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EDITORIAL

Next-generation CAR-NK cell therapy for glioblastoma: strategies to overcome antigen escape and the immunosuppressive tumor microenvironment

Samuel J. Steuart[a,b,c], Eric P. Grewal[a,b], Jing Sun[a,b], Leland G. K. Richardson[a,b], Uriel S. Bulow[c,d,e], Wilfredo F. Garcia-Beltran[c,d,e], William T. Curry Jr.[a,b,c] and Bryan D. Choi[a,b,c]

[a]Brain Tumor Immunotherapy Lab, Mass General Brigham, Boston, MA, USA; [b]Department of Neurosurgery, Mass General Brigham, Boston, MA, USA; [c]Harvard Medical School, Boston, MA, USA; [d]Ragon Institute of Mass General, MIT and Harvard, Cambridge, MA, USA; [e]Department of Pathology, Mass General Brigham, Boston, MA, USA

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1. Introduction

Chimeric antigen receptor (CAR)-NK cells represent a versatile, 'off-the-shelf' immunotherapeutic platform designed to overcome the antigen heterogeneity and immunosuppressive hurdles that have historically limited T-cell therapies in solid tumors. By reviewing NK cell biology alongside clinical challenges like poor infiltration and persistence, this article examines how CAR-NK cells may help overcome antigen escape and the immunosuppressive tumor microenvironment (TME) as a next-generation treatment for glioblastoma (GBM).

GBM is the most common and lethal malignant primary brain tumor, conferring a median survival between 8 and 15 months [1,2]. The standard of care for GBM has seen few advances that meaningfully improve prognosis since the introduction of the Stupp protocol in 2005, which consists of maximal safe resection followed by radiotherapy and temozolomide (TMZ) [2]. Immunotherapy has transformed outcomes in many hematologic and solid malignancies; however, its efficacy in GBM has been hampered given the constraints of antigenic heterogeneity and escape and the immunosuppressive TME [3,4].

CAR-T cells have been studied in patients with GBM, demonstrating feasibility and transient radiographic responses [4,5], though durable responses remain rare [4]. Antigen escape and limited durability have driven emerging strategies emphasizing multiantigen targeting and the exploration of alternative immune effector cell (IEC) sources with distinct biological properties [4–6].

Natural killer (NK) cells are innate, cytotoxic lymphocytes that kill tumor cells by detecting stress-induced ligands and/or the tumor-associated loss of MHC class I expression [7]. Unlike T cells, this enables NK cells to kill antigen-negative tumor cells without relying on the expression of a specific antigen. CAR constructs originally designed for T cells have been transduced into NK cells, generating CAR-NK cells with enhanced tumor specificity and cytotoxicity while simultaneously propelling CARs tailored to NK cell biology [8]. CAR-NK cells combine the antigen-specific cytotoxicity of CARs with the innate, antigen-independent cytotoxicity of NK cells [8]. CAR-NK cell approaches in GBM have thus far been limited by short *in vivo* persistence and susceptibility to the immunosuppressive TME. Here, we review NK cell biology and discuss how CAR-NK cells and their unique properties could be leveraged to overcome these barriers and enhance antitumor efficacy.

2. NK cell biology, mechanisms, and immunotherapeutic potential

NK cells are innate, lymphoid-derived IECs that lack T-cell receptors (TCRs) or CD3 complexes and are classified by surface proteins CD56 and CD16a: $CD56^{bright}CD16^{low/-}$ (immunomodulatory) and $CD56^{dim}CD16^{+}$ (cytotoxicity) [7,8]. When an antibody is present, NK cells mediate antibody-dependent cell cytotoxicity (ADCC) via CD16a, a low-affinity IgG Fc receptor. NK cells have MHC-I-specific inhibitory receptors, including killer cell immunoglobulin-like receptors (KIRs), that inhibit activation upon engagement [7,8]. NK cell activation occurs when stress-induced ligands are upregulated and detected on target cells [7]. Canonical examples of NK cell receptors recognizing stress-induced ligands on tumor cells for activation include NKG2D recognizing MICA/B and ULBPs, 2B4 recognizing CD48, DNAM-1 recognizing PVR and Nectin-2, and natural cytotoxicity receptors like NKp30 and NKp46 recognizing B7-H6 and PCNA or heparan sulfate, respectively (Figure 1) [7,9,10]. Other than CD16a, no single activating receptor can induce NK cell killing, requiring combinations like NKG2D and 2B4 [9]. Once activated, NK cells kill via cytolytic granule releases or FAS ligand-mediated cytotoxicity, and can sequentially engage multiple targets [7]. The balance of NK cells' activating and inhibitory receptors underlies why GBM, which often downregulates MHC class I expression, may be particularly vulnerable to NK cell killing.

Because NK cells lack TCRs or CD3, they can be used as 'off-the-shelf' allogeneic products without causing graft-versus-host disease (GVHD), conferring a favorable safety profile compared to CAR-T cells [8]. NK cells can be derived from several sources, including immortalized NK cell lines (e.g.

CONTACT Bryan D. Choi bchoi@mgh.harvard.edu Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA

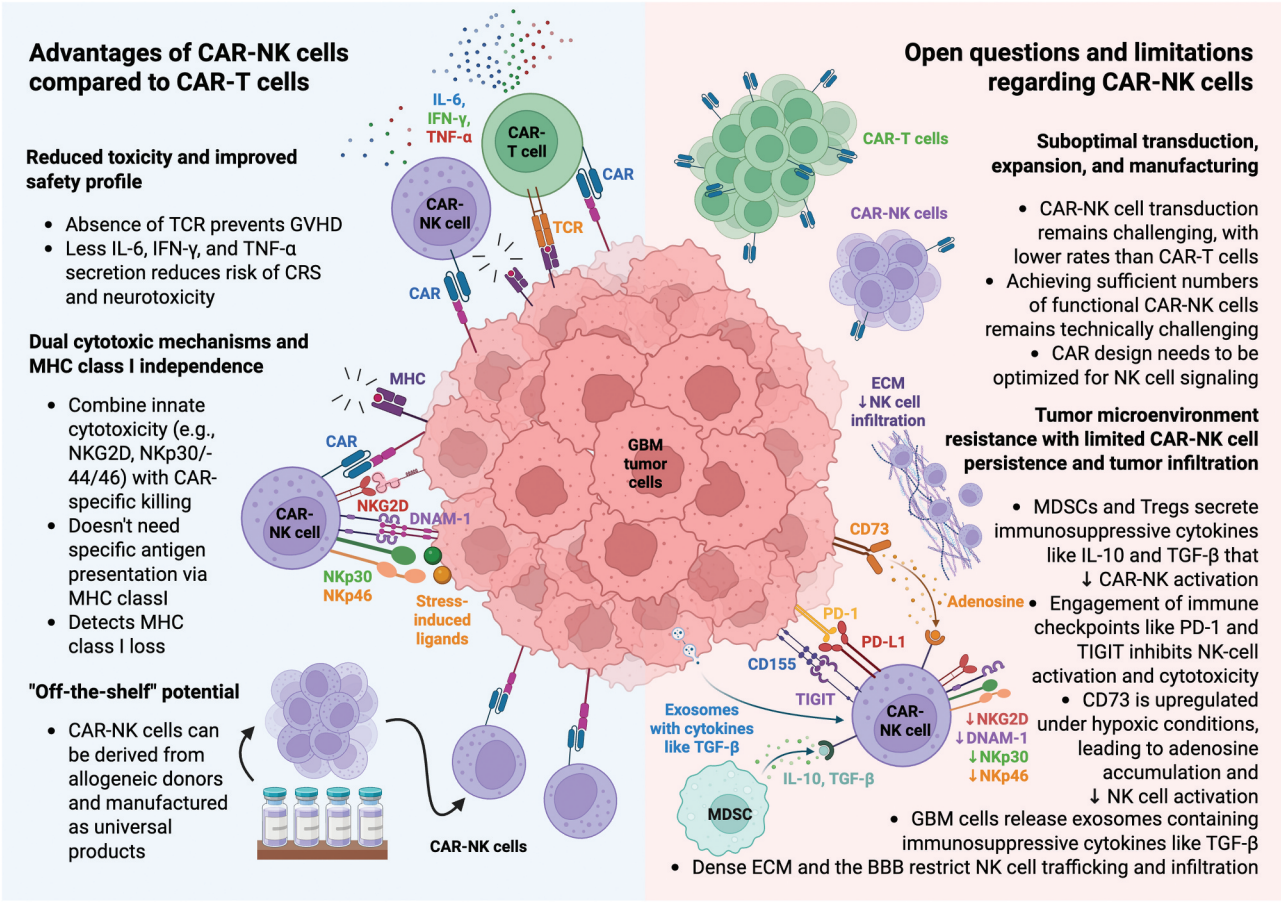

Figure 1. Advantages and limitations of CAR-NK cells in GBM. Created in BioRender. Steuart, S. (2026) https://BioRender.com/0crmub3.

BBB: blood–brain barrier; CAR: Chimeric antigen receptor; CRS: cytokine release syndrome; ECM: extracellular matrix; GVHD: Graft-versus-host disease; MDSC: Myeloid-derived suppressor cell; MHC: major histocompatibility complex; TCR: T-cell receptor. Image created using BioRender.com with reference to [7–13,16].

NK92), peripheral or umbilical cord blood, or induced pluripotent stem cells (iPSCs) [8]. Advancements in *ex vivo* conditioning enable large-scale administration of stimulated NK cells, while ongoing genetic engineering enhancements may help overcome some of the immunosuppressive features of GBM [7].

3. NK cell challenges in treating GBM

In the TME, NK cells' antitumor function is impaired by GBM cells' reduced expression of NK activating ligands and increased inhibitory signals [11]. Immunosuppressive cells in the TME, including myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), secrete cytokines like interleukin-10 (IL-10) and transforming growth factor β (TGF-β), reducing NK-activating receptor expression and anti-tumor cytokine production [10]. GBM cells also secrete exosomes, which carry immunosuppressive molecules like TGF-β [10]. In the hypoxic TME, upregulation of CD39/CD73 leads to adenosine accumulation, suppressing NK cell metabolism and effector function via purinergic signaling [8,12]. Additional factors in the TME that impair NK cell function include immune checkpoints (e.g. PD-1/PD-L1, TIGIT/CD96/CD155, and LAG-3/TIM-3) and extracellular matrix (ECM)-mediated physical barriers to infiltration (Figure 1) [10,13].

GBM stem cells (GSCs) may represent key therapeutic targets due to their role in initiation, progression, and therapeutic resistance [10,14]. Several *in vitro* studies show that NK cells selectively kill GSCs [10,14]. In patient-derived GBM tissue sections, NK cells localize near perivascular niches, suggesting that they may overcome BBB limitations and target GSCs in brain tissue [14]. Together with MDSCs, GSCs generate an immunosuppressive and metabolically hostile TME that impairs NK cell activity, promotes tumor persistence, and gives GSCs a survival advantage [14].

4. CAR-NK cell therapy: structure, function, advantages, and limitations

CARs originally designed for T cells, including CD3ζ-based constructs, have generated CAR-NK cells that safely eliminate tumor cells [8,15]. Designing CARs with transmembrane and intracellular signaling domains based on activating signals associated with NK cell biology improves CAR-NK activation and persistence [8,9,16].

Unlike CAR-T cells, CAR-NK cells combine innate and CAR-mediated cytotoxicity, enabling resistance to antigen escape in tumors with antigen heterogeneity or MHC class I loss [7,8,10]. CAR-NK cells also exhibit lower risks of neurotoxicity and CRS given their altered cytokine profile and absence of

long-term *in vivo* persistence, as well as GVHD via the absence of TCRs, which enables 'off-the-shelf' allogeneic use [8]. Since patients with GBM often have lymphocyte exhaustion that could complicate autologous CAR-T manufacturing, iPSC-derived or banked allogeneic CAR-NK cells offer a practical alternative. iPSC-derived NK cells can be engineered early to integrate synthetic enhancements that target solid tumors better via improved trafficking and persistence [16]. Furthermore, cell-line derived CAR-NK cells, particularly from iPSC sources, facilitate standardized, large-scale manufacturing by offering a homogenous and renewable platform that bypasses the donor-to-donor variability and limited expansion capacity inherent to primary NK cell products [16].

Primary NK cells are intrinsically resistant to lentiviral and retroviral infection, which presents a barrier to genetic engineering [16]. Unlike CAR-T cells, CAR-NK cells exhibit transient *in vivo* persistence even with exogenous cytokine support, compromising long-term tumor control [16]. CAR-NK cells derived from peripheral or umbilical cord donors face inter-donor heterogeneity in receptor repertoire and expansion potential, adding additional complexity to manufacturing and efficacy [16].

5. Preclinical and clinical evidence for CAR-NK cells in GBM

Preclinical studies demonstrate that CAR-NK cells targeting single GBM antigens mediate antitumor effects and prolong survival in murine orthotopic GBM models, although responses are variable and often transient [12,13]. Next-generation CAR-NK cells incorporate features such as dual-targeting receptors for multiple antigen coverage or cytokine-armored CARs (e.g. IL-15) to improve persistence [10,13].

The first clinical trial of CAR-NK cells in GBM delivered HER2-targeting CAR-NK92 cells intracranially to nine patients. The therapy was well tolerated, with no CRS or neurotoxicity and five patients demonstrating radiographically stable disease for 7–37 weeks [17]. This trial established the feasibility of CAR-NK delivery to the CNS and enabled next-generation CAR-NK studies for GBM.

6. Expert opinion

CAR-NK cells could drive next-generation immunotherapies for GBM. Future efforts should improve CAR-NK cells' trafficking, resistance to the immunosuppressive TME, ability to overcome antigen heterogeneity and escape, and integration into multimodal GBM immunotherapy regimens.

6.1. Optimizing CAR-NK cell trafficking

Modulating chemokines to enhance NK cell homing is a promising strategy to improve their trafficking to GBM. Simultaneously engaging multiple chemokine receptors enhances chemotaxis, suggesting CAR-NK cell trafficking could be improved with multi-receptor engineering [10,13]. Locally, combination strategies such as oncolytic viruses (OVs) or radiotherapy may elevate chemokine levels [10,13,18]. Additional approaches include temporarily disrupting the BBB with focused ultrasound and switching from systemic to intraventricular or intratumoral CAR-NK cell delivery [10,13,19]. Optimizing clinical accessibility will require comparative research into the trade-offs between delivery ease and tumor penetration to evaluate whether systemic intravenous infusion augmented by blood–brain barrier disruption can achieve comparable efficacy to localized strategies such as intraventricular, intracavitary, or convection-enhanced delivery. Ultimately, the transition to 'off-the-shelf' allogeneic platforms must be paired with delivery methods that can be standardized across non-academic centers to ensure these therapies reach a broad patient population.

6.2. Resisting GBM's immunosuppressive TME

Strategies must enhance persistence by optimizing CAR-NK cells' balance of activation and inhibition. CAR design should be optimized to reflect activating signals associated with NK cell biology [8], guided by recent evidence that NK receptor-based domains, particularly those involving the synergistic combination of NK receptors, induce potent activation in NK cells [9]. CAR-NK constructs should also avoid tonic signaling to reduce activation-induced functional exhaustion [13]. Persistence can be enhanced by cytokine armoring [16], with IL-15 armoring demonstrating superior persistence in preclinical *in vivo* models when compared to CAR-NK cells alone [8]. CRISPR-based gene editing offers multiple avenues to enhance CAR-NK persistence and resistance to immunosuppression in the TME, including introducing TGF-β or adenosine resistance or knocking out NKG2A or inhibitory checkpoints like LAG-3 [8,10,13].

6.3. Overcoming antigen heterogeneity and escape

Dual- or multi-antigen targeting and logic-gated CAR-NK constructs may be useful strategies to overcome GBM's antigen heterogeneity and escape [8]. CAR-NK cells combining several of these aforementioned features, including multiantigen targeting, cytokine armoring, and localized activation circuits, have overcome antigen heterogeneity and demonstrated enhanced resistance to immunosuppression in preclinical GBM models [12]. Logic-gated CAR constructs may safeguard against on-target/off-tumor activity, broaden antigen coverage, and reduce the risk of antigen escape [13]. Preclinical studies have shown that CAR-NK cells targeting GSCs led to potent tumor reduction and delayed recurrence, suggesting GSC-targeting should be further optimized and included in next-generation GBM therapeutics [10,12,14].

6.4. Exploring combination strategies

Finally, combination strategies may eventually integrate CAR-NK platforms into multimodal GBM immunotherapy regimens. When combined with radiotherapy or TMZ, CAR-NK cell approaches could be enhanced by therapy-induced upregulation of stress-induced ligands and depletion of immunosuppressive populations, while pairing with immune checkpoint inhibitors or monoclonal antibodies may relieve inhibitory signaling and enhance ADCC. Combination with

OV treatment may further augment tumor immunogenicity, chemokine-driven NK cell recruitment, and intratumoral immune activation [10,13,14,16]. Additionally, the combination of CAR-T and CAR-NK cell therapies may offer biological synergy by pairing the long-term persistence and memory of T cells with the rapid, innate-driven cytotoxicity of NK cells to more effectively mitigate antigen escape [8,13]. Ultimately, any combination strategy must be carefully weighed against the increased technical complexity and manufacturing costs, which may undermine the inherent accessibility and 'off-the-shelf' advantages of standalone allogeneic CAR-NK products [16].

7. Conclusion

CAR-NK cells are a versatile immunotherapeutic platform with the potential to overcome key barriers to advancing GBM treatment. Continued refinement of NK-specific engineering and rational integration into multimodal regimens will determine their ultimate clinical impact.

Author contributions

CRediT: **Samuel J. Steuart:** Conceptualization, Writing – original draft, Writing – review & editing; **Eric P. Grewal:** Conceptualization, Supervision, Writing – review & editing; **Jing Sun:** Supervision, Writing – review & editing; **Leland G. K. Richardson:** Supervision; **Uriel S. Bulow:** Writing – review & editing; **Wilfredo F. Garcia-Beltran:** Supervision, Writing – review & editing; **William T. Curry Jr.:** Supervision; **Bryan D. Choi:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Disclosure statement

BD Choi declares advisory roles to Biodexa Pharmaceuticals and Third Rock Ventures; patent filings related to chimeric antigen receptor T cell therapy; and financial interest in Altido Therapeutics, Inc., which is developing CAR T cells as therapeutics for multiple indications based on the CAR-TEAM technology developed at the MGH Cancer Center. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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