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REVIEW

Research Progress of NK Cells in Glioblastoma Treatment

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Abstract: NK cells are a type of antitumor immune cell with promising clinical application, following T cells. The activity of NK cells is primarily regulated by their surface receptors and immune microenvironment. In gliomas, the tumor microenvironment exerts a strong immunosuppressive effect, which significantly reduces the clinical efficacy of NK cell immunotherapy. Therefore, this review aims to discuss the latest research on the role of NK cells in glioma immunotherapy, focusing on aspects such as NK cell development, function, and localization. It summarizes information on the compounds, monoclonal antibodies, and cytokine therapies targeting NK cells while emphasizing the current status and trends of gene-modified NK cells in glioma treatment. Additionally, it explores the molecular mechanisms underlying immune escape in glioma cells, providing a theoretical foundation and new perspectives for NK cell-based immunotherapy in gliomas.

Keywords: glioblastoma, survival rates, therapeutic strategies, inhibitory factors

Introduction

Glioblastoma (GBM) is the most common malignant tumor.[1] The global incidence of GBM is approximately 10 cases per 100,000 people. The median survival for GBM is approximately 12 months, with estimated survival rates at 1, 2, and 5 years being 28.4%, 11.5%, and 3.4%, respectively.[2] Figure 1 illustrates various therapeutic approaches for GBM. Although surgical treatment, postoperative chemoradiotherapy and other treatments have made certain progress, the prognosis of GBM patients is still poor due to multiple factors such as postoperative recurrence and resistance to chemoradiotherapy. Given the poor prognosis of GBM, more effective treatment strategies are urgently needed.

Research on NK cells has progressed over the past 50 years (Figure 2).[3] Figure 3 presents several NK cell-based therapeutic strategies.[4] NK cells possess several activating and inhibiting receptors on their surface, which play critical roles in identifying malignant cells. The activation of NK cells depends on the abundance and expression of these receptor ligands in glioma cells.[5] Furthermore, numerous inhibitory factors within the tumor immune microenvironment (TIM) inhibit NK immune responses. Enhancing NK infiltration into glioma tissue and boosting their activity are key strategies in targeted NK cell-based therapy. Recent studies targeting the TIM and tumor cells, in combination with NK cell therapies, have shown significant potential for tumor treatment. Therefore, the review aims to provide an in-depth summary of the latest advancements in NK cell-based therapies for GBM.

NK Cell Development, Functions, and Localizations

In mice and humans, NK cells undergo distinct stages of development (Figure 4).[6–9] Many NK cells are not only cytotoxic but also produce various molecules, including perforin and granzyme, which can lead to stress-induced cell death. Additionally, NK cells express FASL and TRAIL, allowing them to induce apoptosis in target cells by binding to the corresponding receptors, FAS or TRAILR. Furthermore, NK cells secrete various cytokines, such as interferon IFN-γ, TNF-α, interleukin [IL]-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokines (CCL3, CCL4, CCL5, XCL1).[10]

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5ALA: aminolevulinic acid
PDT: photodynamic therapy
PTT:Photothermal therapy
TTF: tumor-treating fields
LITT:Laser-induced thermal therapy
TMZ:temozolomide
BCNU: carmustine
BEV: bevacizumab

Multimodality treatment against GBM

While the standard therapy consists of maximum safety resection,adjuvant radiotherapy, and chemotherapy with temozolomide, which is collectively termed the Stupp regimen, several promising adjuvant therapies have been used for treating GBM.

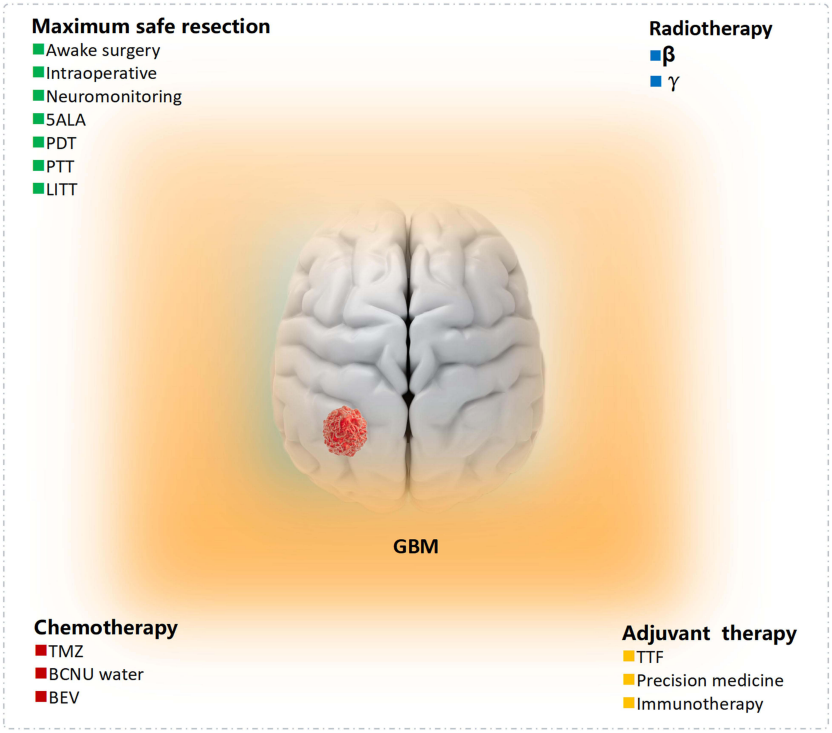

Figure 1 Multiple approaches for GBM treatment.

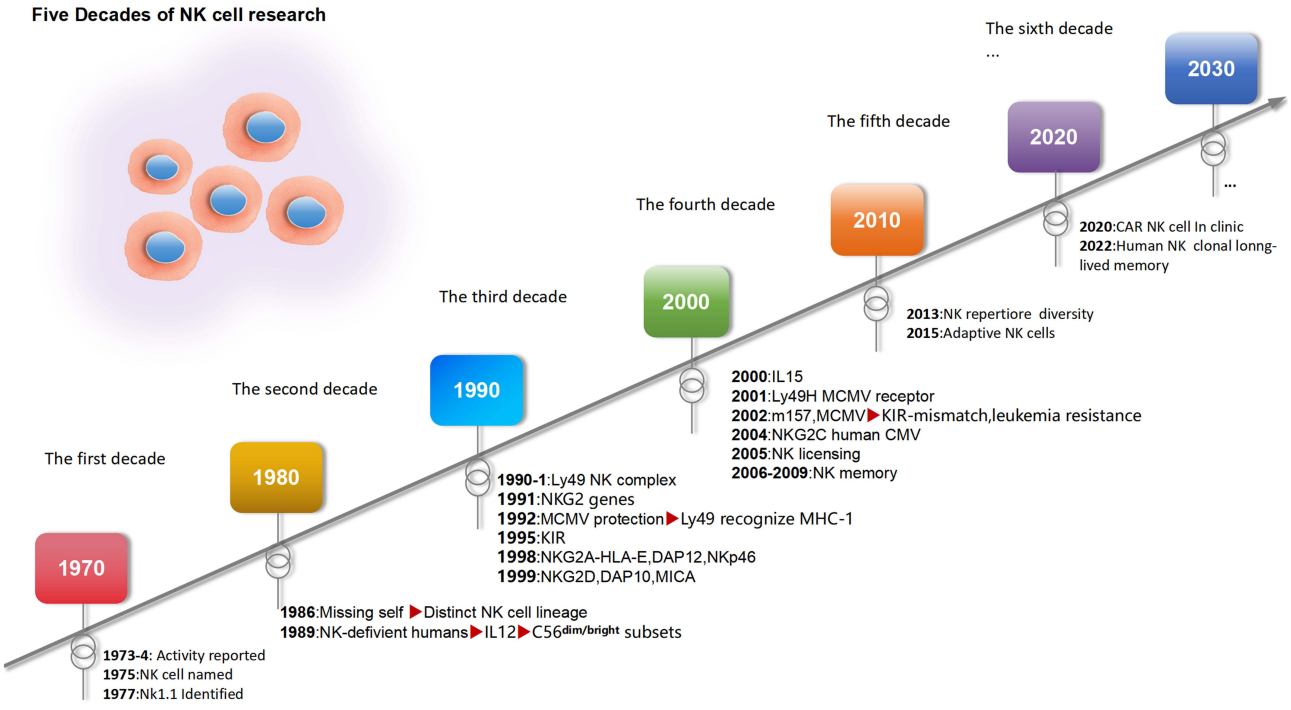

Figure 2 History of NK cells.

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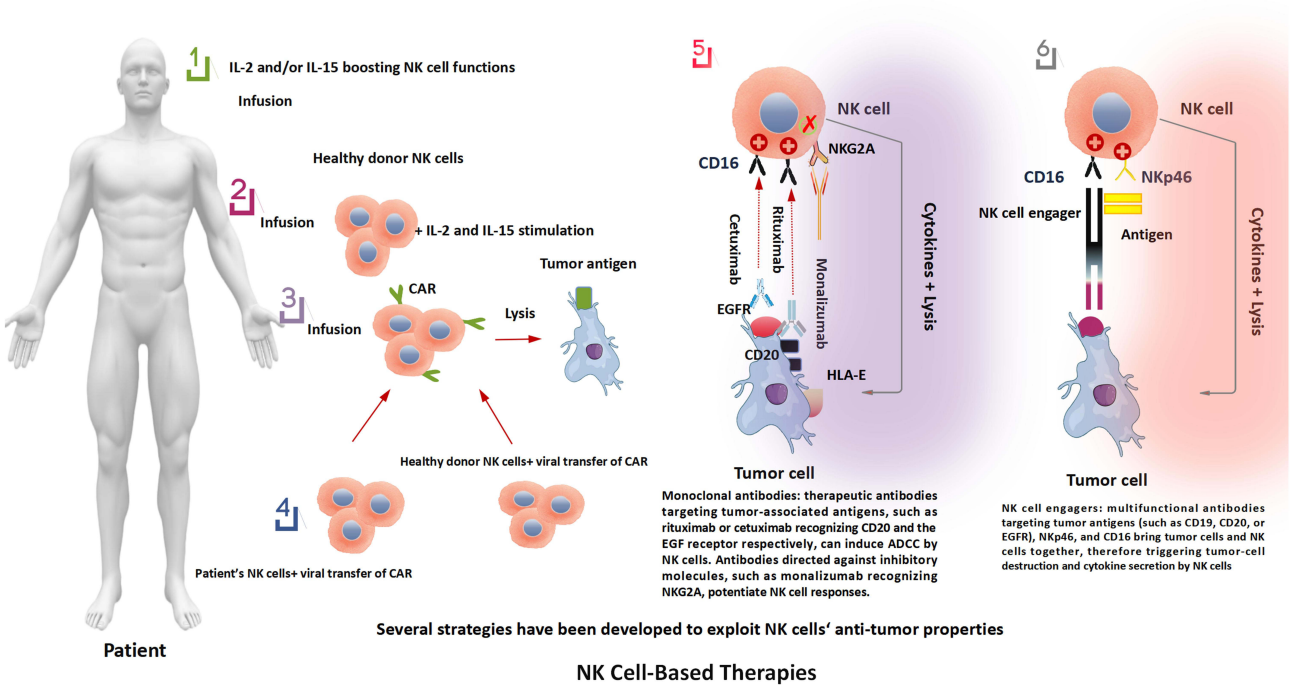

Figure 3 NK cells-based therapies.

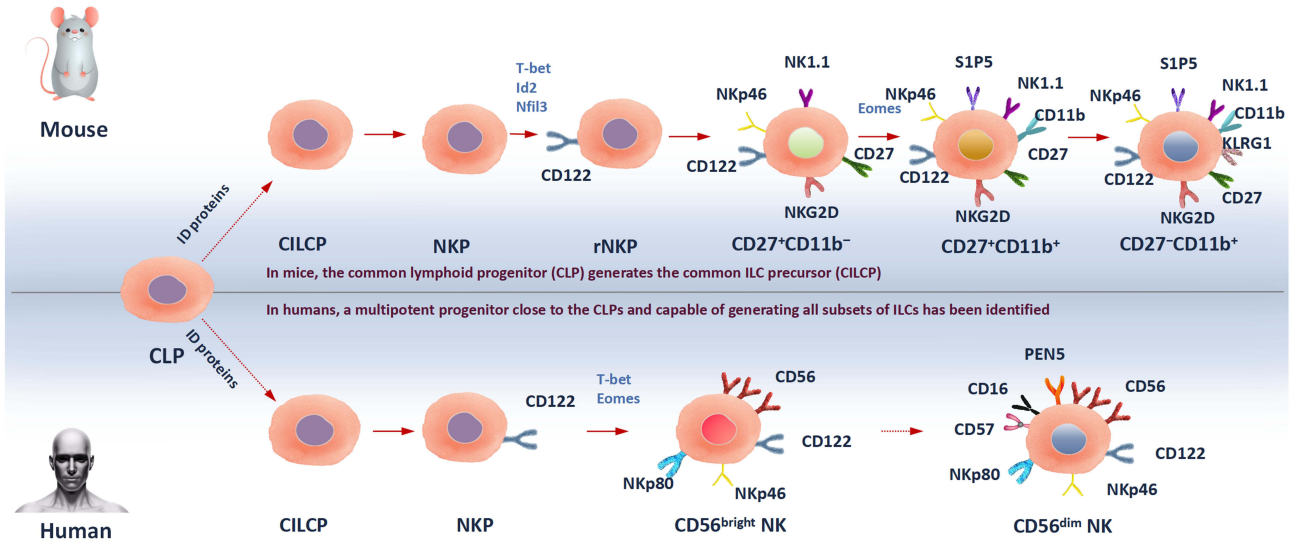

Figure 4 The development process of NK cells.

The regulation of the NK cell effector function is maintained by a dynamic equilibrium between inhibitory and activating cell surface receptors (Figure 5). Additionally, NK cells recognize tumor cells based on the balance between stimulatory and inhibitory receptors[11–21] (Figure 6). These cells operate by killing cancerous cells in an antigen-independent manner (Figure 7).[22–25]

Some previous research shows that NK cells are present in various tissues and organs, with Figure 8 illustrating their localizations.[26]

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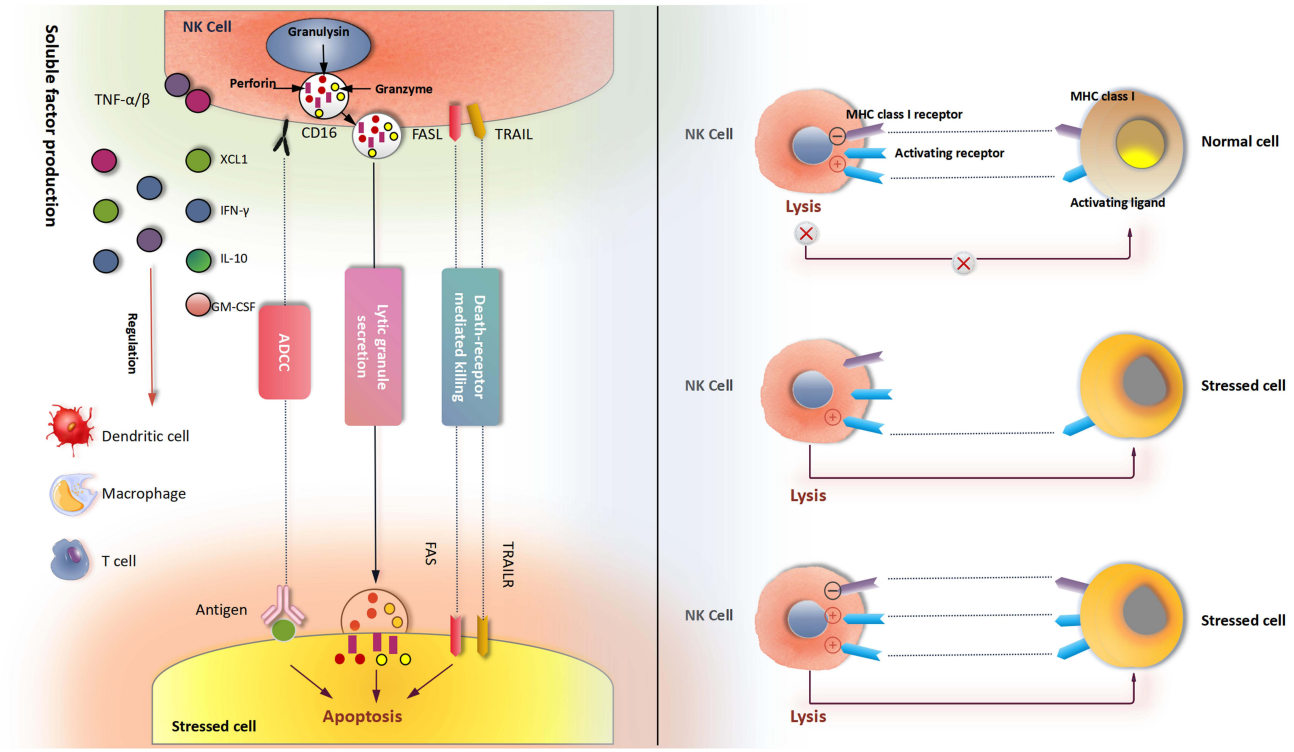

Figure 5 NK Cell Functions.

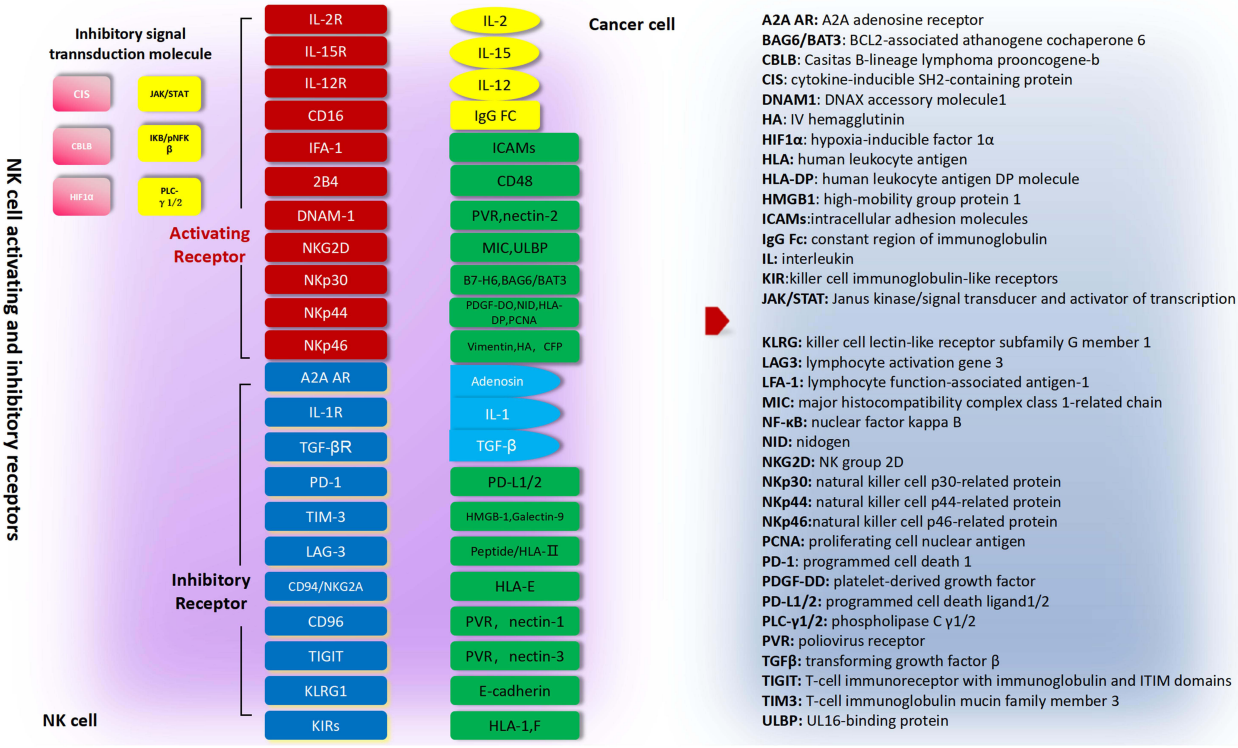

Figure 6 NK cells recognize tumor cells based on a balance between the stimulatory and inhibitory receptors above.

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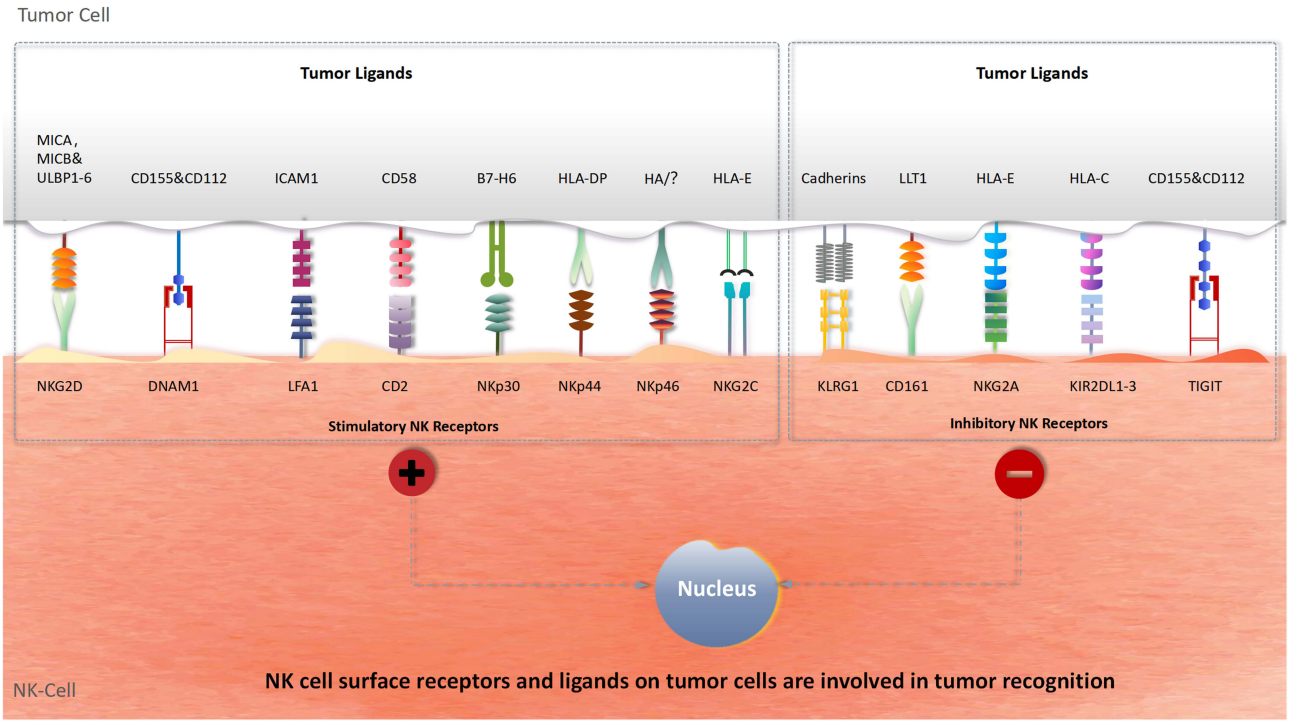

Figure 7 NK cell surface receptors and ligands on tumor cells are involved in tumor recognition. NK cells express a set of stimulatory (or activation) receptors as well as inhibitory receptors to recognize healthy cells and aberrant cells such as virus-infected or a potential tumorigenic cell through MHC-I receptor appearance.

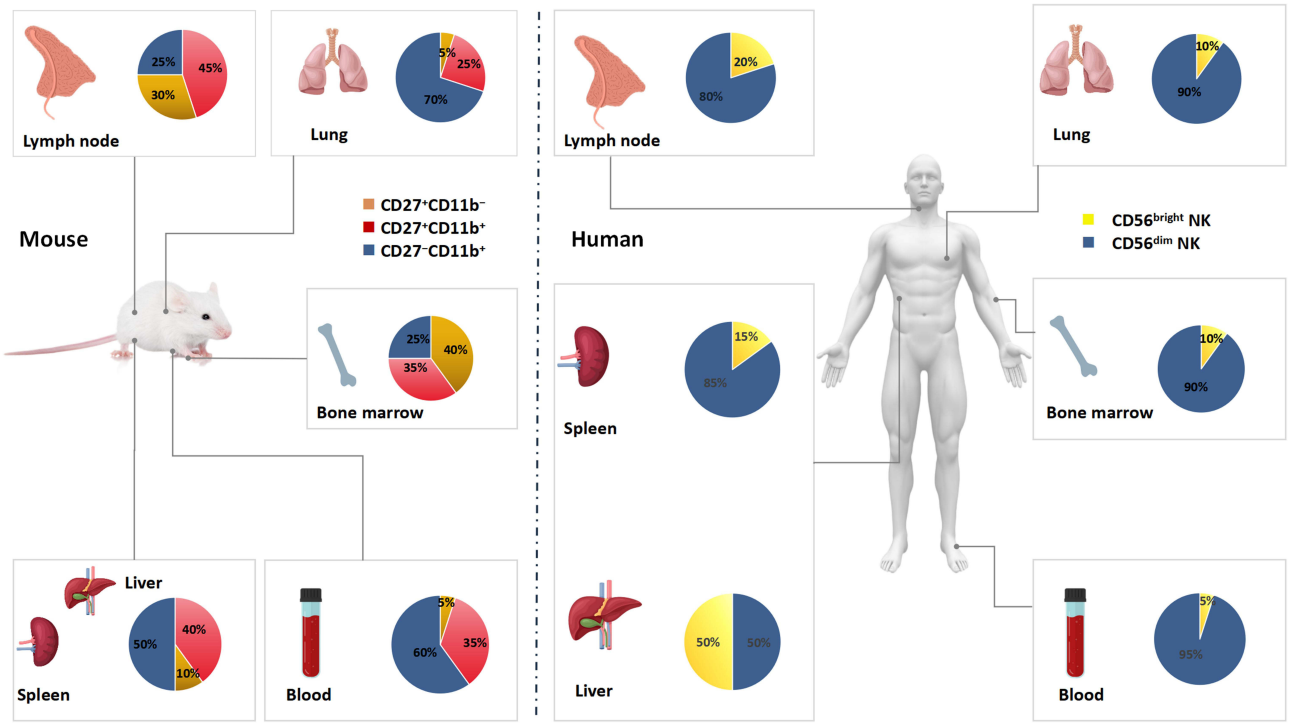

Figure 8 The localization of NK Cells.

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Sources of NK Cells for Immunotherapy

NK cells for clinical treatment can be obtained from umbilical cord blood (UCB),[27,28] peripheral blood (PB),[29,30] stem cells, and NK-92.[31,32] Currently, most clinical trials involving NK cells focus on UCB-NK, PB-NK, and NK-92 cells. However, significant challenges exist in the manufacturing process for the final therapeutic cell doses.[33–37] Figure 9 depicts the specific workflow for isolating NK cells from various donor sources and their subsequent expansion for adaptive transfer.

NK Cell Functionality in the TME

The regulation of NK cell metabolism is influenced by various factors.[38,39] Regarding nutrition, NK cells are competitive with tumor cells and myeloid-derived suppressor cells (MDSCs).[40] Tumor cells not only consume large amounts of glucose but also produce lactic acid, which is transported to NK cells by the transporters SLC16A1 and SLC16A, impairing ATP production.[41] Tumor cells produce extracellular adenosine through the action of extracellular nucleotidases CD39 and CD73, which inhibit oxidative phosphorylation and glycolysis in NK cells.[42] Additionally, TGF-β secreted by tumor cells and MDSCs disrupt the mTOR signaling pathway[43–46] (Figure 10).

NK Cells and Their Interactions in the Glioma Microenvironment

Effect of Glioma on NK Cell Function

Gliomas are classified as immune "cold tumors" due to their low lymphocyte infiltration, and the glioma microenvironment has the capacity to suppress the immune response systematically and locally. MHC-I on the surface of normal cells binds to inhibitory receptors on the surface of NK cells, regulating their killing function. However, overexpression of MHC-I in central nervous system tumors inhibits NK cell activity. Most glioma cells exhibit high levels of MHC-I molecules, which interact with KIRs to inhibit NK cell activity. NKG2D, a non-MHC-I-specific activation receptor for NK cells, plays a crucial role in determining NK cell cytotoxicity against tumor cells, which is dependent on the abundance of NKG2D ligands present in glioma cells. Glioma cells can also regulate the expression of receptors on the

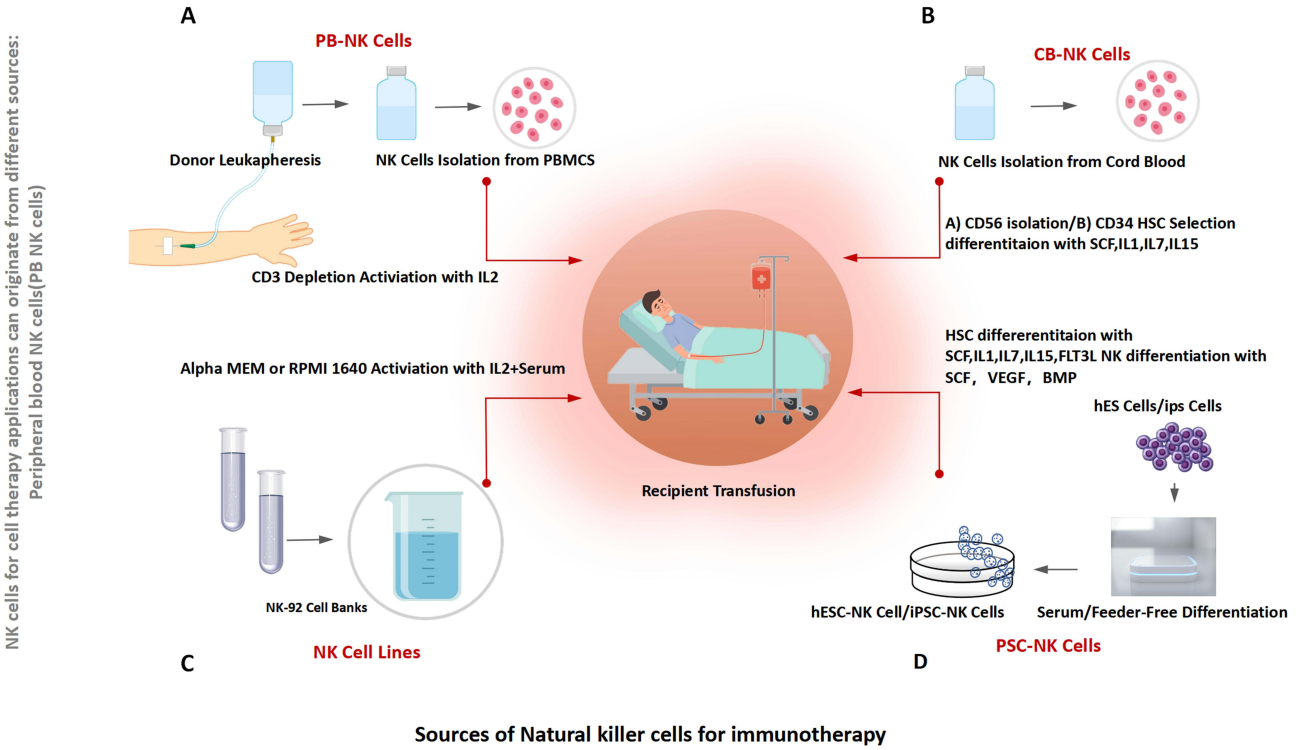

Figure 9 Sources of NK cells for immunotherapy. (**A**) Allogeneic umbilical cord blood NK cells (CB-NK Cells); (**B**) NK cell cancer cell lines (NK-92); (**C**) hESC and iPSCs; (**D**) Merits and demerits with the different NK cell sources vary as described.

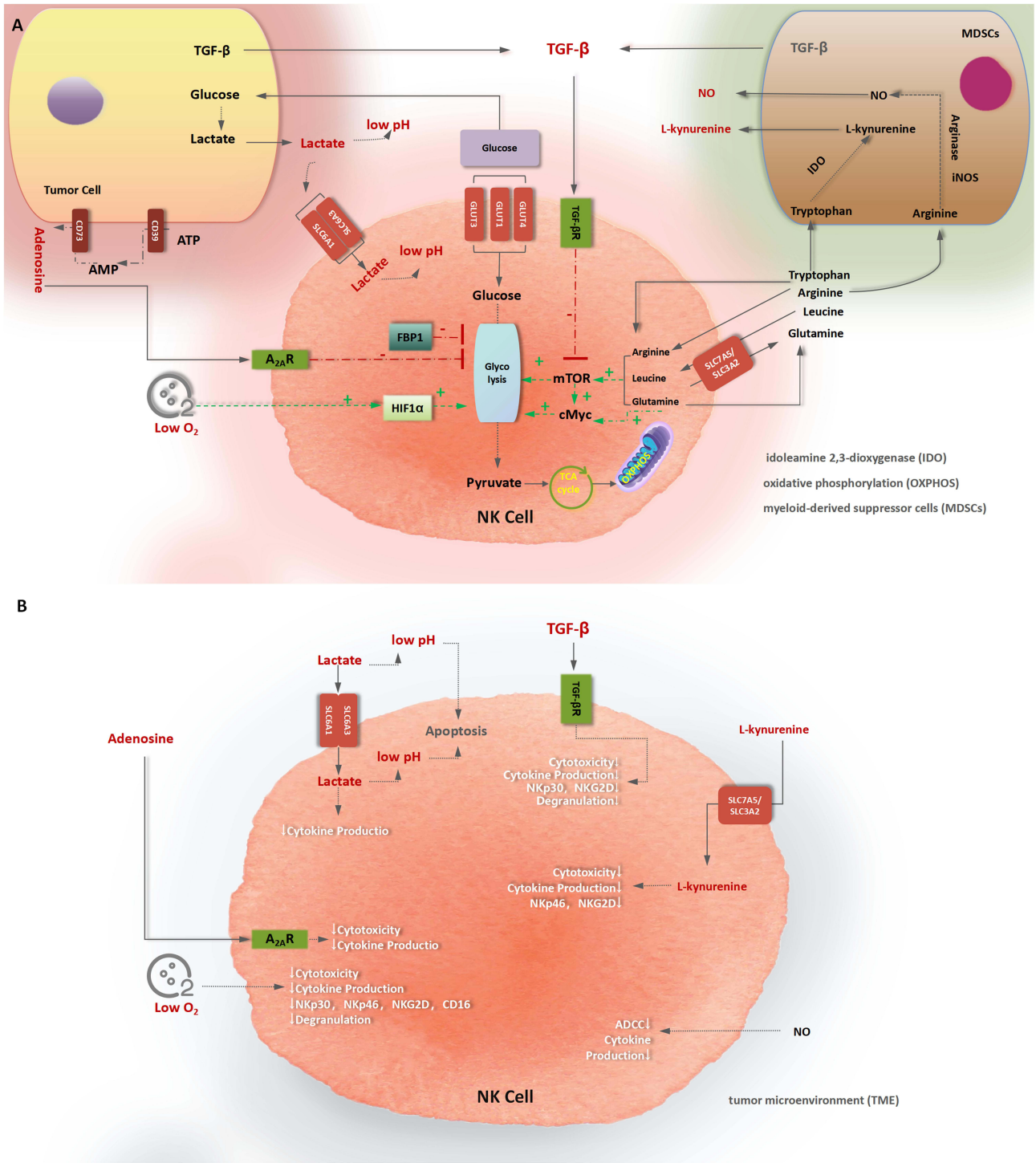

Figure 10 The functions of NK cell in the TME. (**A**) Tumor microenvironment shapes NK cell metabolism and effector functions; (**B**) A schematic representation of several metabolites and other factors present in the TME that limit NK cell effector functions.

surface of NK cells, thereby inhibiting their function. Mutations in the isocitrate dehydrogenase1 1/2 genes in diffuse glioma promote epigenetic reprogramming of various immune genes, including downregulation of NKG2D ligands and resistance to NK cell-mediated cytotoxicity. Studies show that IDH mutant gliomas are more prone to mutations that inactivate their own NKG2D ligands, allowing them to evade NK cell-mediated immune surveillance.[47,48] However,

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other studies suggest that the IDH1-R132H mutation can enhance the recruitment of NK cells to the tumor site by modulating the TIM, potentially improving patient prognosis.[49]

Glioma can impair NK cell function not only by downregulating NKG2D ligands to inactivate NK cell receptors but also by the secretion of cytokines. Glioma stem cells (GSCs) in GBM significantly induce and secrete transforming growth factor β (TGF-β), which inhibits NK cell function in the glioma immune microenvironment.[50] The interaction between αv integrin on GSCs and CD9 and CD103 on NK cells stimulates TGF-β production by GSCs. TGF-β, released by GSCs, then activates transforming growth factor beta receptor 2 on NK cells, blocking their antitumor activity. Combining donor-derived NK cells or allogeneic NK cells with inhibitors targeting αv integrin or TGF-β receptors enhances their antitumor activity.[51] TGF-β also downregulates NKG2D expression in peripheral blood NK cells, inhibiting their function.[11,12,52,53] Patients with GBM frequently exhibit lymphocytopenia due to gliomas activating signal transducer and activator of transcription 3. Hyperphosphorylation of STAT3 induces various soluble immunosuppressive factors, including interleukin-10 (IL-10), prostaglandin E-2, vascular endothelial growth factor, and TGF-β, which collectively inhibit CTL activity and proliferation.[54] Additionally, gliomas may secrete soluble immunosuppressive factors such as carbohydrate-binding proteins and galectin-1, which reduce lymphocyte viability. Galectin1-deficient gliomas are more susceptible to lysis by NK cells and are cleared before they can adapt to the antitumor immune response. Inhibiting galectin-1 in gliomas can restore NK cell immune surveillance and improve survival rates of patients with glioma. Furthermore, glioma cells can upregulate indoleamine 2, 3-Dioxygenase (IDO) to inhibit antitumor immunity. As a key rate-limiting enzyme in tryptophan metabolism, IDO depletes tryptophan and generates kynuridine, a metabolite that inactivates NK cells and fosters the development of regulatory T cells (Tregs), thereby contributing to tumor immunosuppression.

In patients with glioma, the accumulation of MDSCs in peripheral blood is significantly higher than that in healthy individuals.[55,56] MDSC interacts with macrophages to promote IL-10 expression and inhibit IL-12 secretion, leading to the conversion of macrophages into tumor-promoting M2-type cells. Additionally, other chemokines and cytokines, such as CX3CL1 and CCL5, contribute to abnormal tumor angiogenesis in the TME by recruiting glioma-associated macrophages.[57,58] Furthermore, the tumor can gain a survival advantage by depriving surrounding normal cells of nutrients and oxygen, which may inhibit NK cell metabolism and antitumor activity.[59]

Role of NK Cells in Glioma Immunity

The number of NK cells is associated with the prognosis of glioma, as neurons produce the CX3CL1 chemokine, which promotes the accumulation of CX3CR1 + NK cells in the brain.[60] A study shows that GBM exhibits the highest degree of NK cell infiltration among tumors when compared to that of breast cancer and melanoma, suggesting the important role NK cells play in glioma immune monitoring.[61] NK cells are also present in meningiomas and metastatic brain tumors,[62] and they can effectively lyse GBM and medulloblastoma in vitro.[63] Killer cell immunoglobulin-like receptor 2DS2 and KIR2DS4 alleles are linked to the regulation of cytomegalovirus gene expression, which induces platelet-derived growth factor D by enhancing the recruitment of peripheral cells and promoting tumor angiogenesis. Thus, PDGFD expression further stimulates the growth of GBM.[64] Most PDGFD in GBM can bind to the activated NKp44 receptor, enhancing the cytotoxic capability of NK cells to control tumor growth and improve survival rates in patients with GBM. B7-H6 (NKp30 ligand) is highly expressed in human gliomas, potentially inducing tumor development by specifically binding to NKP30 and upregulating IL-32 expression. These studies suggest that NK cells significantly influence the prognosis of patients with glioma.

As key effector cells of the innate immune system of the body, the infiltration of NK cells in the TME serves as a reliable indicator of tumor prognosis. Bioinformatics analyses indicate that patients with glioma with high expression levels of NK cell activation genes exhibit significantly improved prognosis. Compared to higher-grade gliomas, the number of activated NK cells is significantly higher in lower-grade gliomas, suggesting a marked decrease in activated NK cells during the progression from low to high-grade gliomas.[65] Another study shows a significant correlation between the number of activated NK cells and the survival rate of patients with GBM.[66] Therefore, NK cells can prevent the malignant transformation of brain gliomas. They can effectively eliminate undifferentiated GSCs. IFN-γ released by NK cells promotes the differentiation of GSCs, enhancing their sensitivity

to chemotherapy but reducing their susceptibility to NK cell cytotoxicity.[67] Enhancing NK cell-mediated destruction of GSCs while promoting IFN-γ release to induce GSC differentiation represents a potential direction for targeted therapy.[68,69]

Application of NK Cells in Treating Glioma

Activate NK Cell Therapy

Central nervous system tumors typically exhibit poor immunogenicity and high immunosuppression levels, making immunotherapy less effective. The cellular activity of NK cells is influenced by the balance between inhibitory and activating receptors. Chemotherapeutic and molecularly targeted drugs for brain gliomas can upregulate the expression of NK cell-activating receptors and promote the release of cytokines or the induction of NKG2D ligand expression. Temozolomide (TMZ) and radiotherapy can increase NKG2D ligand expression in the glioma tissues of patients with GBM. Animal studies show that TMZ or radiation-induced NKG2D ligand expression prolongs survival in GBM mouse models. Activation of DNA damage repair (DDR) mechanisms can induce the production of NKG2D ligands, enhancing the antitumor function of NK cells. However, the proteasome inhibitor bortezomib (BTZ) can downregulate the expression of activating receptor NKG2D and DNAM-1 ligand by activating DDR pathways, thus promoting NK cell lethality against GBM. In a study involving experimental animals, the combination of autologous NK cells and BTZ significantly inhibits tumor growth and extends survival in 25% of cases.[68] Triple therapy combining BTZ with oncolytic herpes simplex virus (OHSV) and NK cells has shown greater efficacy in treating GBM, as BTZ enhances the expression of the NK cell-activating receptor DNAM-1. OHSV infection can induce NK cells to secrete IFN-γ and TNF-α, resulting in increased tumor cell death and improving survival rates in transplanted tumors in nude mice. Additionally, the demethylating agent decitabine increases NKG2D ligand expression, thereby restoring the cytotoxicity capability of NK cells against IDH mutant glioma cells.[69,70] The histone deacetylase inhibitor mucorin A induces the upregulation of NKG2D ligands, enhancing NK-cell-mediated cleavage of GBM cells.[71]

Recently, triple-functional antibodies that recognize tumor antigens while binding to NKp46 and CD16 have shown superior antitumor effects in vivo compared to that of traditional antibodies targeting tumor antigens.[72] Proteins encoded by the MHC-I chain-related genes A and B (MICA and MICB) are classified as NKG2D ligands. Antibodies targeting MICA and MICB prevent their loss from the surface of tumor cells, promoting the binding between NKG2D and its ligands and inhibiting tumor growth. The antitumor effects of these antibodies are primarily mediated through the activation of NKG2D and CD16.[73] KIRs can recognize MHC-I molecules on tumor cells, inhibiting NK cell activation and promoting tumor cell growth.[74] However, KIRs from allogeneic NK cells do not recognize the MHC-I molecules of the recipient patient, resulting in a lack of inhibitory signals and activating NK cells to effectively kill tumor cells. Utilizing NK cells with KIR2DS2 immune genotypes has proven effective in killing GBM cells and prolonging survival in animal models.[11,75,76]

NK Cell-Based Immunotherapy Against GBM

Given the benefits of immunotherapy utilizing NK cells, this approach is promising for treating GBM. NK cells can target GBM heterogeneity without the need for specific tumor-associated antigen recognition.[77–81] They effectively eliminate tumor cells that downregulate MHC-I expression on their surface,[82] as the interaction between MHC-I and KIRs inhibits NK cell activity and protects healthy autologous cells.[83,84] Given these advantages, Tables 1–3 summarizes current clinical studies investigating NK cell-based therapies for GBM.[85,86] Recent studies show the efficacy of intratumoral G47Δ treatment for residual or recurrent GBM using a stereotactic surgical system.[87,88] This method is particularly effective from a direct delivery standpoint, as it directly influences immune cells within the immunosuppressive GBMTME. Figure 11 illustrates various NK cell therapy approaches, including adoptive NK cell therapy, CAR-NK cell therapy, checkpoint-blocking therapy, and gene-edited NK therapy.

Table 1 Clinical Trials of CAR NK Cell Therapy Against Glioma

Clinical Trials	Phase	Administration	Source	Agent	Type
NCT03383978	I	I.I.	NK-92 cells	Anti-MUC1 CAR NK cells	Glioma
NCT02839954	I/II	I.V.	Autologous NK cells	NK-92/5.28.z cells	GBM

Abbreviations; I.I., Intracranial injection; I.V., Intravenous; MUC1, mucin 1.

Table 2 Clinical Trials of Adoptive NK Cell Therapy Against Glioma

Clinical Trials	Phase	Administration	Source	Agent	Type
NCT00909558	I	I.V.	Autologous NK cells	Autologous NK cells	Glioma
NCT04254419	I	Intra-tumoral injection	Autologous NK cells	NK-92/5.28.z cells	GBM
NCT02100891	II	I.V.	Autologous NK cells	Autologous NK cells with HLA haploidentical HCT	Glioma

Abbreviations: I.V., Intravenous; HCT, hematopoietic cell transplantation.

Table 3 Clinical Trials of LAK Cell Therapy Against Glioma

Clinical Trials	Phase	Administration	Source	Agent	Type
NCT00814593	II	Intra-tumoral injection	Autologous PBMCs	LAK cells	GBM
NCT00003067	II	Intra-tumoral injection	Autologous PBMCs	LAK cells with aldesleukin	Gliomas
NCT00331526	II	Intra-tumoral injection	Autologous PBMCs	LAK cells with aldesleukin	GBM
NCT00005813	I	Intra-tumoral injection	Autologous PBMCs	LAK cells with MDX477 bispecific antibody	GBM

Abbreviations: LAK, lymphokine-activated killer; PBMC, peripheral blood mononuclear cells.

Combination Therapy

The primary factor contributing to the low efficiency of immunotherapy for solid tumors is the immunosuppressive effect of TME. Current research targeting TIM and tumor cells in conjunction with NK cell therapy shows effectiveness in tumor treatment. Recent studies indicate that combining NK cells with monoclonal antibodies or chemotherapeutic agents significantly enhances the survival rate of animals with GBM. Chondroitin sulfate proteoglycan 4, also known as neuron glial antigen 2 (NG2), plays a crucial role in intracellular signaling, activating pathways such as ERK and FAK, which are closely associated with tumor growth and metastasis. Research shows that combining activated NK cells with the NG2-targeting monoclonal antibody mAb9.2.27 can inhibit tumor deterioration and extend the median survival time in GBM models. Studies of GBM antigens show that NK cells regulate the pro-inflammatory environment in GBM primarily through IFN-γ secretion rather than through direct cytotoxic action. The mechanism of the combined treatment involving NK cells and mAb9.2.27 in rat GBM models is based on the conversion of glioma-associated macrophages from an anti-inflammatory M2 phenotype to a pro-inflammatory M1 phenotype. This transformation results in significantly increased levels of IFN-γ and TNF-α in the cerebrospinal fluid of treated rats, while immunosuppressive cytokines such as IL-10, IL-6, and IL-1β are significantly decreased. Cancer adjuvants, including CpG oligonucleotide, can enhance the antitumor immune response by mimicking pathogen-associated molecular patterns and inducing plasmacytoid dendritic cells to produce type I interferon (IFN-I). IFN-I further enhances the antitumor activity of NK cells. Although CpG oligonucleotide therapy has shown promising results in vitro, in vivo studies involving patients with primary and recurrent gliomas, have not shown significant therapeutic effects using CpG oligonucleotide alone. Research shows that NK cells serve as the primary antitumor effector cells in mouse glioma models treated with a repeated low dose of CpG oligonucleotide. Despite their potential, tumor-infiltrating NK cells remain susceptible to local and systemic inhibitory signals, necessitating more effective strategies to enhance NK cell functionality within the brain TME.

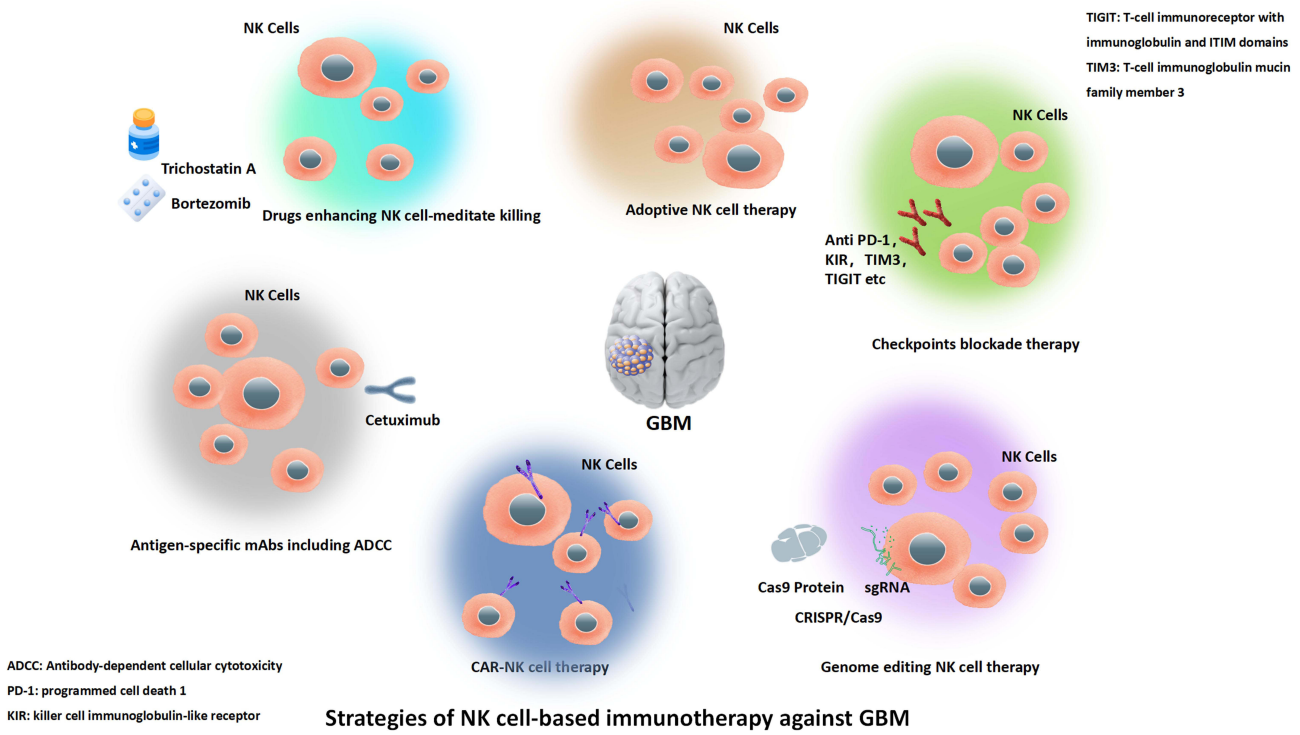

Figure 11 Multi-approaches of NK cell-based immunotherapy against GBM.

Combined treatment with CpG oligonucleotide and Treg elimination can enhance the cytotoxic effects of NK cells against brain glioma.

Malignant glioma-induced binding of human leukocyte antigen E (HLA-E) and lectin-like transcript 1 (LLT1) to CD94/NKG2A and CD161 inhibits NK cell function. Blocking the interaction of HLA-E with CD94/NKG2A or LLT1 with CD161 using small interfering RNAs or blocking antibodies promotes NK cell-mediated lysis of glioma cells. Moreover, combining the humanized anti-NKG2A antibody monalizumab with cetuximab enhances antibody-dependent cellular cytotoxicity (ADCC) in head and neck squamous cell carcinoma.[89] As EGFR serves as a critical target for various tumors, including gliomas, the ability of monalizumab to block NKG2A may augment NK cell-mediated ADCC against gliomas, especially TMZ-resistant gliomas.[90]

Combination therapy using anti-PD-1 and anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) has increased NK cell and CD8+T cell infiltration in the CNS, improving survival rates in GBM mouse models. The blood-brain barrier (BBB), a selectively permeable membrane, protects the brain parenchyma from harmful substances but also limits the entry of immunotherapeutic agents and immune cells. Poly (β-L-malic acid) can covalently bind to anti-PD-1 or anti-CTLA-4 antibodies, facilitating trans-BBB transport and enhancing NK cell infiltration and survival in mice vaccinated with the intracellular GL261 model.[91] Combining these techniques with cytolytic NK cells may help to overcome the challenges posed by BBB selective permeability and the immunosuppressive environment of the brain TME.[92]

Genetically Modified NK Cells

Chimeric antigen receptors (CARs) replace the α and β variable regions of the TCR with tumor-specific antibody single-chain variable regions (SCFV). These are fused to the CD3ζ signaling chain of T-cell or NK cell receptors through a transmembrane junction region. This enables the transduction of somatic toxic T lymphocytes (CAR-T) or NK cells (CAR-NK), allowing for the recognition of tumor-specific antigens through SCFVs, which facilitates targeted tumor cell killing (Figure 12). Adoptive transfer of NKG2D-based CAR T cells in combination with radiotherapy showed therapeutic synergy in a GL261 in situ glioma mouse model. Mice inoculated with GL261 intratuminal CAR T cells exhibited a higher survival rate than those injected with intravenous CAR T cells despite a lower number of CAR T cells

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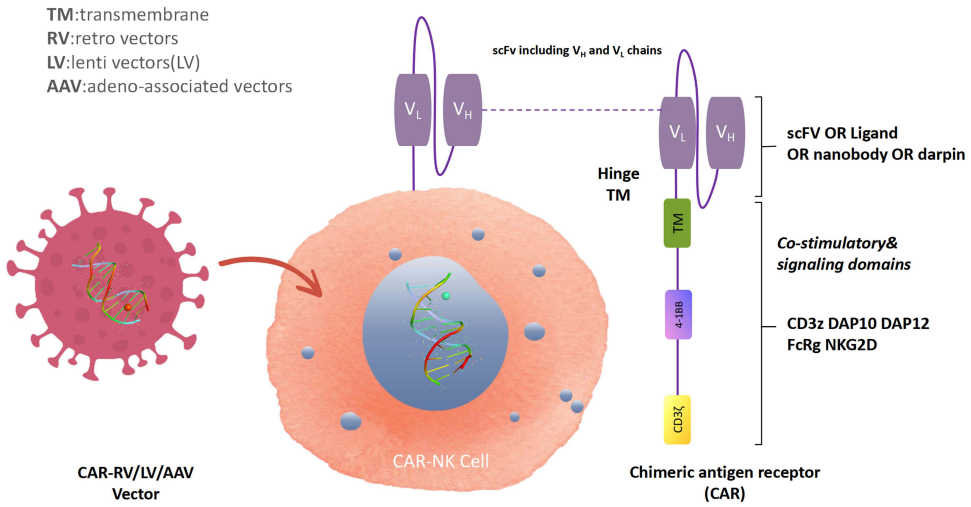

Figure 12 CRA-NK molecule delivery of genetic cargo NK cells with CAR encoding RV, LV or AAV.

being administered directly into the brain parenchyma. However, the continuous in vivo expansion of CAR-T cells can lead to cytokine release syndrome and subsequent graft-versus-host disease. These conditions significantly affect patient quality of life. CAR-NK cells mitigate these issues by targeting various tumor antigens, including EGFR and human epidermal growth factor receptor 2 (HER2, also known as ErbB2). These CAR-NK cells maintain natural cytotoxicity while targeting tumor antigens effectively.

EGFR gene amplification and protein overexpression occur in 40%–60% of GBM cases, while these markers are rarely expressed in normal brain tissue. GBM typically co-expresses EGFR and EGFR type III mutants (EGFRvIII). Different CAR-NK constructs can be designed to specifically target EGFR and EGFRvIII in GBM, depending on the target.[93] Studies confirm that CAR NK-92 cells, which exhibit high expression of NK cell-activating receptors, effectively inhibit the growth of xenografted gliomas that overexpress EGFR. By repeatedly injecting CAR NK-92 cells (which express high levels of NK cell-activating receptors) intracranially into GBM xenografted immunosuppressed mice (NSG mice) with EGFR or EGFRvIII expression, significant tumor growth inhibition and improved overall survival were observed compared to the control group treated with NK-92 cells alone.[94] HER2, a member of the EGFR family, is not expressed in the central nervous system of healthy adults but is present in 80% of GBM cases, where its expression correlates with poor survival outcomes. CAR-NK cells targeting EGFR, EGFRvIII, and HER2 have shown potent cytotoxic effects against primary GBM tumor cells and cell lines in vitro.[95] The injection of HER2-targeted NK cells into NSG mouse models with xenografted GBM cells effectively inhibits tumor progression and prolongs survival. HER2-NK -92/CAR-NK cells continue to exhibit tumor-killing abilities and retain the cytotoxic function of normal NK cells despite potential antigen loss. GSCs are susceptible to allogeneic NK cells, and specific targeting with HER2-CAR NK-92 cells can lyse HER2-positive GSCs. However, prolonged exposure may lead to the elimination of target cells by parental NK-92 cells that lack CAR. Oncolytic viruses (OVs) possess a dual mechanism of action, directly attacking cancer cells while also stimulating a tumor-specific immune response. Combining EGFR-CAR NK cells with OV-IL15C, which highly expresses a human IL15/IL15Rα sushi domain fusion protein, has been utilized to treat GBM in mouse models. The infiltration and activation levels of NK and CD8+ T cells in glioma tissue are significantly higher in the combined treatment group than in the single treatment group. Moreover, the effectiveness of the combined treatment is also significantly higher, resulting in a significant extension of survival time for the mice.[96] Therefore, the activation of CAR-NK cells is determined by the integration of CAR signals and the intrinsic signals of NK cells.

Genome-Editing NK Cell Therapy

This section provides an overview of genome-edited NK cell therapy, excluding CAR-NK therapy. The combination of adoptive NK cell therapy with checkpoint inhibitors presents a promising strategy for treating GBM, but the effectiveness of checkpoint inhibitors is influenced by the timing of administration and delivery efficiency, especially in GBM. Genome-edited NK cells can knock out immunosuppressive receptor genes, continuously blocking the expression of inhibitory receptors.

The CRISPR/Cas9 genetic-editing system is widely used to modify T cells by targeting inhibitory genes such as PD-1 and CTLA-4.[97–101] Conversely, CRISPR/Cas9 genome editing of NK cells has faced challenges, primarily due to low viral transduction efficiency; however, recent advances using Cas9/RNP electroporation have provided new insights.[102–105] Rautela et al describe the CRISPR/Cas9 genome editing of major human NK cells, particularly targeting the CISH gene and the NKp46 receptor.[105] Two groups were used to reveal the use of CRISPR/Cas9 through electroporation to create CD38 knockout NK cells.[106] These CD38 knockout NK cells show an improved metabolic profile, enhancing ADCC against CD38+ multiple myeloma cell lines and patient-derived samples.[106–108] Table 4 presents the CRISPR/Cas9-mediated NK cell-based therapies mentioned above.

For GBM therapy, several studies establish TIM-3 knockout major NK cells, which exhibit enhanced cytotoxicity against GBM.[110] In the experimental design, 106 orders of NK cells per shot were subjected to electroporation. The researchers were able to obtain 4.7×108 genetically modified NK cells from 16 mL of blood in 2 weeks, providing sufficient quantities for clinical application. In comparison, a previous study reports an electroporation setting of 105 orders of NK cells per session with significantly lower expansion efficacy.[105] Huang et al show that CRISPR/Cas9 knocks out multiple genes in major NK cells; however, these NK cells show no significant activation.[109] While CRISPR/Cas9 genome editing in major NK cells currently lags behind that in T cells, ongoing advancement in this technology indicates that it may offer a potential strategy for combating GBM.

Technical Bottleneck and Future Direction of NK Cell Therapy for Glioma

NK cell-based adoptive immunotherapy for cancer is a rapidly evolving field with the potential to benefit patients with solid tumors. This approach includes identifying and effectively blocking NK cell immune checkpoints, as well as utilizing specific killer cell junctions or redirecting NK cell tumor-specific lysis through CAR loading. However, the transgenic efficiency of primary NK cells is only approximately 15%, presenting a significant technical bottleneck of CAR-NK applications. Improving the efficiency of NK cell transgene delivery is an important research focus in CAR-NK cell therapy.[110] Currently, CAR-NK cells are entering clinical trials for the treatment of malignant gliomas.[51] In addition to ongoing studies on engineered CAR NK-92 cells, other allogeneic sources—such as peripheral or umbilical blood NK cells and NK cells differentiated from induced pluripotent stem cells—are also preparing to enter clinical trials for brain tumors. Although GBM shows strong adaptability that allows evasion of immune attacks and counteraction of

Table 4 CRISPR/Cas9 Electroporation Genome-Editing NK Cell-Based Immunotherapy Against GBM

Target	Source	Ref.
PD1	PBMCs isolated from healthy donor	[109]
NKG2A	PBMCs isolated from healthy donor	[2]
TIM3	PBMCs isolated from healthy donor	[109]
TIGIT	PBMCs isolated from healthy donor	[109]
TGF-β	PBMCs isolated from healthy donor	[3]

Abbreviations: GBM, glioblastoma; PD1, programmed death 1; TGF, transforming growth factor; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM3, T-cell immunoglobulin and mucin domain-containing 3; NKG2A, NK group 2A.

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immunosuppression, the heterogeneous expression of CAR-targeted antigens presents a significant challenge for immune escape. For instance, HER2-CAR-T therapy for GBM has shown that these T cells can metastasize into tumors, but the highly effective T cells within the TME lead to rapid selection of antigen-losing variants.[111] CAR-NK cells may encounter similar challenges. However, NK cells naturally exhibit diverse cytotoxic effects, with activated receptors interacting with various ligands expressed by tumor cells, including natural cytotoxic receptors (NKp46, NKp44, and NKp30), NKG2D, and DNAM-1 (CD226). These NK cell receptors typically recognize stress-inducing ligands expressed on immune or tumor cells exposed to prolonged therapeutic stress. Therefore, CAR-NK cells can inhibit glioma cells through CAR-dependent and NK cell receptor-dependent pathways, enabling the elimination of GBM cells that express low or no CAR-targeted antigens. The HER2-NK-92/5.28z CAR NK retains effector function despite potential antigen loss, thereby restoring the cytotoxic capacity of adoptive metastatic cells to that of baseline NK cells. Currently, HER2-CAR-NK therapy is undergoing clinical trials for GBM treatment (CAR2BRAIN; NCT0338978).

In comparison to CAR-T cells, CAR-NK cells exhibit a shorter lifespan and limited in vivo expansion. This results in higher safety during treatment; however, it also restricts long-term efficacy, necessitating repeated treatments to achieve significant effects. The ectopic expression of pro-inflammatory cytokines, such as IL-15 and CAR, enhances the persistence of CAR-NK cells and promotes T-cell penetration and activation. The strategy of inducing endogenous immune memory has emerged as a foundation for CAR-NK cell activation in GBM therapy.[112] In patients with GBM, a strategic combination of adoptive NK cell transfer with conventional therapies—such as radiation therapy, immune checkpoint inhibitors, angiogenesis inhibitors, or myeloid intercellular regulators—can modulate the function of innate and adaptive immune cells in the TME. This approach may effectively harness the cytotoxic effects of CAR-NK cells against GBM.[113–115]

Bio-Material-Based NK Cell Surface Modification Technologies

Up to now, varieties of manipulation techniques for decorating functional moieties onto cell surface and heightening target recognition have been developed[116] (Figure 13). A hydrophobic interaction-mediated ex-vivo cell surface engineering utilizing lipid-based bio-materials may be a most advanced technology that could accomplish efficient cell surface modification by a single way without influence of inherent properties of cells (especially, NK cells).[116] In order to NK cells with target-specificity, synthetic CD30-specific aptamers are modified on cell surfaces to result in aptamer-engineered NK cells (ApEn-NK) without gene mutation or other injuries. Under surface-modified aptamer guide, ApEn-NK specifically bind to CD30-expressing lymphoma cells but do not react to off-target cells. The research results specific cell binding of ApEn-NK triggers higher apoptosis/death rates of lymphoma cells compared to parental NK cells.

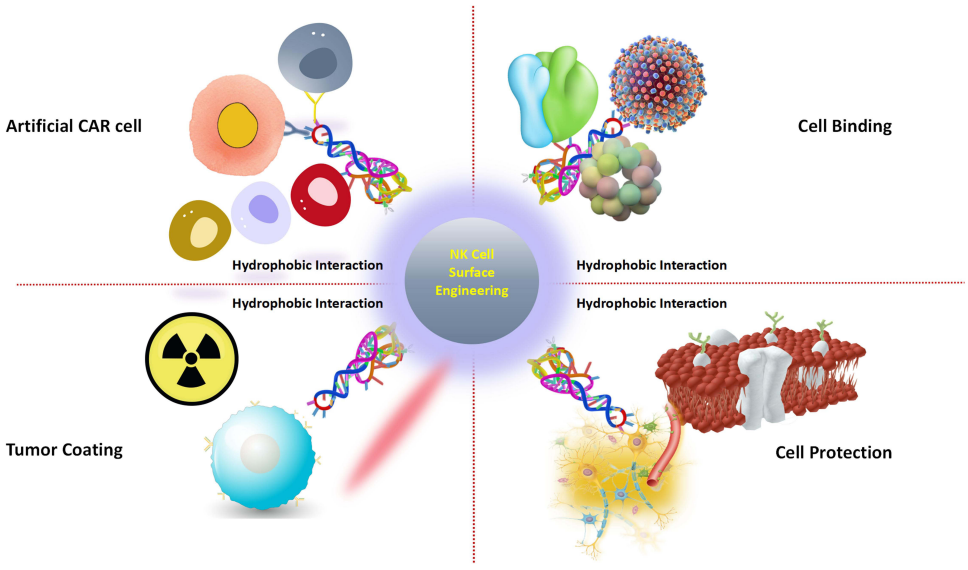

Figure 13 Bio-material-based NK cell surface modification technologies.

Additionally, experiments with primary human NK cells indicated the potential of ApEn-NK to specifically target and kill lymphoma cells, thus providing a novel method for targeted immunotherapy by NK cells.[117] In addition, one study reported a chemoenzymatic glycocalyx editing way to introduce high-affinity and specific CD22 ligands onto NK-92MI and cytokine-induced NK cells to obtain tumor-specific CD22 targeting. These CD22-ligand modified cells proved effectively raised tumor cell binding and killing in vitro without damaging normal B cells.[118] Another study, researcher developed synthetic lipid-folate conjugates, for steady modifying onto NK cell surfaces via hydrophobic interactions, thus increasing folate-mediated ligand-receptor immune interactions with target cancers.[119] Similarly, lipid-PEG conjugated hyaluronic acid (HA) materials (HA-PEG-Lipid) for the simple ex-vivo surface coating of NK cells is developed for 1) lipid-mediated cellular membrane anchoring via hydrophobic interaction and thus 2) fully demonstration of the CD44 ligand (i e, HA) onto NK cells for cancer targeting, without the need for genetic manipulation.[120] Therefore, with the continuous progress of biotechnology, we firmly believe bio-material-based NK cell surface modification will enhance NK cell therapy technologies for tumor treatment (especially, GBM)

Conclusions

NK cells serve as the first line of defense in the body and play a crucial role in combating cancer cells. NK cell infiltration in gliomas correlates with patient prognosis. Analysis of RNA sequencing data from the TCGA database indicates that NK cell infiltration scores in low-grade gliomas and GBMs are higher than those of T cells.[121] However, the glioma microenvironment inhibits NK cell function, leading to their dysfunction. When NK cells are injected into a patient, they often become dysfunctional due to inhibitory signals released by tumor cells or tumor-associated mononuclear cells/macrophages and rapidly lose their ability to recognize and eliminate tumors. Currently, little is understood about the mechanisms underlying immunotherapy resistance in GBM or the intrinsic properties of these tumors. Therefore, an in-depth exploration of NK cell dysfunction and the glioma microenvironment is essential for identifying strategies to reverse NK cell impairment. Dysregulation of N6-methyladenosine (m6A) modification is closely linked to the onset, progression, and drug resistance of gliomas.[122,123] Studies show that in pancreatic ductal adenocarcinoma with a high m6A score, T-cell dysfunction correlates with reduced NK cell presence. As an emerging field, the relationship between m6A modification status and NK cell function, as well as immune cell infiltration in gliomas, remains unclear.[124]

Current findings suggest that combining immunotherapy with other treatments may lead to breakthroughs in GBM therapy. Therefore, future glioma treatments are likely to focus on combining NK cell therapies with molecularly targeted drugs, including anti-angiogenic agents, EGFR inhibitors, and epigenetic modifiers. Currently, NK cell treatments are primarily in the animal experimental phase, and their application in patients is still a distance away. Ongoing clinical trials are expected to explore NK cell combination therapies further, necessitating a reevaluation of clinical trial designs to efficiently assess the potential clinical effect of these immunotherapy approaches. We believe that NK cell-based immunotherapy will exhibit improved efficacy in glioma treatment in the future.

Abbreviations

GBM, glioblastoma; TIM, tumor immune microenvironment; UCB, umbilical cord blood; MDSCs, myeloid-derived suppressor cells; GSCs, Glioma stem cells; IL-10, interleukin-10; IDO, indoleamine 2,3-Dioxygenase; Tregs, regulatory T cells; TMZ, Temozolomide; DDR, DNA damage repair; BTZ, bortezomib; OHSV, oncolytic herpes simplex virus; MICA, MHC-I chain-related genes A; MICB, MHC-I chain-related genes B; NG2, neuron glial antigen 2; IFN-I, type I interferon; HLA-E, human leukocyte antigen E; LLT1, lectin-like transcript 1; ADCC, antibody-dependent cellular cytotoxicity; CTLA-4, anti-cytotoxic T lymphocyte-associated protein 4; BBB, blood-brain barrier; CARs, Chimeric antigen receptors; SCFV, single-chain variable regions; HER2, human epidermal growth factor receptor 2; OVs, Oncolytic viruses.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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References

1. Yang DX, Jing Y, Xu ZM, et al. Primary glioblastoma of cerebellopontine angle in adult mimicking acoustic neuroma. *World Neurosurg*. 2019;122:48–52. doi:10.1016/j.wneu.2018.10.073
2. Davis FG, Smith TR, Gittleman HR, Ostrom QT, Kruchko C, Barnholtz-Sloan JS. Glioblastoma incidence rate trends in Canada and the United States compared with England, 1995–2015. *Neuro Oncol*. 2020;22(2):301–302. doi:10.1093/neuonc/noz203
3. Lanier LL. Five decades of natural killer cell discovery. *J Exp Med*. 2024;221(8):e20231222. doi:10.1084/jem.20231222
4. Crinier A, Narni-Mancinelli E, Ugolini S, Vivier E. SnapShot: natural killer cells. *Cell*. 2020;180(6):1280–1280.e1. doi:10.1016/j.cell.2020.02.029
5. Pan C, Zhai Y, Li G, Jiang T, Zhang W. NK cell-based immunotherapy and therapeutic perspective in gliomas. *Front Oncol*. 2021;11:751183. doi:10.3389/fonc.2021.751183
6. Xu W, Cherrier DE, Chea S, et al. An Id2RFP-reporter mouse redefines innate lymphoid cell precursor potentials. *Immunity*. 2019;50(4):1054–1068.e3. doi:10.1016/j.immuni.2019.02.022
7. Scoville SD, Mundy-Bosse BL, Zhang MH, et al. A progenitor cell expressing transcription factor RORγt generates all human innate lymphoid cell subsets. *Immunity*. 2016;44(5):1140–1150. doi:10.1016/j.immuni.2016.04.007
8. André P, Spertini O, Guia S, et al. Modification of P-selectin glycoprotein ligand-1 with a natural killer cell-restricted sulfated lactosamine creates an alternate ligand for L-selectin. *Proc Natl Acad Sci USA*. 2000;97(7):3400–3405. doi:10.1073/pnas.97.7.3400
9. Crinier A, Milpied P, Escalière B, et al. High-dimensional single-cell analysis identifies organ-specific signatures and conserved NK cell subsets in humans and mice. *Immunity*. 2018;49(5):971–986.e5. doi:10.1016/j.immuni.2018.09.009
10. Vivier E, Raulet DH, Moretta A, et al. Innate or adaptive immunity? The example of natural killer cells. *Science*. 2011;331(6013):44–49. doi:10.1126/science.1198687
11. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. *Nat Rev Cancer*. 2016;16(1):7–19. doi:10.1038/nrc.2015.5
12. Wu SY, Fu T, Jiang YZ, Shao ZM. Natural killer cells in cancer biology and therapy. *Mol Cancer*. 2020;19(1):120. doi:10.1186/s12943-020-01238-x
13. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. *Nat Immunol*. 2016;17(9):1025–1036. doi:10.1038/ni.3518
14. Moretta A, Bottino C, Vitale M, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Ann Rev Immunol*. 2001;19:197–223. doi:10.1146/annurev.immunol.19.1.197
15. Pessino A, Sivori S, Bottino C, et al. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. *J Exp Med*. 1998;188(5):953–960. doi:10.1084/jem.188.5.953
16. Vitale M, Bottino C, Sivori S, et al. NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. *J Exp Med*. 1998;187(12):2065–2072. doi:10.1084/jem.187.12.2065
17. Pende D, Parolini S, Pessino A, et al. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *J Exp Med*. 1999;190(10):1505–1516. doi:10.1084/jem.190.10.1505
18. Brandt CS, Baratin M, Yi EC, et al. The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans. *J Exp Med*. 2009;206(7):1495–1503. doi:10.1084/jem.20090681
19. Binici J, Hartmann J, Herrmann J, et al. A soluble fragment of the tumor antigen BCL2-associated athanogene 6 (BAG-6) is essential and sufficient for inhibition of NKp30 receptor-dependent cytotoxicity of natural killer cells. *J Biol Chem*. 2013;288(48):34295–34303. doi:10.1074/jbc.M113.483602
20. Rosental B, Brusilovsky M, Hadad U, et al. Proliferating cell nuclear antigen is a novel inhibitory ligand for the natural cytotoxicity receptor NKp44. *J Iimmunol*. 2011;187(11):5693–5702. doi:10.4049/jimmunol.1102267
21. Niehrs A, Garcia-Beltran WF, Norman PJ, et al. A subset of HLA-DP molecules serve as ligands for the natural cytotoxicity receptor NKp44. *Nat Immunol*. 2019;20(9):1129–1137. doi:10.1038/s41590-019-0448-4
22. Bryceson YT, March ME, Barber DF, Ljunggren HG, Long EO. Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. *J Exp Med*. 2005;202(7):1001–1012. doi:10.1084/jem.20051143

23. Gunesch JT, Angelo LS, Mahapatra S, et al. Genome-wide analyses and functional profiling of human NK cell lines. *Mol Immunol*. 2019;115:64–75. doi:10.1016/j.molimm.2018.07.015
24. Dogra P, Rancan C, Ma W, et al. Tissue determinants of human NK cell development, function, and residence. *Cell*. 2020;180(4):749–763.e13. doi:10.1016/j.cell.2020.01.022
25. Angelo LS, Banerjee PP, Monaco-Shawver L, et al. Practical NK cell phenotyping and variability in healthy adults. *Immunol Res*. 2015;62(3):341–356. doi:10.1007/s12026-015-8664-y
26. Björkström NK, Ljunggren HG, Michaëlsson J. Emerging insights into natural killer cells in human peripheral tissues. *Nat Rev Immunol*. 2016;16(5):310–320. doi:10.1038/nri.2016.34
27. Verneris MR, Miller JS. The phenotypic and functional characteristics of umbilical cord blood and peripheral blood natural killer cells. *Br J Haematol*. 2009;147(2):185–191. doi:10.1111/j.1365-2141.2009.07768.x
28. Heipertz EL, Zynda ER, Stav-Noraas TE, et al. Current perspectives on "Off-The-Shelf" Allogeneic NK and CAR-NK cell therapies. *Front Immunol*. 2021;12:732135. doi:10.3389/fimmu.2021.732135
29. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood*. 2005;105(8):3051–3057. doi:10.1182/blood-2004-07-2974
30. Romee R, Rosario M, Berrien-Elliott MM, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci Trans Med*. 2016;8(357):357ra123. doi:10.1126/scitranslmed.aaf2341
31. Huang Q, Huang M, Meng F, Sun R. Activated pancreatic stellate cells inhibit NK cell function in the human pancreatic cancer microenvironment. *Cell Mol Immunol*. 2019;16(1):87–89. doi:10.1038/s41423-018-0014-2
32. Klingemann H, Boissel L, Toneguzzo F. Natural killer cells for immunotherapy—advantages of the NK-92 cell line over blood NK cells. *Front Immunol*. 2016;7:91. doi:10.3389/fimmu.2016.00091
33. Passweg JR, Tichelli A, Meyer-Monard S, et al. Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. *Leukemia*. 2004;18(11):1835–1838. doi:10.1038/sj.leu.2403524
34. Tonn T, Schwabe D, Klingemann HG, et al. Treatment of patients with advanced cancer with the natural killer cell line NK-92. *Cytotherapy*. 2013;15(12):1563–1570. doi:10.1016/j.jcyt.2013.06.017
35. Knorr DA, Ni Z, Hermanson D, et al. Clinical-scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy. *Stem Cells Transl Med*. 2013;2(4):274–283. doi:10.5966/sctm.2012-0084
36. Ni Z, Knorr DA, Clouser CL, et al. Human pluripotent stem cells produce natural killer cells that mediate anti-HIV-1 activity by utilizing diverse cellular mechanisms. *J Virol*. 2011;85(1):43–50. doi:10.1128/JVI.01774-10
37. Woll PS, Grzywacz B, Tian X, et al. Human embryonic stem cells differentiate into a homogeneous population of natural killer cells with potent in vivo antitumor activity. *Blood*. 2009;113(24):6094–6101. doi:10.1182/blood-2008-06-165225
38. Terrén I, Orrantia A, Vitallé J, Zenarruzabeitia O, Borrego F. NK cell metabolism and tumor microenvironment. *Front Immunol*. 2019;10:2278. doi:10.3389/fimmu.2019.02278
39. Myers JA, Miller JS. Exploring the NK cell platform for cancer immunotherapy. *Nat Rev Clin Oncol*. 2021;18(2):85–100. doi:10.1038/s41571-020-0426-7
40. Tarazona R, Lopez-Sejas N, Guerrero B, et al. Current progress in NK cell biology and NK cell-based cancer immunotherapy. *Cancer Immunol Immunother*. 2020;69(5):879–899. doi:10.1007/s00262-020-02532-9
41. Granzin M, Wagner J, Köhl U, Cerwenka A, Huppert V, Ullrich E. Shaping of natural killer cell antitumor activity by ex vivo cultivation. *Front Immunol*. 2017;8:458. doi:10.3389/fimmu.2017.00458
42. Pelletier A, Nelius E, Fan Z, et al. Resting natural killer cell homeostasis relies on tryptophan/NAD+ metabolism and HIF-1α. *EMBO Rep*. 2023;24(6):e56156. doi:10.15252/embr.202256156
43. Muñoz M, Coveñas R. Glioma and neurokinin-1 receptor antagonists: a new therapeutic approach. *Anticancer Agents Med Chem*. 2019;19(1):92–100. doi:10.2174/1871520618666180420165401
44. Cao Y, Feng YH, Gao LW, et al. Artemisinin enhances the anti-tumor immune response in 4T1 breast cancer cells in vitro and in vivo. *Int Immunopharmacol*. 2019;70:110–116. doi:10.1016/j.intimp.2019.01.041
45. Han F, Guo S, Huang C, et al. Gastric cancer mesenchymal stem cells inhibit natural killer cell function by up-regulating FBP1. *Cent-Eur J Immunol*. 2021;46(4):427–437. doi:10.5114/ceji.2021.111753
46. Nachef M, Ali AK, Almutairi SM, Lee SH. Targeting SLC1A5 and SLC3A2/SLC7A5 as a potential strategy to strengthen anti-tumor immunity in the tumor microenvironment. *Front Immunol*. 2021;12:624324. doi:10.3389/fimmu.2021.624324
47. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol*. 2003;3(10):781–790. doi:10.1038/nri1199
48. Cho U, Yang SH, Yoo C. Estimation of the occurrence rates of IDH1 and IDH2 mutations in gliomas and the reconsideration of IDH-wildtype anaplastic astrocytomas: an institutional experience. *Int J Med Res*. 2021;49(6):3000605211019258. doi:10.1177/03000605211019258
49. Ren F, Zhao Q, Huang L, et al. The R132H mutation in IDH1 promotes the recruitment of NK cells through CX3CL1/CX3CR1 chemotaxis and is correlated with a better prognosis in gliomas. *Immunol Cell Biol*. 2019;97(5):457–469. doi:10.1111/imcb.12225
50. Gonzalez-Junca A, Driscoll KE, Pellicciotta I, et al. Autocrine TGFβ is a survival factor for monocytes and drives immunosuppressive lineage commitment. *Cancer Immunol Res*. 2019;7(2):306–320. doi:10.1158/2326-6066.CIR-18-0310
51. Shaim H, Shanley M, Basar R, et al. Targeting the αv integrin/TGF-β axis improves natural killer cell function against glioblastoma stem cells. *J Clin Invest*. 2021;131(14):e142116. doi:10.1172/JCI142116
52. Castriconi R, Cantoni C, Della Chiesa M, et al. Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. *Proc Natl Acad Sci USA*. 2003;100(7):4120–4125. doi:10.1073/pnas.0730640100
53. Close HJ, Stead LF, Nsengimana J, et al. Expression profiling of single cells and patient cohorts identifies multiple immunosuppressive pathways and an altered NK cell phenotype in glioblastoma. *Clin Exp Immunol*. 2020;200(1):33–44. doi:10.1111/cei.13403
54. Wei J, Barr J, Kong LY, et al. Glioblastoma cancer-initiating cells inhibit T-cell proliferation and effector responses by the signal transducers and activators of transcription 3 pathway. *Mol Cancer Ther*. 2010;9(1):67–78. doi:10.1158/1535-7163.MCT-09-0734
55. Raychaudhuri B, Rayman P, Ireland J, et al. Myeloid-derived suppressor cell accumulation and function in patients with newly diagnosed glioblastoma. *Neuro Oncol*. 2011;13(6):591–599. doi:10.1093/neuonc/nor042

56. Rodrigues JC, Gonzalez GC, Zhang L, et al. Normal human monocytes exposed to glioma cells acquire myeloid-derived suppressor cell-like properties. *Neuro Oncol*. 2010;12(4):351–365. doi:10.1093/neuonc/nop023
57. Laudati E, Currò D, Navarra P, Lisi L. Blockade of CCR5 receptor prevents M2 microglia phenotype in a microglia-glioma paradigm. *Neurochem Int*. 2017;108:100–108. doi:10.1016/j.neuint.2017.03.002
58. Held-Feindt J, Hattermann K, Müerköster SS, et al. CX3CR1 promotes recruitment of human glioma-infiltrating microglia/macrophages (GIMs). *Exp Cell Res*. 2010;316(9):1553–1566. doi:10.1016/j.yexcr.2010.02.018
59. O'Brien KL, Finlay DK. Immunometabolism and natural killer cell responses. *Nat Rev Immunol*. 2019;19(5):282–290. doi:10.1038/s41577-019-0139-2
60. Dekker LJM, Verheul C, Wensveen N, et al. Effects of the IDH1 R132H mutation on the energy metabolism: a comparison between tissue and corresponding primary glioma cell cultures. *ACS Omega*. 2022;7(4):3568–3578. doi:10.1021/acsomega.1c06121
61. Holl EK, Frazier VN, Landa K, Beasley GM, Hwang ES, Nair SK. Examining peripheral and tumor cellular immunome in patients with cancer. *Front Immunol*. 2019;10:1767. doi:10.3389/fimmu.2019.01767
62. Griessmair L, Pirringer L, Mountford S, et al. Expression of IL-37 correlates with immune cell infiltrate and fibrosis in pediatric autoimmune liver diseases. *J Pediatr Gastroenterol Nutr*. 2022;74(6):742–749. doi:10.1097/MPG.0000000000003443
63. Sivori S, Parolini S, Marcenaro E, et al. Involvement of natural cytotoxicity receptors in human natural killer cell-mediated lysis of neuroblastoma and glioblastoma cell lines. *J Neuroimmunol*. 2000;107(2):220–225. doi:10.1016/s0165-5728(00)00221-6
64. Krenzlin H, Behera P, Lorenz V, et al. Cytomegalovirus promotes murine glioblastoma growth via pericyte recruitment and angiogenesis. *J Clin Invest*. 2019;129(4):1671–1683. doi:10.1172/JCI123375
65. Lu J, Li H, Chen Z, et al. Identification of 3 subpopulations of tumor-infiltrating immune cells for malignant transformation of low-grade glioma. *Can Cell Inter*. 2019;19:265. doi:10.1186/s12935-019-0972-1
66. Mostafa H, Pala A, Högel J, et al. Immune phenotypes predict survival in patients with glioblastoma multiforme. *J Hematol Oncol*. 2016;9(1):77. doi:10.1186/s13045-016-0272-3
67. Tseng HC, Inagaki A, Bui VT, et al. Differential targeting of stem cells and differentiated glioblastomas by NK cells. *J Cancer*. 2015;6(9):866–876. doi:10.7150/jca.11527
68. Zeng X, Yao D, Liu L, et al. Terminal differentiation of bone marrow NK cells and increased circulation of TIGIT+ NK cells may be related to poor outcome in acute myeloid leukemia. *Asia-Pac J Clini Oncol*. 2022;18(4):456–464. doi:10.1111/ajco.13723
69. Razavi SM, Lee KE, Jin BE, Aujla PS, Gholamin S, Li G. Immune evasion strategies of glioblastoma. *Front Surg*. 2016;3:11. doi:10.3389/fsurg.2016.00011
70. Zhang X, Kim WJ, Rao AV, et al. In vivo efficacy of decitabine as a natural killer cell-mediated immunotherapy against isocitrate dehydrogenase mutant gliomas. *Neurosurg Focus*. 2022;52(2):E3. doi:10.3171/2021.11.FOCUS21489
71. Höring E, Podlech O, Silkenstedt B, Rota IA, Adamopoulou E, Naumann U. The histone deacetylase inhibitor trichostatin a promotes apoptosis and antitumor immunity in glioblastoma cells. *Anticancer Res*. 2013;33(4):1351–1360.
72. Gauthier L, Morel A, Anceriz N, et al. Multifunctional natural killer cell engagers targeting NKp46 trigger protective tumor immunity. *Cell*. 2019;177(7):1701–1713.e16. doi:10.1016/j.cell.2019.04.041
73. Ferrari de Andrade L, Tay RE, Pan D, et al. Antibody-mediated inhibition of MICA and MICB shedding promotes NK cell-driven tumor immunity. *Science*. 2018;359(6383):1537–1542. doi:10.1126/science.aao0505
74. Grabowski MM, Sankey EW, Ryan KJ, et al. Immune suppression in gliomas. *J Neuro-Oncol*. 2021;151(1):3–12. doi:10.1007/s11060-020-03483-y
75. Petitprez F, Meylan M, de Reyniès A, Sautès-Fridman C, Fridman WH. The tumor microenvironment in the response to immune checkpoint blockade therapies. *Front Immunol*. 2020;11:784. doi:10.3389/fimmu.2020.00784
76. Maskalenko NA, Zhigarev D, Campbell KS. Harnessing natural killer cells for cancer immunotherapy: dispatching the first responders. *Nat Rev Drug Discov*. 2022;21(8):559–577. doi:10.1038/s41573-022-00413-7
77. Bergmann N, Delbridge C, Gempt J, et al. The intratumoral heterogeneity reflects the intertumoral subtypes of glioblastoma multiforme: a regional immunohistochemistry analysis. *Front Oncol*. 2020;10:494. doi:10.3389/fonc.2020.00494
78. Qazi MA, Vora P, Venugopal C, et al. Intratumoral heterogeneity: pathways to treatment resistance and relapse in human glioblastoma. *Ann Oncol*. 2017;28(7):1448–1456. doi:10.1093/annonc/mdx169
79. Shen SH, Woroniecka K, Barbour AB, Fecci PE, Sanchez-Perez L, Sampson JH. CAR T cells and checkpoint inhibition for the treatment of glioblastoma. *Expert Opin Biol Ther*. 2020;20(6):579–591. doi:10.1080/14712598.2020.1727436
80. Böttcher JP, Bonavita E, Chakravarty P, et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell*. 2018;172(5):1022–1037.e14. doi:10.1016/j.cell.2018.01.004
81. Zhou X, Yu J, Cheng X, et al. The deubiquitinase Otub1 controls the activation of CD8+ T cells and NK cells by regulating IL-15-mediated priming. *Nat Immunol*. 2019;20(7):879–889. doi:10.1038/s41590-019-0405-2
82. Bix M, Liao NS, Zijlstra M, Loring J, Jaenisch R, Raulet D. Rejection of class I MHC-deficient haemopoietic cells by irradiated MHC-matched mice. *Nature*. 1991;349(6307):329–331. doi:10.1038/349329a0
83. Nicholson SE, Keating N, Belz GT. Natural killer cells and anti-tumor immunity. *Mol Immunol*. 2019;110:40–47. doi:10.1016/j.molimm.2017.12.002
84. Li J, Hu H, Lian K, et al. CAR-NK cells in combination therapy against cancer: a potential paradigm. *Heliyon*. 2024;10(5):e27196. doi:10.1016/j.heliyon.2024.e27196
85. Lamers-Kok N, Panella D, Georgoudaki AM, et al. Natural killer cells in clinical development as non-engineered, engineered, and combination therapies. *J Hematol Oncol*. 2022;15(1):164. doi:10.1186/s13045-022-01382-5
86. Ishikawa E, Takano S, Ohno T, Tsuboi K. Adoptive cell transfer therapy for malignant gliomas. *Adv Exp Med Biol*. 2012;746:109–120. doi:10.1007/978-1-4614-3146-6_9
87. Smith C, Lineburg KE, Martins JP, et al. Autologous CMV-specific T cells are a safe adjuvant immunotherapy for primary glioblastoma multiforme. *J Clin Invest*. 2020;130(11):6041–6053. doi:10.1172/JCI138649
88. Izumoto S, Tsuboi A, Oka Y, et al. Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. *J Neurosurg*. 2008;108(5):963–971. doi:10.3171/JNS/2008/108/5/0963

89. André P, Denis C, Soulas C, et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. *Cell*. 2018;175(7):1731–1743.e13. doi:10.1016/j.cell.2018.10.014
90. Meng X, Zhao Y, Han B, et al. Dual functionalized brain-targeting nanoinhibitors restrain temozolomide-resistant glioma via attenuating EGFR and MET signaling pathways. *Nat Commun*. 2020;11(1):594. doi:10.1038/s41467-019-14036-x
91. Galstyan A, Markman JL, Shatalova ES, et al. Blood-brain barrier permeable nano immunoconjugates induce local immune responses for glioma therapy. *Nat Commun*. 2019;10(1):3850. doi:10.1038/s41467-019-11719-3
92. Barrow AD, Colonna M. Tailoring Natural Killer cell immunotherapy to the tumour microenvironment. *Semin Immunopathol*. 2017;31:30–36. doi:10.1016/j.smim.2017.09.001
93. Liu C, He Y, Feng X, Li J, Wang J. Expression of EPHA5 in lung adenocarcinoma is associated with lymph node metastasis and EGFR mutation. *APMIS*. 2022;130(6):338–345. doi:10.1111/apm.13222
94. Genßler S, Burger MC, Zhang C, et al. Dual targeting of glioblastoma with chimeric antigen receptor-engineered natural killer cells overcomes heterogeneity of target antigen expression and enhances antitumor activity and survival. *Oncoimmunology*. 2015;5(4):e1119354. doi:10.1080/2162402X.2015.1119354
95. Shams A, Shabani R, Asgari H, et al. In vitro elimination of EL4 cancer cells from spermatogonia stem cells by miRNA-143- and 206-loaded folic acid-conjugated PLGA nanoparticles. *Nanomedicine*. 2022;17(8):531–545. doi:10.2217/nnm-2021-0210
96. Ma R, Lu T, Li Z, et al. An oncolytic virus expressing IL15/IL15Rα combined with off-the-shelf EGFR-CAR NK cells targets glioblastoma. *Cancer Res*. 2021;81(13):3635–3648. doi:10.1158/0008-5472.CAN-21-0035
97. Zhao Z, Shi L, Zhang W, et al. CRISPR knock out of programmed cell death protein 1 enhances anti-tumor activity of cytotoxic T lymphocytes. *Oncotarget*. 2017;9(4):5208–5215. doi:10.18632/oncotarget.23730
98. Zhang W, Shi L, Zhao Z, et al. Disruption of CTLA-4 expression on peripheral blood CD8 + T cell enhances anti-tumor efficacy in bladder cancer. *Cancer Chemother Pharmacol*. 2019;83(5):911–920. doi:10.1007/s00280-019-03800-x
99. Yahata T, Mizoguchi M, Kimura A, et al. Programmed cell death ligand 1 disruption by clustered regularly interspaced short palindromic repeats/Cas9-genome editing promotes antitumor immunity and suppresses ovarian cancer progression. *Cancer Sci*. 2019;110(4):1279–1292. doi:10.1111/cas.13958
100. Su S, Zou Z, Chen F, et al. CRISPR-Cas9-mediated disruption of PD-1 on human T cells for adoptive cellular therapies of EBV positive gastric cancer. *Oncoimmunology*. 2016;6(1):e1249558. doi:10.1080/2162402X.2016.1249558
101. Nakazawa T, Natsume A, Nishimura F, et al. Effect of CRISPR/Cas9-Mediated PD-1-disrupted primary human third-generation CAR-T cells targeting EGFRvIII on in vitro human glioblastoma cell growth. *Cells*. 2020;9(4):998. doi:10.3390/cells9040998
102. Naeimi Kararoudi M, Tullius BP, Chakravarti N, et al. Genetic and epigenetic modification of human primary NK cells for enhanced antitumor activity. *Semin hematol*. 2020;57(4):201–212. doi:10.1053/j.seminhematol.2020.11.006
103. Kararoudi MN, Likhite S, Elmas E, et al. CRISPR-targeted CAR gene insertion using Cas9/RNP and AAV6 enhances anti-AML activity of primary NK cells. bioRxiv. 2021.
104. Naeimi Kararoudi M, Nagai Y, Elmas E, et al. CD38 deletion of human primary NK cells eliminates daratumumab-induced fratricide and boosts their effector activity. *Blood*. 2020;136(21):2416–2427. doi:10.1182/blood.2020006200
105. Rautela J, Surgenor E, Huntington ND. Drug target validation in primary human natural killer cells using CRISPR RNP. *J Leukoc Biol*. 2020;108(4):1397–1408. doi:10.1002/JLB.2MA0620-074R
106. Gurney M, Stikvoort A, Nolan E, et al. CD38 knockout natural killer cells expressing an affinity optimized CD38 chimeric antigen receptor successfully target acute myeloid leukemia with reduced effector cell fratricide. *Haematologica*. 2022;107(2):437–445. doi:10.3324/haematol.2020.271908
107. Zhu H, Blum RH, Bernareggi D, et al. Metabolic reprogramming via deletion of CISH in human iPSC-derived NK cells promotes in vivo persistence and enhances anti-tumor activity. *Cell Stem Cell*. 2020;27(2):224–237.e6. doi:10.1016/j.stem.2020.05.008
108. Pomeroy EJ, Hunzeker JT, Kluesner MG, et al. A genetically engineered primary human natural killer cell platform for cancer immunotherapy. *Mol Ther*. 2020;28(1):52–63. doi:10.1016/j.ymthe.2019.10.009
109. Huang RS, Lai MC, Shih HA, Lin S. A robust platform for expansion and genome editing of primary human natural killer cells. *J Exp Med*. 2021;218(3):e20201529. doi:10.1084/jem.20201529
110. Morimoto T, Nakazawa T, Matsuda R, et al. CRISPR-Cas9-Mediated TIM3 knockout in human natural killer cells enhances growth inhibitory effects on human glioma cells. *Int J Mol Sci*. 2021;22(7):3489. doi:10.3390/ijms22073489
111. Brown CE, Badie B, Barish ME, et al. Bioactivity and safety of IL13Rα2-redirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. *Clin Cancer Res*. 2015;21(18):4062–4072. doi:10.1158/1078-0432.CCR-15-0428
112. Zhang C, Burger MC, Jennewein L, et al. ErbB2/HER2-specific NK cells for targeted therapy of glioblastoma. *J National Cancer Inst*. 2015;108(5):10.1093/jnci/djv375. doi:10.1093/jnci/djv375
113. Dar TB, Henson RM, Shiao SL. Targeting innate immunity to enhance the efficacy of radiation therapy. *Front Immunol*. 2019;9:3077. doi:10.3389/fimmu.2018.03077
114. Sevenich L. Turning "Cold" into "Hot" tumors-opportunities and challenges for radio-immunotherapy against primary and metastatic brain cancers. *Front Oncol*. 2019;9:163. doi:10.3389/fonc.2019.00163
115. Gras Navarro A, Espedal H, Joseph JV, et al. Pretreatment of glioblastoma with bortezomib potentiates natural killer cell cytotoxicity through TRAIL/DR5 mediated apoptosis and prolongs animal survival. *Cancers*. 2019;11(7):996. doi:10.3390/cancers11070996
116. Kim S, Kim K. Lipid-mediated ex vivo cell surface engineering for augmented cellular functionalities. *Biomater Adv*. 2022;140:213059. doi:10.1016/j.bioadv.2022.213059
117. Yang S, Wen J, Li H, et al. Aptamer-engineered natural killer cells for cell-specific adaptive immunotherapy. *Small*. 2019;15(22):e1900903. doi:10.1002/smll.201900903
118. Hong S, Yu C, Wang P, et al. Glycoengineering of NK cells with glycan ligands of CD22 and selectins for B-cell lymphoma therapy. *Angew Chem*. 2021;60(7):3603–3610. doi:10.1002/anie.202005934
119. Kim S, Li S, Gajendiran M, et al. Lipid anchor-mediated NK cell surface engineering for enhanced cancer immunotherapy. *Chem Eng J*. 2023;473:145211. doi:10.1016/j.cej.2023.145211

120. Kim S, Li S, Jangid AK, et al. Surface engineering of natural killer cells with CD44-targeting ligands for augmented cancer immunotherapy. *Small*. 2024;20(24):e2306738. doi:10.1002/smll.202306738
121. Cózar B, Greppi M, Carpentier S, Narni-Mancinelli E, Chiossone L, Vivier E. Tumor-infiltrating natural killer cells. *Cancer Discov*. 2021;11(1):34–44. doi:10.1158/2159-8290.CD-20-0655
122. Tassinari V, Cesarini V, Tomaselli S, et al. ADAR1 is a new target of METTL3 and plays a pro-oncogenic role in glioblastoma by an editing-independent mechanism. *Genome Biol*. 2021;22(1):51. doi:10.1186/s13059-021-02271-9
123. Chang YZ, Chai RC, Pang B, et al. METTL3 enhances the stability of MALAT1 with the assistance of HuR via m6A modification and activates NF-κB to promote the malignant progression of IDH-wildtype glioma. *Cancer Lett*. 2021;511:36–46. doi:10.1016/j.canlet.2021.04.020
124. Zhou Z, Zhang J, Xu C, et al. An integrated model of N6-methyladenosine regulators to predict tumor aggressiveness and immune evasion in pancreatic cancer. *EBioMedicine*. 2021;65:103271. doi:10.1016/j.ebiom.2021.103271