu J, Ouyang Q, et al. Immune Checkpoint Inhibition Overcomes ADCP-Induced Immunosuppression by Macrophages. Cell. 2018;175(2):442-57.e23.
190.Strassheimer F, Strecker MI, Alekseeva T, Macas J, Demes MC, Mildenberger IC, et al. OS12.6.A Combination therapy of CAR-NK-cells and anti-PD-1 results in high efficacy against advanced-stage glioblastoma in a syngeneic mouse model and induces protective anti-tumor immunity in vivo. Neuro-Oncology. 2021.
191.Liu WN, So WY, Harden SL, Fong SY, Wong MXY, Tan WWS, et al. Successful targeting of PD-1/PD-L1 with chimeric antigen receptor-natural killer cells and nivolumab in a humanized mouse cancer model. Sci Adv. 2022;8(47):eadd1187.
192.A Phase II Study of Immunotherapy Combination: Irradiated PD-L1 CAR-NK Cells Plus Pembrolizumab Plus N-803 for Subjects With Recurrent/Metastatic Gastric or Head and Neck Cancer [Internet]. 2021. Available from: https://clinicaltrials.gov/study/NCT04847466.
193.Klapdor R, Wang S, Morgan MA, Zimmermann K, Hachenberg J, Büning H, et al. NK Cell-Mediated Eradication of Ovarian Cancer Cells with a Novel Chimeric Antigen Receptor Directed against CD44. Biomedicines. 2021;9(10).
194.Klapdor R, Wang S, Hacker U, Büning H, Morgan M, Dörk T, et al. Improved Killing of Ovarian Cancer Stem Cells by Combining a Novel Chimeric Antigen Receptor-Based Immunotherapy and Chemotherapy. Hum Gene Ther. 2017;28(10):886-96.
195.Lin X, Liu Z, Dong X, Wang K, Sun Y, Zhang H, et al. Radiotherapy enhances the anti-tumor effect of CAR-NK cells for hepatocellular carcinoma. J Transl Med. 2024;22(1):929.
196.Liu E, Ang SOT, Kerbauy L, Basar R, Kaur I, Kaplan M, et al. GMP-Compliant Universal Antigen Presenting Cells (uAPC) Promote the Metabolic Fitness and Antitumor Activity of Armored Cord Blood CAR-NK Cells. Frontiers in immunology. 2021;12:626098.
197.Page A, Chuvin N, Valladeau-Guilemond J, Depil S. Development of NK cell-based cancer immunotherapies through receptor engineering. Cellular & molecular immunology. 2024;21(4):315-31.
198.Zhao Y, Stepto H, Schneider CK. Development of the First World Health Organization Lentiviral Vector Standard: Toward the Production Control and Standardization of Lentivirus-Based Gene Therapy Products. Human gene therapy methods. 2017;28(4):205-14.
199.Allan DSJ, Wu C, Mortlock RD, Chakraborty M, Rezvani K, Davidson-Moncada JK, et al. Expanded NK cells used for adoptive cell therapy maintain diverse clonality and contain long-lived memory-like NK cell populations. Molecular therapy oncolytics. 2023;28:74-87.
200.Marin D, Li Y, Basar R, Rafei H, Daher M, Dou J, et al. Safety, efficacy and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19+ B cell tumors: a phase 1/2 trial. Nature medicine. 2024;30(3):772-84.
201.Li T, Niu M, Zhang W, Qin S, Zhou J, Yi M. CAR-NK cells for cancer immunotherapy: recent advances and future directions. Front Immunol. 2024;15:1361194.
202.Pang Z, Wang Z, Li F, Feng C, Mu X. Current Progress of CAR-NK Therapy in Cancer Treatment. Cancers. 2022;14(17).
203.Tang Y, Yin H, Zhao X, Jin D, Liang Y, Xiong T, et al. High efficacy and safety of CD38 and BCMA bispecific CAR-T in relapsed or refractory multiple myeloma. Journal of Experimental & Clinical Cancer Research. 2022;41(1):2.
204.Wang K, Wang L, Wang Y, Xiao L, Wei J, Hu Y, et al. Reprogramming natural killer cells for cancer therapy. Molecular Therapy. 2024.
205.Wang K, Wang L, Wang Y, Xiao L, Wei J, Hu Y, et al. Reprogramming natural killer cells for cancer therapy. Molecular therapy : the journal of the American Society of Gene Therapy. 2024.