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CAR-T Cell Therapy for Glioblastoma: Obstacles and Advances

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ABSTRACT

Glioblastoma (GBM) is the most aggressive primary malignancy of the central nervous system. Chimeric antigen receptor T (CAR-T) cell therapy has shown promising therapeutic potential against GBM, yet its efficacy remains constrained by multiple barriers, including physical barriers imposed by the blood–brain barrier and extracellular matrix, the immunosuppressive tumor microenvironment, spatiotemporal antigen heterogeneity, and safety concerns. In this review, we summarize the major obstacles limiting CAR-T therapy in GBM and discuss emerging strategies to overcome these challenges. Next-generation engineered CAR-T cells—through armored modifications, logic-gated regulation, and dual-targeting approaches—enhance specificity, persistence, and controllability. Concurrently, combinatorial approaches leveraging biomaterials enable localized delivery and sustained release of CAR-T cells, while physical modalities, such as focused ultrasound and thermal modulation, can transiently disrupt the blood–brain barrier or induce immunogenic cell death. Integration with real-time imaging further enables dynamic monitoring of therapeutic responses. Together, these synergistic strategies may enhance antitumor efficacy while minimizing systemic toxicity, paving the way for future CAR-T-based therapies in glioblastoma.

1. Introduction

Glioblastoma (GBM) is the most aggressive primary brain tumor. Its location within the central nervous system, immunosuppressive microenvironment, and antigen heterogeneity pose major challenges for standard therapies (Louis et al., 2021; Tan et al., 2020). The current standard—maximal surgical resection, radiotherapy, and temozolomide chemotherapy—offers limited survival benefit. Median overall survival is only 12–15 months (Stupp et al., 2005). Chimeric antigen receptor T (CAR-T) cell therapy has emerged as a promising immunotherapeutic approach for addressing this unmet clinical need. Yet, its application in GBM faces four main barriers: (i) physical barriers imposed by the blood–brain barrier (BBB) and dense extracellular matrix (ECM), which block immune cell infiltration(Huang et al., 2022; Montoya et al., 2024); (ii) an immunosuppressive environment maintained by regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and inhibitory cytokines like TGF-β and IL-10(Mi et al., 2020); (iii) heterogeneous antigen expression, allowing immune evasion(Barish et al., 2022; Park et al., 2024); and (iv) safety risks, including on-target/off-tumor toxicity and immune effector cell-associated neurotoxicity syndrome (ICANS), which narrow the therapeutic window(Grant et al., 2022).

CAR-T therapy has undergone four generations of development, demonstrating remarkable benefits in hematological malignancies (Dagar et al., 2023; Patel et al., 2025). However, in the context of solid tumors such as GBM, conventional CAR-T architectures exhibit intrinsic limitations, underscoring the need for next-generation designs to enhance antitumor potency, improve tumor-targeted infiltration, refine antigen specificity, and mitigate off-target toxicity (Hamieh et al., 2023; Kwon and Chen, 2025). Nonetheless, engineering CAR-T cells alone remains insufficient to overcome obstacles such as low delivery efficiency and limited local persistence. Therefore, supplementary approaches are being investigated (Chen et al., 2022; Vo et al., 2025).

Combining smart biomaterials with CAR-T therapy represents an emerging approach for precision immunotherapy in GBM. Biomaterials exhibit good biocompatibility and can modulate immune responses. They can enable localized delivery and sustained release of therapeutic agents (Chao et al., 2023; Logun et al., 2025). Physical modalities such as focused ultrasound can temporarily disrupt the BBB. Advanced

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imaging techniques such as MRI and MPI enable noninvasive monitoring of CAR-T distribution and therapeutic responses (Sabbagh et al., 2021; Wu et al., 2023).

In this review, we first summarize the biological barriers that limit CAR-T efficacy in GBM. We then examine two main strategies: (i) engineering CAR-T cells with logic-gated circuits, armored modifications, and enhanced tumor infiltration capacity to create "intelligent" cells, and (ii) building biomaterial-based platforms for precise delivery and local remodeling, supported by physical interventions. Finally, we discuss challenges associated with clinical use and future research directions to facilitate the clinical translation of CAR-T therapy for GBM.

2. Literature Search Strategy

A literature search was performed using PubMed, Web of Science, and Scopus databases to identify studies related to CAR-T cell therapy for glioblastoma. Search terms were selected based on previously published studies and adjusted during the literature screening process. The following combinations of keywords were used: ("glioblastoma" OR "glioma" OR "high-grade glioma") AND ("chimeric antigen receptor T cell" OR "CAR-T" OR "CAR T-cell therapy") AND ("immunotherapy" OR "adoptive cell therapy").

Additional searches were performed using keywords related to major biological barriers and emerging therapeutic strategies, including "blood–brain barrier", "tumor microenvironment", "antigen heterogeneity", "biomaterials", "drug delivery", "focused ultrasound", and "imaging". Relevant original research articles, clinical studies, and review articles published up to January 2026 were considered. Studies addressing the limitations of CAR-T therapy, strategies for improving therapeutic efficacy, and clinical applications in glioblastoma were included. Reference lists of selected publications were also screened to identify additional relevant studies.

3. CAR-T Cell Therapy

CAR-T cell therapy has emerged as a well-established modality for hematological malignancies. Upon intravenous infusion, effector T cells circulate systemically to specifically recognize and eliminate tumor cells. This approach has demonstrated remarkable efficacy in hematological malignancies. Notably, in 2017, the U.S. Food and Drug Administration (FDA) approved Tisagenlecleucel for the treatment of relapsed or refractory acute lymphoblastic leukemia (ALL) (Maude et al., 2018), followed by the approval of Axicabtagene Ciloleucel for large B-cell lymphoma, marking the clinical advent of CAR-T therapy in hematologic malignancies (Nastoupil et al., 2020). The impressive outcomes observed in these contexts have spurred interest in extending CAR-T strategies to solid tumors. However, solid tumors such as glioblastoma face unique obstacles, including a profoundly immunosuppressive microenvironment, restrictive physiological barriers, and pronounced tumor heterogeneity, which collectively limit the efficacy of CAR-T interventions (Patel et al., 2025).

3.1. What is CAR-T?

Chimeric antigen receptor T (CAR-T) cells are genetically engineered immune cells. They contain a single-chain variable fragment (scFv), a CD8 hinge, a transmembrane domain (TMD), and an intracellular CD3ζ signaling domain (ICD). CAR-T cells recognize and kill target cells without relying on MHC antigen presentation. Their specific binding to tumor-associated antigens enables the selective targeting and destruction of malignant cells (Patel et al., 2025).

CAR-T designs have evolved through four generations (Fig. 1). The first generation comprised only the scFv–CD3ζ construct, second- and third-generation CARs incorporated one or more co-stimulatory domains (e.g., CD28, 4–1BB) to enhance T cell activation, persistence, and antitumor efficacy. Fourth-generation CARs, also known as TRUCKs (T cells redirected for universal cytokine-mediated killing), are further engineered to secrete immunomodulatory cytokines, thereby augmenting their therapeutic potential (Patel et al., 2025).

The first clinical evaluation of CAR-T therapy in recurrent glioblastoma (GBM) was conducted in 2015 by Brown et al., who administered autologous CD8 + cytotoxic T cells targeting IL13Rα2 directly into the tumor in three patients (Brown et al., 2015). In two patients, no tumor recurrence was observed at the infusion sites; MRI analysis of one patient showed increased necrotic volume at the treatment site. The third patient underwent surgical intervention due to disease progression, with pathology revealing an overall decrease in IL13Rα2 expression. Only transient adverse events were reported in two patients, indicating preliminary safety of intracavitary CAR-T delivery. This early study showed that CAR-T therapy could be used for GBM but also identified ongoing challenges: the immunosuppressive tumor microenvironment, antigen heterogeneity, and delivery limitations (Brown et al., 2015).

3.2. Challenges of CAR-T therapy in GBM

Glioblastoma (GBM) is pathologically distinct and more challenging than blood cancers. Systemically administered CAR-T cells must cross the blood–brain barrier (BBB) and traverse the dense extracellular matrix (ECM) to reach the tumor. Inside the brain, effector cells must work in a strongly immunosuppressive environment. Tumor cells also exhibit high heterogeneity, often mutating or losing antigens to evade immune attack. Safety risks during therapy further complicate treatment. In this section, we discuss the biology behind these four main challenges and outline why next-generation CAR-T or combined strategies are needed.

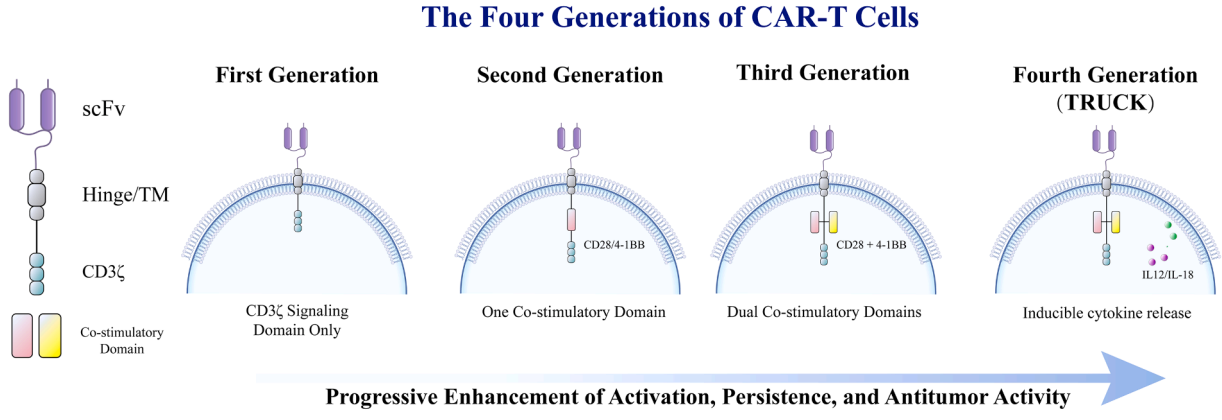

Fig. 1. The four generations of CAR-T cells.

3.2.1. Physical barriers

In hematologic malignancies, CAR-T cells are typically administered intravenously, allowing systemic circulation to facilitate efficient tumor cell contact and clearance, thereby achieving durable disease remission. By contrast, in GBM, intravenously delivered CAR-T cells must cross the highly specialized BBB, composed of endothelial cells, astrocytes, pericytes, and specialized tight junction structures. Although GBM-associated invasion can partially disrupt BBB integrity, this effect is variable and generally insufficient for effective CAR-T delivery. The first clinical trial evaluating intravenously administered EGFRvIII-targeted CAR-T cells in recurrent GBM (NCT02209376) demonstrated that, while CAR-T cells could penetrate the BBB and infiltrate tumors, infiltration levels varied markedly between patients, and clinical efficacy was limited (O'Rourke et al., 2017).

Beyond the BBB, CAR-T cells encounter a second physical obstacle: the abnormally dense GBM ECM. This matrix, enriched in collagen and hyaluronic acid (HA) and remodeled by cancer-associated fibroblasts (CAFs), forms a fibrotic barrier that significantly impedes immune cell penetration and migration. Preclinical studies indicate that hyaluronidase (HAase)-mediated HA degradation can enhance intratumoral CAR-T infiltration (Collado et al., 2024; Kiyokawa et al., 2021). However, uncontrolled HA degradation may release low-molecular-weight fragments that paradoxically promote tumor invasiveness (Bhattacharyya et al., 2023; Unnikandam Veettil et al., 2021).

Given the dual impediment of the BBB and ECM, systemic administration alone often fails to achieve sufficient local CAR-T accumulation. Consequently, researchers have explored direct tumor-targeted delivery strategies, including intratumoral (ICT) and intraventricular (ICV) injection, postoperative cavity infusion, and convection-enhanced delivery (CED). These approaches substantially increase local CAR-T concentration, reduce systemic exposure, and in some cases, elicit distal antitumor immune responses. For instance, Brown and colleagues demonstrated that intraventricular administration of IL13Rα2-targeted CAR-T cells in recurrent multifocal GBM induced local tumor regression comparable to intratumoral delivery, with notable differences in control of distant and metastatic lesions (Brown et al., 2016). In a 2024 Phase I study evaluating three regional delivery approaches (ICT, ICV, and dual ICT/ICV) in 65 patients, all routes were well-tolerated without dose-limiting toxicities (DLTs) (Brown et al., 2024). Median overall survival (mOS) was 7.7 months, 50% of patients achieved disease stabilization (SD) for at least 2 months, and two patients each achieved partial (PR) and complete remission (CR). These findings confirm the safety and feasibility of regional CAR-T administration and suggest that dual delivery, combined with optimized T cell manufacturing, may confer a survival benefit.

In summary, the BBB and ECM constitute the primary physical barriers limiting CAR-T efficacy in GBM (Fig. 2). Future strategies should focus on optimizing delivery routes, modulating ECM composition, and developing BBB-disruption techniques. While systemic administration alone yields limited clinical benefit in GBM monotherapy, integration with these approaches holds significant translational promise.

3.2.2. Immunosuppressive tumor microenvironment

Even after successfully infiltrating the tumor, CAR-T cells must operate within a profoundly immunosuppressive microenvironment. GBM is a prototypical "cold" tumor, characterized by a paucity of tumor-infiltrating lymphocytes (TILs) and a marked enrichment of immunosuppressive cell populations (Grabowski et al., 2021).

At the cellular level, the inhibitory network comprises regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs). Both peripheral blood and tumor tissues from GBM patients exhibit elevated MDSC levels (Gielen et al., 2015; Raychaudhuri et al., 2011). MDSCs suppress T cell proliferation through arginase-mediated arginine depletion and induce apoptosis via nitric oxide (NO)-mediated disruption of the Jak3/STAT5 signaling pathway (Bingisser et al., 1998; Rodriguez et al., 2005). Additionally, MDSCs promote macrophage polarization toward a tumor-supportive M2 phenotype through direct cell–cell contact and IL-10 secretion (Ostrand-Rosenberg et al., 2012).

At the molecular level, immunosuppressive signaling is equally complex. Transforming growth factor-beta (TGF-β), a key inhibitory cytokine within the tumor microenvironment (TME), suppresses effector T cell differentiation via the SMAD pathway while promoting Treg expansion (Li et al., 2006). Immune checkpoint pathways, notably PD−1/PD-L1, are highly active in GBM. Chronic exposure of CAR-T cells to antigen stimulation and immunosuppressive factors upregulates

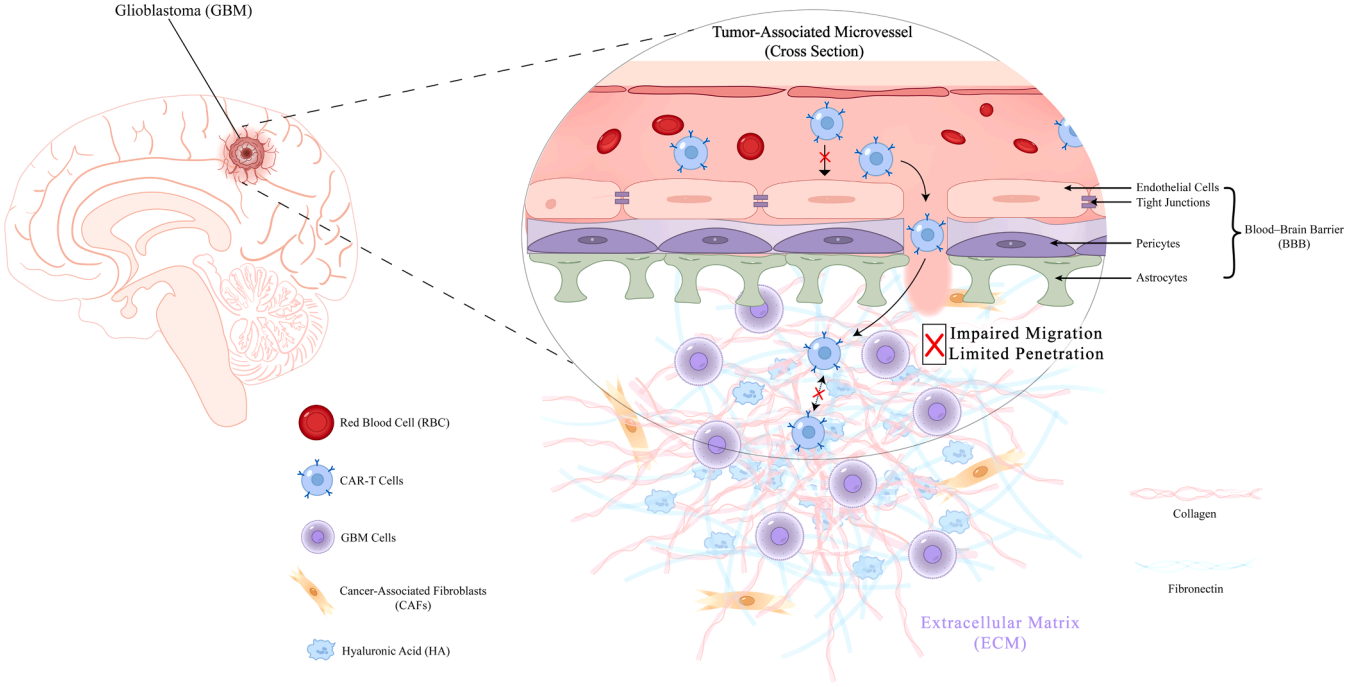

Fig. 2. Physical barriers to CAR-T cells in glioblastoma.

inhibitory receptors such as PD−1, which, upon engagement with PD-L1 on tumor cells, triggers signaling cascades that induce T cell exhaustion (Ouyang et al., 2024).

Aberrant tumor metabolism further exacerbates immunosuppression. Hypoxia, nutrient deprivation, lactate accumulation from aerobic glycolysis, and tryptophan metabolites such as kynurenine directly inhibit T cell function (Chang and Pearce, 2016) (see Table 1 for a detailed summary).

Collectively, these observations indicate that simply enhancing the intrinsic cytotoxicity of CAR-T cells is insufficient to overcome the suppressive TME. Effective strategies require combinatorial approaches to modulate the tumor microenvironment and restore immune functionality.

3.2.3. Antigen spatiotemporal heterogeneity

Antigenic heterogeneity—variation in the type and level of antigen expression across tumor cells over space and time—is a central mechanism driving immune escape in CAR-T therapy.

Temporal heterogeneity reflects dynamic changes in antigen expression during tumor progression (Fig. 3). Early in tumor development, immune surveillance selectively eliminates antigen-positive cells, allowing antigen-negative subclones to expand—a “Darwinian selection” process that confers resistance to immune recognition and clearance (Dunn et al., 2002). Spatial heterogeneity manifests as distinct antigen expression profiles across different regions of the same tumor (Fig. 3). Using single-cell sequencing, Neftel et al. classified GBM into four principal malignant cell states: NPC-like, OPC-like, AC-like, and MES-like. The proportion of these states varies not only between patients but also across intratumoral regions (Neftel et al, 2019). Greenwald et al. further demonstrated that NPC-like cells predominate in the tumor core, whereas AC-like cells are enriched at the invasive margin. Proliferation indices decrease from the tumor core toward the periphery, indicating a spatial gradient in tumor cell proliferation (Greenwald et al, 2024).

The ACT IV clinical trial illustrates the clinical consequences of antigenic heterogeneity. This study evaluated the EGFRvIII-targeted peptide vaccine Rindopepimut in combination with standard therapy in newly diagnosed GBM patients (Weller et al., 2017). Despite inducing robust immune responses, the trial was terminated early due to lack of survival benefit, with median overall survival essentially identical between the experimental and control arms (20.1 vs. 20.0 months). These findings were corroborated in O’Rourke’s CAR-T study, which reported that, post-infusion, excised tumors exhibited specific loss or downregulation of EGFRvIII, with marked intratumoral spatial variation in expression (O’Rourke et al., 2017).

The interplay of temporal and spatial heterogeneity underlies these observations: spatially, different regions and cell states (e.g., NPC-like versus AC-like) exhibit distinct EGFRvIII expression; temporally, EGFRvIII-positive clones are eliminated under therapeutic pressure, whereas preexisting or newly arising negative subclones expand. This dynamic leads to immune escape via antigen loss, explaining why single-antigen therapies frequently fail due to both spatial clustering and temporal evolution of antigen-negative subclones. These insights underscore the need for future strategies targeting multiple antigens or overcoming adaptive microenvironmental resistance.

3.2.4. Safety risks and additional challenges

Compared with hematologic malignancies, the therapeutic safety window for GBM is considerably narrower. On-target/off-tumor toxicity arises when target antigens are expressed at low levels in normal tissues (Fig. 4). For example, B7-H3 is highly expressed in gliomas but also exhibits detectable expression in normal tissues (Meeus et al., 2024). CAR-T cells targeting B7-H3 demonstrate potent cytotoxicity in vitro but induce significant dose-limiting toxicity in vivo due to cross-reactivity with low-level antigen expression in normal tissues (Meeus et al., 2024).

Immune effector cell-associated neurotoxicity syndrome (ICANS) exhibits diverse clinical manifestations in GBM patients, ranging from mild symptoms such as confusion and aphasia to severe complications including cerebral edema, seizures, and neurological deficits (Grant et al., 2022; Wei et al., 2020). Mechanistically, ICANS results from a neuroinflammatory cascade triggered by CAR-T cell activation: effector cells release IFN-γ and other cytokines, activating innate immune cells, which in turn produce inflammatory mediators such as IL-6. This process disrupts the blood–brain barrier and induces vasogenic edema. Given the limited intracranial compliance, edema progression can elevate intracranial pressure and increase the risk of herniation. Cytokine release syndrome (CRS) is another clinically significant complication in GBM, often intertwined with neurotoxicity, creating a complex syndrome (Wei et al., 2020).

The presence of these safety risks necessitates the incorporation of controllable features in CAR-T therapy. Strategies include safety-switch designs, logic-gated activation, and localized delivery to minimize systemic exposure.

In addition, limited CAR-T cell persistence and the time-intensive nature of manufacturing remain major hurdles in the current therapeutic landscape, highlighting the need for optimized production pipelines and strategies to prolong in vivo durability.

4. Next-generation CAR-T cell design

CAR-T therapy for glioblastoma (GBM) faces multiple challenges, including delivery, functionality, specificity, and safety. Limitations

Table 1
Key immunosuppressive features of the glioblastoma tumor microenvironment (Ding et al., 2021; X.-Y. Gu et al., 2025; Jackson et al., 2025; Shan et al., 2022).

Category	Type	Characteristics	Mechanism
Immunosuppressive cells	Tumor-associated macrophages (TAMs)	The most abundant immune cells in the microenvironment, predominantly M2 phenotype (30%–50%)	Secrete immunosuppressive factors; inhibit T cell activation
	Myeloid-derived suppressor cells (MDSCs)	Significantly enriched	Secrete IL-10, TGF-β; inhibit T cell proliferation
	Regulatory T cells (Tregs)	Significantly enriched	Secrete CTLA−4, PD−1; suppress T cell function
	Tumor-infiltrating T cells, NK cells	Low numbers and functionally exhausted	Suppressed; low activity and functional exhaustion
Immunosuppressive molecules	TGF-β, IL-10, IL-6	Immunosuppressive cytokines	Inhibit activation of T cells/NK cells
	PD-L1, CTLA−4, B7-H3, TIM−3, LAG−3	Immune checkpoint molecules	Induce T cell exhaustion
	CXCL12, CXCL1/2, CCL2, CCL5	Chemokines	Recruit immunosuppressive myeloid cells
Metabolic microenvironment	Hypoxia	High expression of HIF−1α	Upregulates PD-L1; recruits MDSCs
	Lactate accumulation	Enhanced glycolysis	Inhibits T cell function; promotes M2 macrophage polarization
	Tryptophan depletion	High expression of IDO1	Suppresses T cells

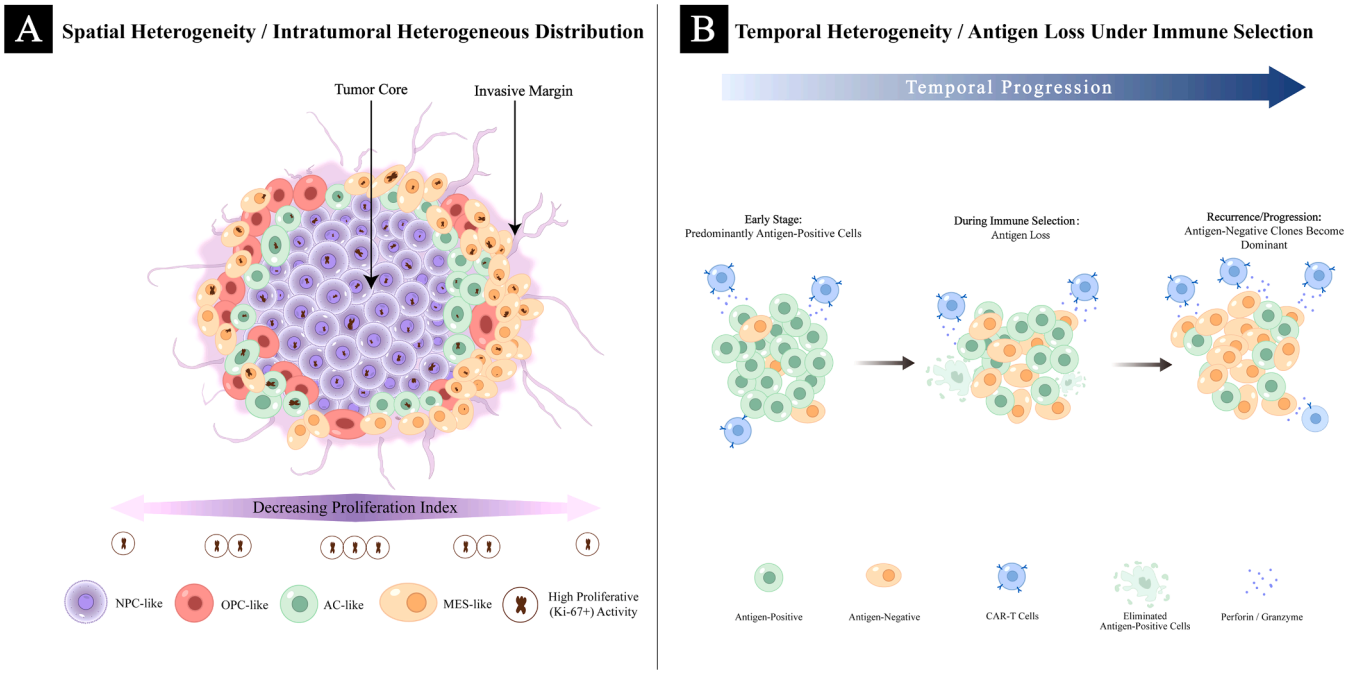

Fig. 3. Spatial and Temporal dimensions of antigen heterogeneity in glioblastoma (A) Spatial heterogeneity. Distinct glioblastoma cellular states, including NPC-like, OPC-like, AC-like, and MES-like populations, coexist within the same tumor and display region-specific distributions. Tumor cell proliferative activity decreases progressively from the tumor core toward the invasive margin. (B) Temporal heterogeneity. Under immune selection pressure, antigen-positive tumor cells are preferentially eliminated by CAR-T cells, whereas antigen-negative subclones survive and progressively expand.

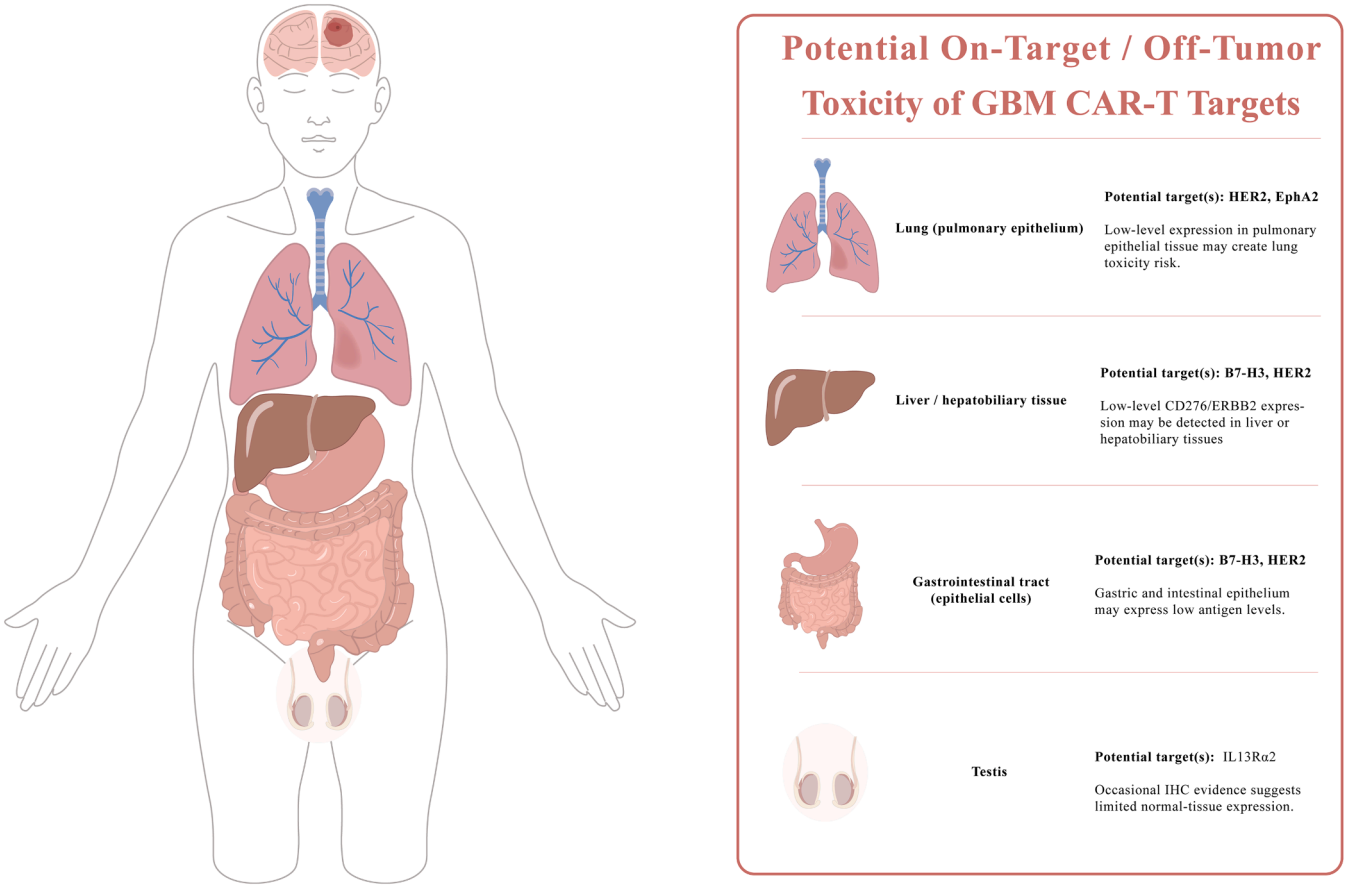

Fig. 4. Potential on-target / off-tumor toxicity of GBM CAR-T targets.

inherent to CAR-T cells have prompted the development of next-generation constructs to enhance antitumor efficacy, address antigen heterogeneity, and promote tumor infiltration. An overview of representative next-generation CAR-T engineering approaches is presented in Fig. 5.

4.1. Enhancing antitumor potency

The immunosuppressive tumor microenvironment (TME) of GBM, enriched with inhibitory cytokines such as TGF-β and IL-10, as well as immune checkpoint ligands such as PD-L1, severely limits the efficacy of adoptive T-cell therapies. To counteract these limitations, "armored" CAR-T cells have been engineered to secrete immunomodulatory cytokines locally within the tumor, thereby reshaping the TME (Kwon and Chen, 2025). For instance, Shih et al. developed IL13Rα2-targeted CAR-T cells that secrete IL-12 and IL-18(Shih et al., 2025). These armored CAR-T cells not only mediated tumor cell lysis but also amplified endogenous $CD4^+$ and exogenous $CD8^+$ T-cell populations, recruited NK cells and monocytes, and elicited multidimensional adaptive immune responses—effects not observed with conventional CAR-T cells.

Alternative strategies involve in situ secretion of immune checkpoint inhibitors. Antibody receptor-modified (ARMed) CAR-T cells, for example, release PD−1 micro-antibodies within the brain, enhancing antitumor activity in murine subcutaneous and intracranial GBM models (Cook et al., 2025). However, direct comparisons with conventional CAR-T combined with systemic checkpoint blockade are lacking, leaving the relative contributions of local antibody secretion and additive effects unresolved.

Another approach leverages dominant-negative TGF-β receptors (dnTGFβRII) expressed on CAR-T cells to sequester immunosuppressive ligands in the TME. Li et al. demonstrated that a tri-modular CAR-T construct (CART-EGFR-IL13Rα2-dnTGFβRII) not only reduced the likelihood of antigen escape but also effectively neutralized TGF-β–mediated immune suppression (Li et al., 2024). Functional assays showed superior T-cell persistence, effector memory formation, and proliferative capacity compared with TGF-β–sensitive CAR-T controls. Safety and efficacy were confirmed in preclinical GBM models, providing a strong rationale for clinical translation.

Targeting intrinsic T-cell exhaustion pathways is another promising avenue. CRISPR/Cas9-mediated knockout of the NR4A transcription factor family (Nr4a1, Nr4a2, Nr4a3) delayed exhaustion under chronic antigen stimulation. Chen et al. demonstrated that NR4A triple-knockout CAR-T cells exhibited sustained effector function and enhanced tumor clearance in solid tumor models (Chen et al., 2019), highlighting epigenetic and transcriptional modulation as a key strategy to counteract TME-mediated inhibition.

4.2. Overcoming antigen escape and heterogeneity

Tumor antigen heterogeneity inevitably drives antigen escape. Clinical trials with IL13Rα2-targeted autologous $CD8^+$ CAR-T cells revealed post-treatment reductions in IL13Rα2 expression, underscoring resistance mechanisms mediated by antigen downregulation or outgrowth of antigen-negative clones.

Dual-targeting strategies yield a promising solution. EGFR and IL13Rα2 are highly expressed in GBM, making them attractive targets for combination therapy (Lassman et al., 2019; O'Rourke et al., 2017). Clinical data demonstrated that EGFRvIII/IL13Rα2 CAR-T cells produced rapid reductions in tumor volume and contrast enhancement, despite manageable early-onset neurotoxicity (Bagley et al., 2025). Tri-modular CAR-T cells incorporating dnTGFβRII further reinforced this dual-targeted design, yielding superior antitumor efficacy relative to single-target CAR-T cells (Li et al., 2024).

Logic-gated CAR-T designs offer enhanced specificity by equipping T cells with Boolean logic circuits that activate only under defined conditions. AND-gated circuits require simultaneous recognition of two antigens to trigger cytotoxicity. A representative example is the synNotch system: in 2016, Roybal et al. developed the first dual-antigen AND-gate circuit, in which a synNotch receptor recognizing the first antigen (e.g., CD19) undergoes proteolytic cleavage, releasing a transcriptional activator (tTA), which induces CAR gene expression under a TRE promoter. Only upon CAR expression and recognition of the second antigen (e.g., mesothelin) is full T cell activation achieved (Roybal et al., 2016). Building on this concept, Choe et al. (2021) developed EGFRvIII-synNotch-induced EphA2/IL-13Rα2 CAR-T cells (E-SYNC), using EGFRvIII as a priming signal to induce CAR expression targeting EphA2/IL-13Rα2, which is homogeneously expressed. This strategy achieved complete remission in heterogeneous PDX models and entered Phase I clinical trials in 2024 (NCT06186401)(Choe et al., 2021). Similarly, Simic et al. engineered a BCAN-synNotch CAR-T system (B-SYNC) to induce CAR expression locally in the CNS using BCAN, a brain-specific extracellular matrix protein, resulting in complete tumor clearance in orthotopic GBM PDX models (Simic et al, 2024).

Beyond AND-gated CARs, NOT-gated CARs provide protection for normal tissues. Fedorov et al. constructed iCARs targeting PD−1 and CTLA−4 to deliver inhibitory signals upon recognizing antigens expressed on healthy tissues, thereby preventing off-tumor activation (Fedorov et al., 2013). OR-gated CARs activate T cells upon recognition of either antigen A or B, effectively mitigating tumor antigen loss.

Complex multi-logic circuits are being explored to integrate AND, OR, and NOT gates. For example, Williams et al. developed an AND-NOT circuit that activates cytotoxicity only when recognizing the ideal target ($GFP^+CD19^+HER2^-$) while inducing apoptosis upon encountering non-ideal targets co-expressing the exclusion antigen ($GFP^+CD19^+HER2^+$) (Williams et al., 2020).

Despite these advances, clinical translation in glioblastoma remains challenging due to the limited availability of antigen pairs that are highly expressed in tumors but absent or minimally expressed in normal tissues. Artificial intelligence can accelerate antigen combination discovery by integrating single-cell RNA-seq, TCGA, and human proteome datasets to identify tumor-specific co-expression patterns, predict off-target toxicity, and model antigen escape under therapeutic pressure. Stanford University's SCAN-ACT platform represents the first systematic integration of single-cell data with logic-gated CAR-T target discovery, potentially reducing target identification time. Initially applied to soft tissue sarcomas, this platform is expected to extend to GBM, evaluating its generalizability across tumor types (Testa et al., 2025).

4.3. Promoting tumor infiltration

Chemokine-receptor axes critically govern T-cell trafficking to tumors. GBM expresses high levels of CXCR2 ligands (CXCL1, CXCL2, CXCL5, CXCL8) (Han et al., 2021), and engineering CAR-T cells to express corresponding receptors (CXCR1/2) enhances intratumoral migration and antitumor efficacy in GBM, ovarian, and pancreatic models (Jin et al., 2019). Such CXCR2-engineered CAR-T products have entered early-phase clinical trials (NCT05353530).

Similarly, co-expression of IL-15 and CCL19 on CAR-T cells promotes recruitment of T cells and dendritic cells via CCR7, increasing intratumoral T-cell infiltration in preclinical models (Chen et al., 2025). Whether this effect is predominantly due to CCL19-mediated chemotaxis or IL-15-driven proliferation remains to be clarified.

5. Combinatorial CAR-T Cell Therapeutic Strategies

While adaptive engineering of CAR-T cells represents a promising avenue for solid tumor therapy, translational challenges remain, including cellular and molecular complexity, cost, and safety considerations. In glioblastoma (GBM), combining CAR-T therapy with conventional treatments—surgical resection, chemoradiotherapy, immune checkpoint inhibitors, or targeted therapies—has demonstrated

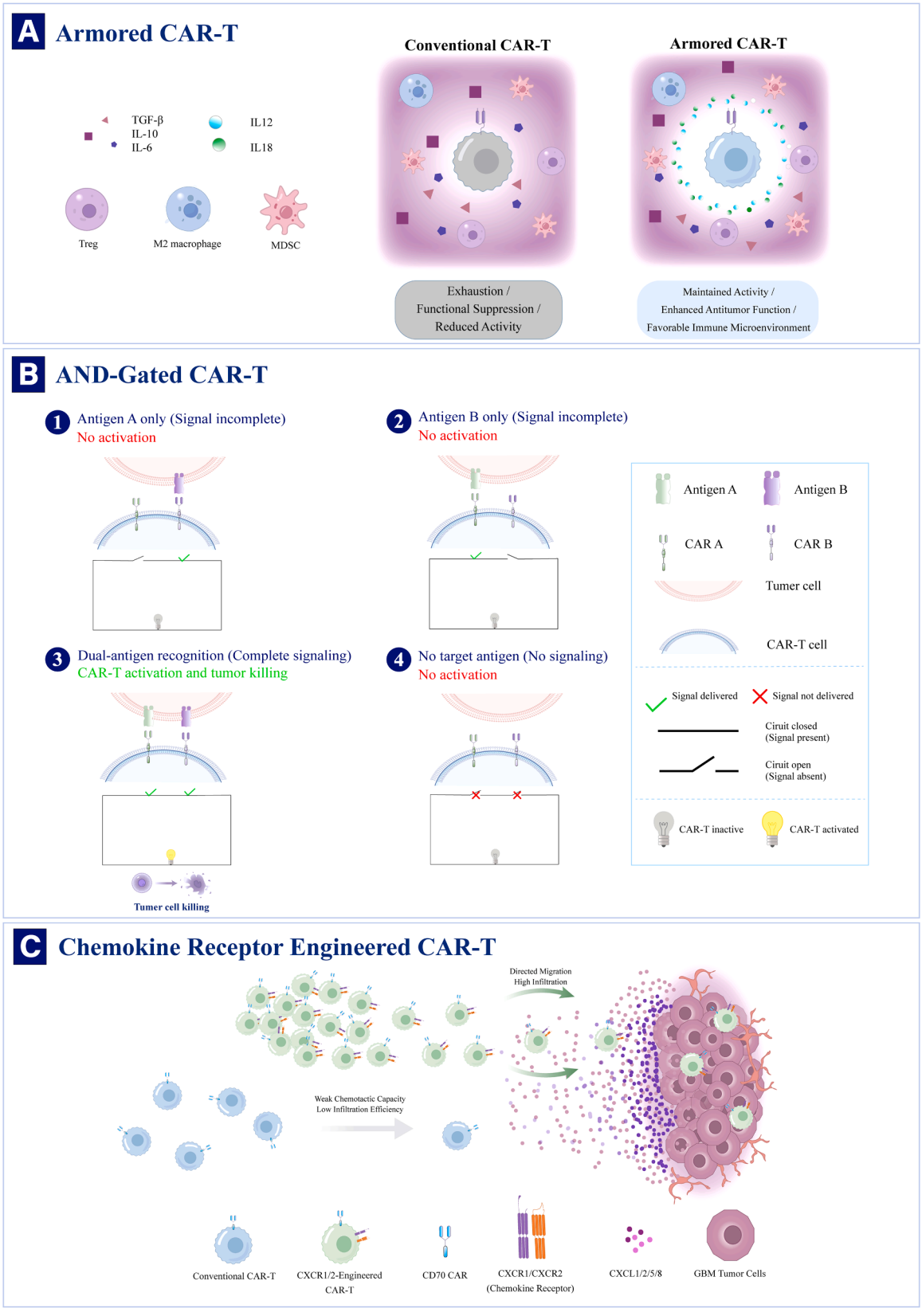

Fig. 5. (A) Armored CAR-T cells. Engineered CAR-T cells secrete immunostimulatory cytokines such as IL-12 and IL-18 to counteract the immunosuppressive tumor microenvironment, maintain effector function, and enhance antitumor activity. (B) AND-gated CAR-T cells. CAR-T activation is triggered only when two tumor-associated antigens are simultaneously recognized, thereby improving tumor specificity and reducing off-target toxicity. (C) Chemokine receptor-engineered CAR-T cells. Forced expression of chemokine receptors such as CXCR1/CXCR2 enhances responsiveness to tumor-derived chemokines, promoting tumor homing, infiltration, and antitumor efficacy within glioblastoma.

enhanced therapeutic outcomes. Concurrently, the convergence of bioengineering and clinical oncology has expanded the research landscape to include biomaterials, ultrasound, electromagnetic fields, and real-time visualization, facilitating more precise therapeutic modulation while enabling minimally invasive intervention (Fig. 6).

5.1. Biomaterials for CAR-T delivery

5.1.1. Local delivery

Injectable hydrogels and implantable scaffolds have emerged as potent vehicles to bypass the blood-brain barrier, achieve high intratumoral CAR-T concentrations, reduce systemic toxicity, and mimic the extracellular matrix to preserve T-cell functionality and longevity.

Post-surgical GBM cavities pose challenges due to irregular geometry and rapid CAR-T cell exhaustion. Thermoresponsive PEG-g-chitosan hydrogels (PCgel), developed by Tsao et al., exemplify a promising platform: liquid at low temperatures for injection, rapidly gelling at physiological temperature, facilitating in situ CAR-T retention and controlled release (Tsao et al., 2014). Similarly, decellularized porcine brain extracellular matrix (dECM) loaded with EGFRvIII-targeted CAR-T cells demonstrated significantly prolonged survival in murine post-resection models, with persistent $CD3^+$ T-cell infiltration at 60 days post-injection, outperforming systemic or irrelevant CAR-T delivery (Logun et al., 2025). Both PCgel and dECM maintained cellular viability and cytolytic activity superior to traditional matrices like Matrigel.

Fibrin gels provide additional advantages by combining biocompatibility, hemostatic properties, and angiogenic support (Pereira et al., 2023). Ogunnaike et al. embedded B7-H3-targeted CAR-T cells within in situ forming fibrin gels, achieving long-term tumor-free survival in 64% of treated immunodeficient mice, compared with 20% in free-injection controls (Ogunnaike et al., 2021). Flow cytometry confirmed enhanced T-cell infiltration within residual tumor tissue, and in vitro release kinetics indicated sustained CAR-T delivery over 5 days, with complete gel absorption within 2 weeks, providing a critical therapeutic window post-surgery.

Nanomaterials designed for systemic administration further complement local strategies. Hyaluronic acid (HA)–rich extracellular matrices in GBM restrict immune cell infiltration. ROS-responsive HAase nanogels, conjugated to CAR-T cell surfaces, allow selective degradation of HA in high-ROS tumor regions, enhancing T-cell penetration while mitigating systemic HA breakdown (Zhao et al., 2025). Such microenvironment-responsive systems exploit GBM-specific pathological features, representing a potential strategy for improving CAR-T infiltration.

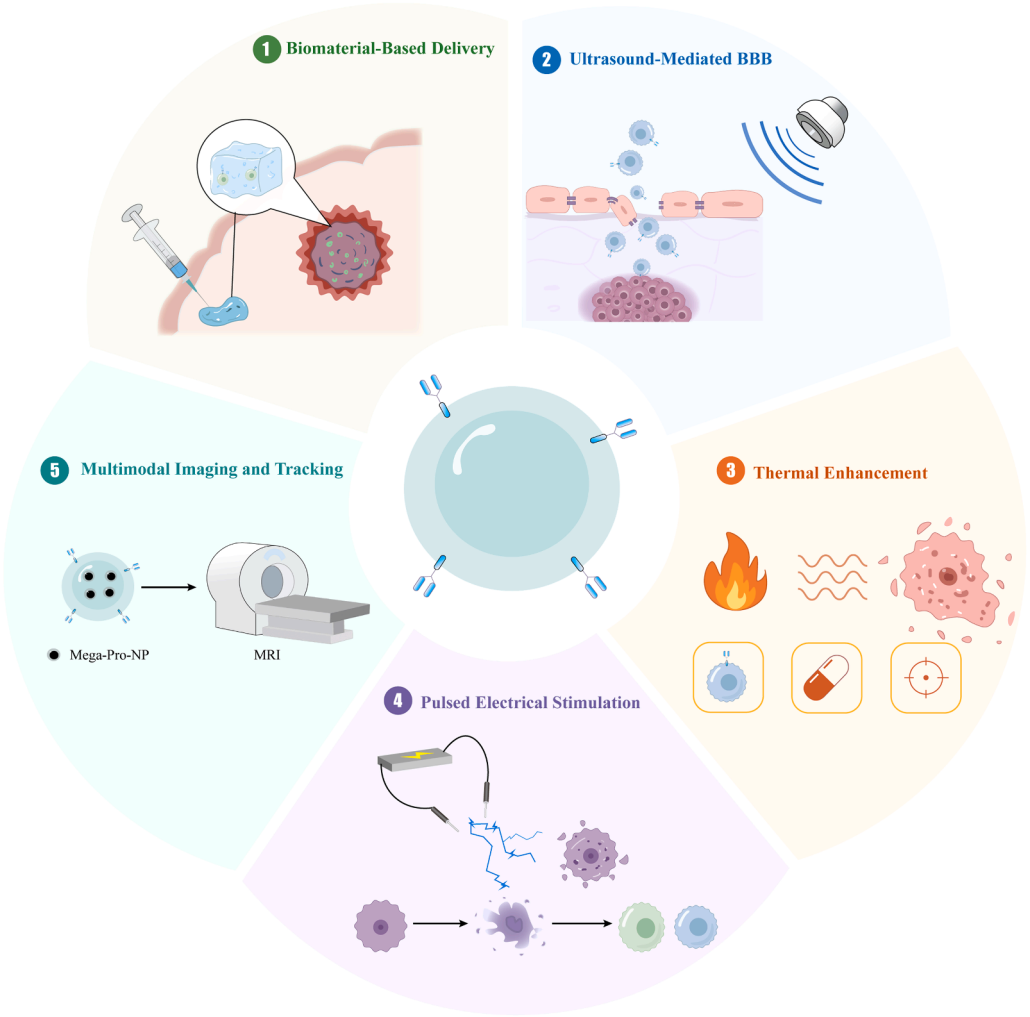

Fig. 6. Bioengineering strategies enhancing CAR-T cell therapy for glioblastoma (1) Biomaterial-based delivery. Injectable hydrogels enable localized CAR-T cell delivery and improve cell retention within the tumor microenvironment. (2) Ultrasound-mediated blood–brain barrier opening. Focused ultrasound transiently disrupts the blood–brain barrier, facilitating CAR-T cell trafficking into intracranial tumors. (3) Thermal enhancement. Thermal interventions exert antitumor effects through immune activation, chemo-sensitization, and direct tumor cell killing. (4) Pulsed electrical stimulation. Pulsed electric fields induce immunogenic cell death and enhance antitumor immune responses. (5) Multimodal imaging and tracking. Superparamagnetic iron oxide nanoparticle (MegaPro-NP)-labeled CAR-T cells can be monitored by MRI and MPI, enabling noninvasive visualization without impairing viability or cytotoxic function.

5.1.2. Immunomodulatory strategies

Hydrogels serve not only as delivery vehicles but also as multifunctional immunomodulatory platforms, capable of locally releasing CAR-T cells, co-delivering immune adjuvants (e.g., IL-2, IL-12, IL-15), and checkpoint inhibitors (e.g., anti-PD-L1). Previous studies administering CAR-T cells intratumorally, intracavitarily (ICT), or intraventricularly (ICV) demonstrated safety and feasibility; however, limited CAR-T persistence often resulted in inconsistent therapeutic outcomes. This is primarily due to T cell exhaustion driven by the immunosuppressive tumor microenvironment, chronic antigen stimulation, and metabolic constraints. Strategies combining microenvironmental modulation may thus enhance CAR-T efficacy.

Hydrogel systems can be loaded with small molecule inhibitors, cytokines, or checkpoint blockade agents to enable multicomponent co-delivery and controlled release, thereby reshaping the tumor microenvironment. For example, Wang et al. reported a fibrin hydrogel co-delivering the HIF−1α inhibitor PX−478 and anti-PD−1 antibody (Meng et al., 2025), achieving differential release kinetics: rapid release of PX−478 within 24 h attenuated hypoxia-induced immunosuppression, while sustained PD−1 blockade over 96 h aligned with post-operative immune reconstitution. In a postoperative GBM model, this hydrogel delayed tumor recurrence and prolonged median survival, while also reducing intraoperative bleeding time.

Cytokine incorporation further enhances CAR-T persistence. IL-15, unlike IL-2, minimally expands regulatory T cells while promoting stem-like memory T cell formation, improving mitochondrial fitness, delaying exhaustion, and enhancing anti-tumor activity (Alizadeh et al., 2019; Pandiyan et al., 2007; Waldmann, 2006). In GL261 orthotopic GBM models, IL-15-loaded polymer-nanoparticle hydrogels maintained local cytokine concentrations for over 7 days, significantly boosting CAR-T expansion, memory phenotype acquisition ($CD62L^+CD44^+$), and IFN-γ secretion, thereby extending median survival substantially (Grosskopf et al., 2022).

Importantly, such biomaterial-assisted delivery not only augments CAR-T function but also stimulates host immunity, facilitating antigen spreading. For instance, localized IFN-α can recruit endogenous T cells to target tumor antigens beyond the CAR-T-specific target (B7-H3) (Rossari et al., 2025). Similarly, hydrogels co-delivering chemokines (CXCL10) and PD-L1 inhibitors enhance cytotoxic T cell recruitment and effector function post-surgery (Li et al, 2025). Hydrogels incorporating dying tumor cells and adjuvants can activate dendritic cells, polarize macrophages toward an M1 phenotype, and synergize with exogenous CAR-T cells to suppress tumor recurrence (Zhang et al., 2025). Collectively, biomaterials provide a versatile platform for CAR-T cell delivery, persistence, proliferation, and anti-tumor function.

5.1.3. In Situ CAR-T production

Conventional autologous CAR-T manufacturing, which requires ex vivo isolation, genetic modification, expansion, and reinfusion over 3–6 weeks, limits its applicability for rapidly progressing patients (Xu et al., 2025). In vivo CAR-T generation strategies—using viral or non-viral vectors to deliver CAR-encoding genetic material directly to endogenous T cells—streamline production, reduce costs, and improve accessibility (Bui et al., 2024).

Clinical feasibility has been demonstrated in relapsed/refractory multiple myeloma. ESO-T01, a nanobody-targeted lentiviral vector, produced BCMA-specific CAR-T cells in situ following single intravenous administration, achieving systemic expansion and tumor remission (J et al., 2025). Non-viral lipid nanoparticles (LNPs) have enabled mRNA- or DNA-mediated CAR expression in T cells, with potential for permanent in vivo programming (Wang et al., 2026).

This in situ generation strategy can also be extended to other immune cells. Jiang and colleagues employed an MLNP-circRNA delivery system to generate macrophages expressing CAR and SIGLEC9 chimeric receptors (CSR) directly in vivo (Fu et al., 2026). These CAR-M cells efficiently phagocytose tumor cells while converting immunosuppressive signals from the tumor microenvironment into activating cues via the CSR, leading to sustained secretion of proinflammatory cytokines such as TNF-α and IL-1β. This mechanism synergistically activates both innate and adaptive immunity, significantly inhibiting glioma growth and prolonging survival.

Similarly, Zhou et al. developed a strategy using enucleated mesenchymal stem cells as carriers that retain tumor-homing capacity but lose proliferative potential (L. Zhou et al., 2025). After loading CAR-encoding plasmids and systemic administration, these cell carriers accumulate in GBM lesions. The carriers undergo programmed apoptosis, exposing signals recognized and engulfed by tumor-associated macrophages, enabling high-efficiency CAR gene delivery and in situ generation of functional CAR macrophages within the tumor microenvironment. This approach not only suppresses tumor growth but also remodels the immunosuppressive microenvironment. When combined with CD47 blockade antibodies, it achieves long-term survival in murine models.

5.2. Physical modulation and real-time tracking of CAR-T cells

5.2.1. Ultrasound-mediated blood–brain barrier modulation

The blood–brain barrier (BBB) remains a major obstacle for systemically administered CAR-T cells in central nervous system tumors. Although glioblastoma (GBM) invasion can locally compromise the BBB, the disruption is heterogeneous and insufficient for efficient CAR-T infiltration. Ultrasound-mediated BBB opening (FUS-BBBO) has emerged as a promising, reversible strategy to enhance CAR-T delivery.

Carpentier et al. developed an implantable pulsed ultrasound device (SonoCloud) that achieved repeated BBB opening in recurrent GBM patients at acoustic pressures up to 1.1 MPa without significant adverse effects (Carpentier et al., 2016). Mechanistically, ultrasound-induced oscillation of intravenously administered microbubbles generates localized fluid dynamics and transient mechanical stress on endothelial tight junctions, enhancing vascular permeability while modulating inflammatory pathways to facilitate T-cell trafficking (Guo et al., 2024). Preclinical studies using low-intensity pulsed ultrasound (LIPU) demonstrated a several-fold increase in intratumoral CAR-T infiltration compared with intravenous infusion alone, without detectable neurotoxicity or cerebral edema, improving median survival by over 129% (Sabbagh et al., 2021). Magnetic resonance-guided focused ultrasound (MRgFUS) further enables precise, real-time monitored BBB opening, and preclinical pediatric studies have shown enhanced B7-H3-targeted CAR-T infiltration into diffuse midline gliomas (DMG, H3K27M) (Teis et al., 2025). Collectively, ultrasound-mediated BBB modulation provides a “systemic administration, local targeting” approach that complements the limitations of conventional intratumoral delivery.

Advanced strategies integrate FUS with synthetic gene circuits to achieve spatiotemporally controlled CAR expression. For example, EchoBack-CAR-T cells employ hyper-sensitive heat shock promoters and positive feedback loops activated by intermittent FUS (L. Liu et al., 2025). In murine GBM models, this system achieved 100% survival in treated mice without off-target toxicity, enhanced cytotoxicity, and reduced exhaustion compared to conventional CAR-T cells, demonstrating broad applicability across CAR scaffolds and solid tumor targets.

5.2.2. Thermal and pulsed electrical stimulation

Hyperthermia exerts antitumor effects via direct cytotoxicity, immune activation, and chemosensitization (Gu et al, 2025). In vitro GBM models, heat-induced CAR-T cells enhanced infiltration and reversed stiffness-mediated immunosuppressive features, improving antitumor efficacy (Tang et al., 2024). Chen et al. anchored near-infrared photosensitizers on CAR-T surfaces, producing localized thermal energy to promote deep tumor infiltration and cytokine secretion, effectively remodeling the immune microenvironment (Chen et al., 2021).

Magnetic hyperthermia therapy (MHT) leverages magnetic nanoparticles (MNPs) to convert energy from an alternating magnetic field

(AMF) into local heat (Xiong et al., 2025). Loading MNPs onto CAR-NK cells enables targeted thermal delivery and immune-mediated cytotoxic synergy, minimizing collateral tissue damage.

Pulsed electrical stimulation has been shown to induce immunogenic tumor cell death. Li et al. combined triboelectric nanogenerator (TENG)-based pulsed currents with CAR-T therapy, generating peak currents of 35 μA via manual sliding (H. Li et al., 2025). The resultant immunogenic cell death enhanced dendritic cell antigen presentation, polarized tumor-associated macrophages toward an M1 phenotype, reduced Treg differentiation, increased CAR-T tumor infiltration, and induced adaptive memory differentiation. This drug-free combinatorial approach eliminated ~60% of NALM6 solid tumor mass while maintaining favorable biosafety.

5.2.3. *Dynamic monitoring of CAR-T cells*

Real-time tracking of CAR-T distribution is essential for evaluating therapeutic efficacy, optimizing delivery, and minimizing off-target toxicity. MRI provides high-resolution, noninvasive imaging for intratumoral CAR-T monitoring. Wu et al. labeled CD70-targeted CAR-T cells with superparamagnetic iron oxide nanoparticles (MegaPro-NP), achieving multimodal MRI and magnetic particle imaging (MPI) tracking in murine GBM models (Wu et al., 2023). Labeled cells retained cytotoxic function, and T2 signal hypointensity correlated with intratumoral T-cell density. Although this approach remains at the preclinical stage, it demonstrates the feasibility of nanoparticle-assisted CAR-T monitoring and provides a promising strategy for future clinical translation. Beyond their application as cellular imaging labels, superparamagnetic iron oxide nanoparticles have also been explored as multifunctional platforms for cancer imaging and combination therapy, suggesting potential opportunities for integrating imaging and therapeutic functions to further optimize CAR-T monitoring and treatment strategies in glioblastoma (Haider et al., 2025).

Reporter gene-based PET imaging enables indirect, noninvasive monitoring of CAR-T activity. HSV1-tk-expressing CAR-T cells can be tracked using [18 F]FHBG PET, providing a quantitative evaluation of therapeutic kinetics (Keu et al., 2017). However, the immunogenicity of exogenous viral proteins must be considered.

Tumor microenvironmental changes can also serve as functional biomarkers. Following CAR-T infusion, transient increases in tumor oxygen partial pressure (pO_2) can be monitored using perfluorocarbon (PFC) probes combined with ^{19}F MRI, providing real-time insight into infiltration efficiency, treatment response, and optimal timing for repeat administration (Chapelin et al., 2021).

6. Clinical Translation and Future Perspectives

CAR-T cell therapy for glioblastoma faces multiple challenges, including the restrictive properties of the blood–brain barrier (BBB), immunosuppressive tumor microenvironment, antigen heterogeneity, and safety concerns. While strategies to overcome the BBB and immunosuppression are increasingly developed, tumor antigen heterogeneity remains a formidable obstacle. GBM-associated antigens such as EGFRvIII, HER2, IL13Rα2, CD70, B7-H3, EphA2, and GD2 have been targeted in preclinical and early clinical studies, showing preliminary efficacy (Mariniello and Migliorini, 2026). However, the highly heterogeneous expression of these antigens limits the coverage of any single target, and antigen loss or downregulation during treatment can undermine the efficacy of monovalent CAR-T therapies. Identification of novel antigens with high tumor specificity and low expression in normal tissues, or broad-spectrum targets covering multiple subclones, is therefore critical. Artificial intelligence (AI) may provide new opportunities in this domain, integrating single-cell RNA sequencing, proteomics, and spatial transcriptomics to identify tumor-specific antigens and design logic-gated circuits that minimize off-tumor toxicity (Walton et al., 2025). AI-driven antigen discovery and logic-gated CAR designs may accelerate the translation of personalized targeting strategies.

Promoting antigen spreading represents another important strategy to achieve durable tumor control. Antigen spreading occurs when initial CAR-T-mediated tumor cell killing stimulates endogenous T-cell responses against additional tumor antigens (Conde et al., 2021). Various approaches aim to induce this effect. For instance, combining CAR-T therapy with conventional radiotherapy or chemotherapy promotes immunogenic cell death and release of damage-associated molecular patterns, enhancing antigen presentation. Alternatively, CAR-T cells can be combined with vaccines carrying the same antigen; in GBM mouse models, EGFRvIII-targeted CAR-T cells co-administered with corresponding vaccines not only enhanced CAR-T efficacy but also elicited endogenous T-cell responses against other tumor antigens, potentially compensating for low or absent antigen expression in tumor subclones and establishing long-term immune memory (Ma et al., 2023). This strategy offers translational potential for reducing relapse after CAR-T therapy.

Next-generation CAR platforms extend beyond T cells to alternative immune effectors, including natural killer (NK) cells, macrophages, and neutrophils (Alrehaili et al., 2025). NK cells possess innate allogeneic properties, enabling scalable "off-the-shelf" production from healthy donors, umbilical cord blood, or induced pluripotent stem cells without stringent HLA matching, reducing manufacturing time and costs. Macrophages actively infiltrate solid tumors, degrade tumor-associated stroma, secrete pro-inflammatory cytokines, and convert immunologically "cold" tumors into "hot" microenvironments conducive to immune attack. Neutrophils can traverse the BBB and infiltrate the brain parenchyma (Walton et al., 2025). CAR-NK and CAR-macrophage platforms, combined with standardized biomaterial scaffolds, may transform personalized therapies into more conventional, scalable drug-like products.

Biomaterials, including hydrogels and nanoparticles, continue to play a pivotal role in CAR-T combination therapies. Preclinical studies demonstrate synergistic potential (Huang et al., 2025), yet clinical translation faces challenges in standardization, safety, and regulatory approval. Hydrogel gelation kinetics, degradation rates, and pore structure are influenced by raw materials, crosslinking conditions, and sterilization methods, lacking GMP-compliant manufacturing protocols (Huang et al., 2025). Consistency in cell distribution, density, and viability within materials remains unstandardized. Safety concerns extend beyond conventional CAR-T risks (cytokine release syndrome, neurotoxicity, off-target effects) to include potential local chronic inflammation from hydrogel degradation, nanoparticle biocompatibility issues, uncontrolled release from responsive materials, and excessive immune activation that can trigger autoimmunity. Establishing appropriate evaluation frameworks and long-term monitoring plans is essential for regulatory approval (Huang et al., 2025).

Dynamic monitoring of CAR-T cells is critical for assessing efficacy and minimizing off-target toxicity. Future multimodal imaging is expected to integrate synthetic biology and nanotechnology: nanosensors under development can simultaneously enhance drug delivery and imaging precision, while genetically encoded circuits in CAR-T cells can trigger imaging signals upon antigen recognition, allowing direct visualization of tumor-targeted activity. AI and machine learning algorithms can analyze large datasets from multimodal imaging to build predictive models of CAR-T activity and therapeutic outcomes (J. Liu et al., 2025). Multimodal in vivo tracking holds high clinical potential to monitor CAR-T migration, proliferation, and tumor interactions in real time, addressing central challenges in GBM immunotherapy (Ijaz et al., 2025). Recent studies integrating PET imaging with genomic analyses demonstrate the ability to optimize CAR design, enhance tumor infiltration, and prolong in vivo persistence (Morath et al., 2025). As imaging technologies advance, multimodal tracking is poised to become a cornerstone for next-generation CAR-T translation in refractory glioblastoma.

7. Conclusions

CAR-T cell therapy represents a promising approach for the treatment of glioblastoma. Compared with conventional modalities, CAR-T cells offer precise tumor targeting, and their combination with other therapeutic strategies may enhance therapeutic efficacy. Next-generation CAR-T designs—including armored modifications, logic-gated control circuits, and precise delivery systems—are advancing the intrinsic functionality of the cells. Biomaterials provide versatile platforms for CAR-T production, delivery, and local retention, while external physical modulation opens new avenues for in vivo control and real-time tracking. These combination strategies should not be viewed in isolation but integrated into a logical therapeutic framework in future clinical studies to truly benefit patients with glioblastoma.

The synergy between biomaterials and engineered cells offers the potential to establish closed-loop therapeutic systems capable of sensing, responding, and regulating activity. Engineered cells can recognize tumor cells expressing specific antigens via logic-gated receptors, while responsive materials detect pathological cues to trigger drug release or structural transformation. Upon antigen sensing, CAR-T cells initiate cytotoxic functions, and the material platform concurrently releases immunomodulators to support cellular effector activity. Imaging-based tracking provides feedback on cellular distribution and tumor accumulation, while external modulation systems allow dynamic adjustment of cellular activity in response to therapeutic intervention.

In summary, the multifaceted challenges facing CAR-T therapy for glioblastoma may be progressively addressed through the systematic integration of these multidimensional strategies. Rigorous clinical trials and further innovations in cellular and material engineering remain critical for advancing CAR-T therapy toward meaningful and durable clinical benefit for glioblastoma patients.

8. Critical View

This review focuses on how glioblastoma-specific barriers influence the development of CAR-T-cell therapies. Instead of attempting to catalogue all available targets and clinical studies, we concentrate on four closely related challenges: limited delivery across the blood–brain barrier and extracellular matrix, an immunosuppressive tumor microenvironment, spatial and temporal antigen heterogeneity, and treatment-related toxicity. We then consider how cellular engineering, biomaterial-assisted delivery, physical modulation, and imaging may help address these barriers. Bringing these areas together allows the strengths and limitations of each strategy to be assessed within the same translational context.

A major challenge in the field is that the growing complexity of CAR-T-cell platforms has not yet been matched by evidence of durable clinical benefit. Armored and logic-gated CAR-T cells, biomaterial-assisted delivery systems, externally regulated approaches, and imaging-based strategies have shown promise, but most remain at the preclinical or early clinical stage. In addition, commonly used experimental models only partly reproduce the antigenic diversity, immune suppression, previous treatment exposure, and intracranial safety constraints seen in patients with glioblastoma. Tumor regression in these models therefore does not necessarily predict acceptable toxicity, sustained CAR-T-cell activity, or improved survival in patients.

Future studies should give greater priority to selecting modifications according to the main barrier in a given treatment setting. Tumors with poor CAR-T-cell delivery may be better suited to regional administration, biomaterial-based retention, or focused ultrasound. Marked antigen heterogeneity may justify dual-targeting or logic-gated recognition, whereas severe local immune suppression may require armored CAR-T cells or locally delivered immunomodulators. These barriers often coexist, but this does not mean that all available modifications should be combined in a single product. Each added component should have a defined biological purpose, a measurable pharmacodynamic effect, an appropriate safety control, and a manufacturing process that can be implemented under good manufacturing practice conditions.

Imaging may also play a more active role than conventional response assessment alone. It could help determine whether CAR-T cells reach the tumor, persist within the brain, and produce the intended biological effect. Such information would be particularly valuable when deciding whether treatment failure reflects inadequate delivery, loss of cellular function, antigen escape, or unacceptable toxicity.

This review emphasizes the need to link treatment design with treatment monitoring. The key clinical questions are whether CAR-T cells reach the relevant tumor compartments, whether they remain functional after arrival, which tumor populations escape treatment, and when therapy should be intensified, redirected, or stopped. Addressing these questions may be more useful for guiding rational combinations than increasing technological complexity without clear biological or clinical justification.

CRediT authorship contribution statement

Lin Chen: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Zhenwei Zou:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language refinement, improvement of clarity, and organization of the manuscript text. After using this tool, the authors reviewed, edited, and verified the content as needed and take full responsibility for the content of the publication.

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