Cancer Immunology, Immunotherapy (2025) 74:363
https://doi.org/10.1007/s00262-025-04222-w

REVIEW

CAR-T cells immunotherapy in the treatment of glioblastoma

Aleksandra Królikowska[1] · **Maciej Tarnowski**[1]

Received: 4 October 2025 / Accepted: 28 October 2025

Abstract
Glioblastoma multiforme (GBM) is highly lethal brain tumor with limited benefit from standard treatment, such as surgery, radiotherapy, and chemotherapy. Its location within the central nervous system, together with the blood–brain barrier, and immunosuppressive niche restricts access and efficacy of therapies. This review examines the current progress of the chimeric antigen receptor (CAR) T cell therapy in GBM, emphasizing therapeutically significant target antigens, delivery strategy and innovations designed to improve safety and persistence. Evidence from preclinical research and early phase clinical trials was assessed to identify key antigen, evaluate routes of administration, and summarize next-generation engineering concepts. Clinical experiences demonstrate that locoregional delivery can enhance tumor penetration compared with systemic infusion. Moreover, CAR-T cells engineered to recognize epidermal growth factor receptor variant III, interleukin-13 receptor subunit alpha-2, human epidermal growth factor receptor 2, or disialoganglioside have shown biological activity in GBM. Emerging platforms, such as dual-target CARs, synNotch, and cytokine-releasing "armored" T cells, develop specificity and overcome barriers posed by tumor heterogeneity and immune suppression. CAR-T therapy in GBM has moved beyond proof-of-concept, with encouraging but preliminary signals of efficacy. Future success will require multi-target approaches, integration with modulators of tumor microenvironment, and optimized delivery systems to achieve durable clinical benefit.

Keywords CAR-T cells · GBM · Cell therapy · Tumor microenvironment

Abbreviations

GBM	Glioblastoma multiforme
CAR-T	Chimeric Antigen Receptor T cells
ACT	Adoptive Cell Transfer
TME	Tumor Microenvironment
BBB	Blood–Brain Barrier
CNS	Central Nervous System
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
GFAP	Glial Fibrillary Acidic Protein
IDH1/2	Isocitrate Dehydrogenase 1/2
MGMT	O6-Methylguanine-DNA-Methyltransferase
EGFR/EGFRvIII	Epidermal Growth Factor Receptor/variant III
TAAs	Tumor-associated antigens
MHC	Major Histocompatibility Complex
AVIL	Advillin
HER2	Human Epidermal Growth Factor Receptor 2
IL13Rα2	Interleukin-13 Receptor subunit alpha-2
CD70, CD133, B7-H3 (CD276)	Cluster of differentiation markers
EphA2	Ephrin type-A receptor 2
GD2	Disialoganglioside
NKG2DL	NKG2D ligands
PDGFRA	Platelet-Derived Growth Factor Receptor Alpha
NF1	Neurofibromin 1
SMARCB1	SWI/SNF-related chromatin regulator
PD-1/PD-L1	Programmed Death-1/Ligand 1

✉ Maciej Tarnowski
maciej.tarnowski@pum.edu.pl

[1] Department of Physiology in Health Sciences, Faculty of Health Sciences, Pomeranian Medical University in Szczecin, Szczecin, Poland

Published online: 06 November 2025

CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
TILs	Tumor-Infiltrating Lymphocytes
NK	Natural Killer (cells)
TCR	T Cell Receptor
IPSCs	Induced Pluripotent Stem Cells
B-ALL	B-cell Acute Lymphoblastic Leukemia
NHL	Non-Hodgkin Lymphoma
OS	Overall Survival
ICV	Intraventricular delivery
ICT	Intracavitary/Intratumoral delivery
IV	Intravenous delivery
PBMCs	Peripheral Blood Mononuclear Cells
APCs	Antigen-Presenting Cells
MACS	Magnetic-Activated Cell Sorting
ScFv	Single-chain Variable Fragment
CD3ζ	T cell surface glycoprotein zeta chain
FcεRiγ	High affinity IgE receptor gamma subunit
IL-2, IL-7, IL-12, IL-15, IL-18	Interleukins
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
SynNotch	Synthetic Notch
TALENs	Transcription Activator-Like Effector Nucleases
TNF-α	Tumor Necrosis Factor-alpha
IFN-γ/IFN-α2	Interferon-gamma/Interferon alpha-2
CXCL11, CXCR1/2	Chemokines and receptors
TRUCK	T cells Redirected for Universal Cytokine Killing
GAMs	Glioma-Associated Macrophages
TAMs	Tumor-Associated Macrophages
TSAs	Tumor-specific antigens
ICANS	Immune Effector Cell-Associated Neurotoxicity Syndrome
HPA	Human Protein Atlas
TCGA, CGGA	The Cancer Genome Atlas/ Chinese Glioma Genome Atlas
RNA-seq	RNA sequencing
IHC	Immunohistochemistry
scRNA-seq	Single-cell RNA-seq
CSF	Cerebrospinal fluid
LIPU	Low-intensity pulsed focused ultrasound
MB	Microbubbles
E-SYNC	EGFRvIII-synNotch–primed CAR-T
B-SYNC	BCAN-syn-Notch → IL13Rα2/ EphA2
PMBCL	Primary mediastinal large B-cell lymphoma
AAPC	Artificial antigen-presenting cell

Introduction

GBM, classified as a grade IV astrocytoma, is among the most aggressive primary brain tumors, characterized by rapid growth, high invasiveness, and poor outcome [1–3]. Globally, the incidence of GBM is approximately 3.19 per 100,000 individuals per year, with a male predominance (male-to-female ratio 1.6:1) and a substantially lower incidence in pediatric populations [4]. The current standard-of-care includes maximal safe resection, followed by radiotherapy and concomitant as well as adjuvant temozolomide chemotherapy [5]. Despite these multimodal interventions, the median overall survival (OS) remains only 12–15 months, with a 5-year survival rate below 5% [4, 6]. The poor prognosis reflects the highly infiltrative nature of GBM, its profound historical and molecular heterogeneity, and its location within the CNS.

Diagnosis relies on neuroimaging, particularly magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), histopathological examination, and increasingly, molecular profiling [2, 4, 7]. Histologically, GBM is characterized by pleomorphic, poorly differentiated, astrocytic cells with high mitotic activity, microvascular proliferation, necrosis, and an elevated Ki-67 proliferation index. Immunohistochemistry typically demonstrates glial fibrillary acidic protein (GFAP), vimentin, and S100 positivity. Molecular markers, such as isocitrate dehydrogenase 1/2 (IDH1/2) mutations, O6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation, and 1p/19q codeletion, provide prognostic and therapeutic guidance [4, 8–11]. The 2021 WHO classification integrates histopathology with molecular diagnostics, improving glioma grading and prediction of treatment response. IDH-mutant gliomas generally present in younger patients with more favorable outcomes, whereas IDH-wildtype GBMs exhibit aggressive biology

and poor survival. MGMT promoter methylation predicts sensitivity to alkylating agents such as temozolomide. In contrast, genetic alterations, including epidermal growth factor receptor (EGFR) amplifications, platelet-derived growth factor receptor alpha (PDGFRA) mutations, neurofibromin 1 (NF1) loss, and chromatin remodeling disruptions such as SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (SMARCB1) contribute to tumor aggressiveness and immune evasion [9–13].

A hallmark barrier to treatment is the immunosuppressive TME, which restricts T cell infiltration, persistence, and effector function, thereby limiting the efficacy of conventional immunotherapies. Chimeric antigen receptor T cell therapy offers a promising strategy to bypass these constraints by genetically engineering autologous T cells to recognize tumor-associated antigens (TAAs) independently of major histocompatibility complex (MHC) restriction. Early studies indicate that CAR-T therapy can induce tumor regression, enhance immune activation, and, when combined with gene-editing, cytokine support, or immune checkpoint inhibition, improve T cell persistence in the TME [14, 15]. Novel targets, such as Advillin (AVIL), show selective expression in GBM cells, providing potential for safer and more effective CAR-T strategies [16].

Despite progress, major challenges persist, including GBM heterogeneity, antigen loss, immunosuppressive TME, and the blood–brain barrier. Ongoing research aims to optimize CAR-T delivery, improve antigen selection, and develop combination strategies to overcome these limitations. The aim of this review is to expose current clinical experiences with CAR-T cell therapy in GBM, provide an overview of the evolving clinical trial landscape, and discuss challenges and future perspectives. Particular attention is given to recent engineering innovations aimed at overcoming the barriers imposed by the GBM microenvironment.

The role of immunotherapy

General background of cancer immunotherapy

The origins of cancer immunotherapy date back to the late nineteenth century, when William Coley observed tumor regression in patients treated with bacterial cultures, later named "Coley's toxin." Subsequently, the capacity of the immune system to recognize and eradicate malignant cells was established [17]. Over the past decades, immunotherapy has emerged as a central strategy in oncology, achieving durable responses in multiple cancer types [18]. Major breakthroughs include immune checkpoint inhibitors (e.g., programmed cell death protein 1 or programmed death-ligand 1, PD-1/PD-L1 blockade), monoclonal antibodies, adoptive T cell therapies, and therapeutic cancer vaccines [19, 20].

Main forms of immunotherapy

Immunotherapy may be employed as either a complementary or, in certain malignancies, a primary therapeutic approach. It is traditionally categorized into active, passive, and adoptive forms, based on their mechanisms of action [17, 19]: (1) Active immunotherapy stimulates the patient's own immune system to recognize and attack tumor cells, often establishing immune memory for long-term protection; (2) passive immunotherapy enhances pre-existing immune responses through the administration of monoclonal antibodies or cytokines (e.g., tumor necrosis factor–alpha, TNF-α); (3) adoptive immunotherapy involves the isolation of autologous immune cells, their ex vivo expansion or genetic modification and subsequent reinfusion to enhance tumor-targeting capacity. This approach includes tumor-infiltrating lymphocytes (TILs), T cell receptor (TCR)-engineered lymphocytes, and CAR-T cells [17, 19].

Anticancer vaccines

Therapeutic cancer vaccines are designed to induce tumor-specific immune response against established malignancies while establishing long-lasting immune memory. These vaccines primarily aim to activate cytotoxic CD8+ T cells capable of recognizing and eradicating recurrent tumor cells [21]. Recent advances in mRNA-based and neoantigen-directed vaccine technologies, accelerated by COVID-19 mRNA platforms, have enabled the development of multi-epitope formulations; however, their efficacy in GBM remains under investigation [22]. A notable example in GBM is SurVaxM, a synthetic peptide mimicking the human survivin protein, which has been tested in clinical trials to elicit a targeted immune response against tumor cells [23, 24]. Anticancer vaccines can be designed as personalized formulations, tailored to individual tumor neoantigens, or as universal vaccines, targeting shared tumor-associated antigens [25–27]. Despite these advances, the overall clinical efficacy of cancer vaccines remains limited compared with immune checkpoint inhibitors or adoptive T cell therapies. Ongoing research continues to refine vaccine formulations, delivery platforms, and adjuvant strategies to enhance therapeutic impact [17]. Detailed information on personalized cancer vaccines is provided in the Supplementary Table (Table S1).

Adoptive cell transfer and CAR-T cells

Adoptive Cell Transfer is an immunotherapeutic strategy in which autologous or allogeneic immune cells are collected, modified, or expanded ex vivo, and reinfused to enhance

anti-tumor activity. Adoptive cell transfer (ACT) encompasses various approaches, including TILs, natural killer (NK) cells, and γδ T cells; however, CAR-T cells are the most developed and clinically advanced [28, 29]. CAR-T cells are genetically engineered T lymphocytes that express CARs to recognize tumor antigens independently of MHC presentation, thereby providing potent and targeted cytotoxicity [19, 28, 30]. CAR-T therapy has demonstrated remarkable efficacy in hematologic malignancies, particularly in B-cell leukemia's and lymphomas, though its application in solid tumors, such as GBM, remains limited by biological and technical barriers [28, 31, 32]. Several ACT-based modalities under investigation are summarized in the Supplementary Table (Table S2) [30].

CAR-T cell therapy

CAR-T cell therapy in hematologic and solid tumors

CAR-T therapy has revolutionized hematologic oncology, with tisagenlecleucel becoming the first FDA-approved product in 2017 for relapsed/refractory pediatric and young adult ALL. Since then, additional CAR-T products have been approved for B-cell non-Hodgkin lymphoma and multiple myeloma (Table S3). Clinical data confirm that CAR-T can induce durable remissions and even apparent cures in subsets of patients [33, 34]. Translating this success to solid tumors has proven more difficult due to tumor heterogeneity, antigen escape, and immunosuppressive TMEs [1]. Nonetheless, early trials suggest GBM may be among the more responsive solid tumors, with encouraging signals reported in first-in-human studies (O'Rourke et al., 2017) [35].

Structure and generations of CAR-T cells

CAR-T treatment concept

Tumor progression reflects not only intrinsic oncogenic changes but also complex interactions within the TME, in which immune suppression plays a central role [9]. CAR-T therapy harnesses the cytotoxic potential of T lymphocytes by redirecting them to specifically recognize TAAs in an HLA-independent manner. Unlike conventional T cells, which rely on TCR recognition of antigens presented by MHC molecules, CAR-T cells are engineered to express synthetic receptors that combine an extracellular antigen-binding domain—derived from the single-chain variable fragment (scFv) of an antibody—with intracellular signaling motifs that trigger T cell activation, proliferation, and cytolytic activity. This design allows CAR-T cells to bypass tumor immune evasion strategies based on antigen processing or MHC downregulation [36, 37]. The therapeutic efficacy of CAR-T therapy lies in its ability to generate durable responses in otherwise treatment-refractory malignancies. By expanding ex vivo and reintroducing genetically modified T cells, the immune system is effectively rearmed with a pool of tumor-targeted effector cells capable of sustained cytotoxicity. Clinical experience in hematological cancers has demonstrated that such an approach can achieve long-term remission, reshaping the paradigm of cancer treatment [38].

CAR architecture and generational evolution

A CAR is a recombinant fusion protein composed of an extracellular scFv for antigen recognition, a hinge region, a transmembrane domain, and intracellular signaling modules. The hinge provides flexibility, while costimulatory domains (e.g., cluster of differentiation markers, CD28 and 4-1BB) enhance persistence and function (Fig. 1) [19, 38, 39]. CAR evolution has progressed through four generations: The first generation of functional CARs is characterized by the single signal molecule; the most common is a T cell surface glycoprotein CD3 zeta chain (CD3ζ). It contains scFv fused to CD3ζ or high affinity immunoglobulin epsilon receptor subunit gamma (FcεRiγ). The second generation of CAR has the ability to generate double signals through the CD3z and a costimulatory endodomain. Second-generation CARs incorporated both CD3ζ and costimulatory domains, such as CD28 or 4-1BB, which enhanced proliferation, interleukin-2 (IL-2) secretion, and in vivo activity. The third generation of CARs is composed of two costimulatory domains combined with CD3z. Third-generation CARs combined multiple costimulatory domains, but results remain inconsistent due to excessive cytokine secretion and safety concerns [40]. The fourth includes T cells redirected for universal cytokine killing (TRUCK) or CAR-T with suicide genes. CAR-T cells armed with additional modules such as inducible cytokine secretion, suicide switches, or immune-modulatory genes, designed to remodel the TME and enhance safety. Early clinical applications in ovarian cancer (MOv-γ CAR) and metastatic renal carcinoma (G250 CAR) demonstrated feasibility but limited efficacy due to activation-induced cell death and inadequate persistence, highlighting the need for continued receptor optimization [38, 41]. More detailed data of CAR molecules and their function are provided in the Supplementary Table (Table S4).

Sources of cells

Manufacturing workflow

The manufacturing of CAR-T cells involves several critical steps, beginning with the collection of autologous or, in experimental settings, allogeneic T cells. Autologous

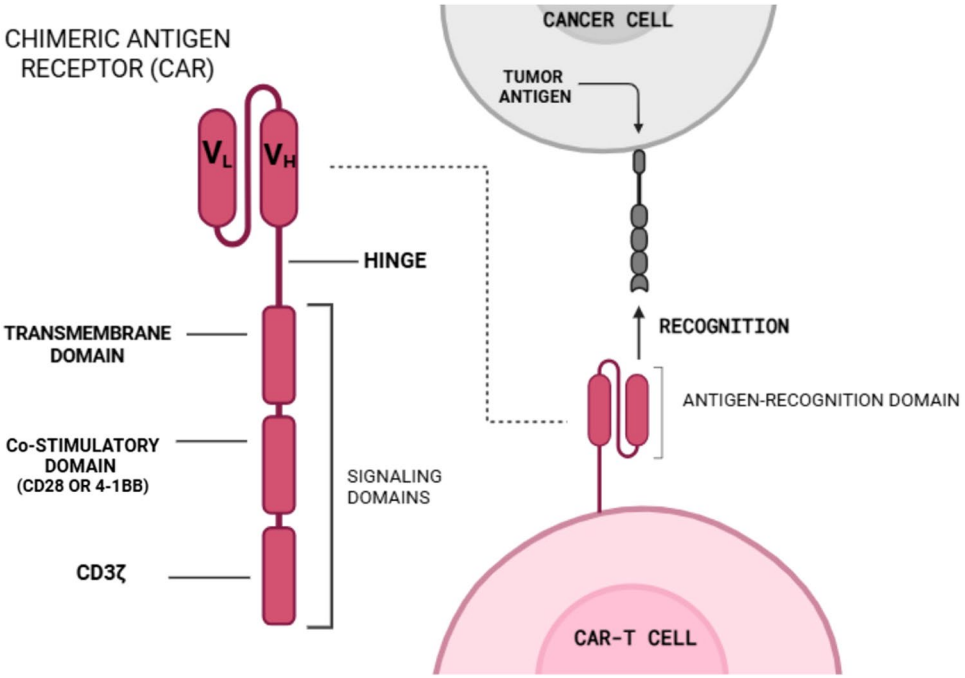

Fig. 1 Structure of CAR-T cell. The CAR-T structure contains an extracellular antigen recognition domain, hinges, transmembrane domain, and signaling domain. Its exact structure, as a group, mainly influences the specificity, activation, and function of CARs. Created with BioRender.com, based on [32]

approaches remain the gold standard, as they minimize the risk of graft-versus-host disease and immune rejection, though they are limited by the patient's prior exposure to chemotherapy and disease-related T cell dysfunction [17, 42]. CAR-T production typically involves: (1) leukapheresis to collect patient peripheral blood mononuclear cells (PBMCs), (2) isolation of CD3+ T cells, (3) genetic modification to introduce the CAR construct, (4) ex vivo expansion, and (5) reinfusion following product release testing for safety, purity, identity, potency, and stability [17, 43].

Isolation, activation and expansion

PBMCs are enriched for CD3+ T cells by centrifugation-based separation or marker-dependent methods such as magnetic-activated cell sorting (MACS). Sustained activation, usually 7–14 days, is supported by cytokines (IL-2, IL-7, IL-15) and artificial stimulation. The most common method employs anti-CD3/CD28-coated magnetic beads, which simultaneously activate and enrich T cells, providing standardized and scalable conditions. While antigen-presenting cells (APCs) can mimic physiological activation, they are impractical for clinical manufacturing. In contrast, artificial antigen-presenting cell (AAPC) systems offer a great control of T cell activation and expansion, while also ensuring consistency and practicality. Recent studies indicate that AAPCs can effectively support both in vitro T cell expansion and in vivo immune priming, making them a promising tool for large-scale adoptive cell therapy manufacturing. Activation strategy significantly influences the phenotype, persistence, and functionality of the final product [43–46].

Methods of genetic engineering

Genetic modification of T cells can be achieved by two principal strategies: the introduction of TCRs or the engineering of CARs. TCR-modified T cells are capable of recognizing intracellular antigens presented by HLA molecules, thereby granting access to the full cellular proteome. However, their efficacy is restricted by HLA polymorphism and tumor-driven downregulation of antigen presentation. In contrast, CAR-T cells are designed to recognize antigens directly on the cell surface in an HLA-independent manner. This bypasses one of the major immune escape mechanisms but limits their applicability to extracellular or secreted antigens [47, 48].

For durable and clinically effective therapy, stable integration of the CAR construct into the T cell genome is essential. To date, retroviral and lentiviral vectors remain the most widely used platforms and are employed in all FDA-approved CAR-T products [49]. These viral systems ensure high efficiency and long-term expression, although their manufacturing cost and the potential risks of insertional mutagenesis have motivated the exploration of alternative technologies. Non-viral approaches, such as transposon-based systems including Sleeping Beauty and PiggyBac, allow integration of large genetic cassettes at lower cost and have shown efficacy even in naïve T cells [50–52]. Electroporation offers another non-viral method, delivering DNA, RNA, or ribonucleoprotein complexes directly

into T cells through transient membrane permeabilization by electrical pulses. Although associated with higher cytotoxicity and reduced efficiency, electroporation has become indispensable for protocols requiring clustered regularly interspaced short palindromic repeats (CRISPR)/Cas-based editing [46, 50].

CRISPR technology has introduced a new level of precision in T cell engineering. Beyond simple CAR integration, CRISPR enables targeted disruption of inhibitory checkpoint molecules such as PD-1, insertion of cytokine support genes, or the introduction of "safety switches" to improve control over infused cells. Recent variants such as Cas12a, Cas13, and Cas14 expand editing possibilities and improve safety profiles. Alongside CRISPR, other nuclease-based systems, including transcription activator-like effector nucleases (TALENs), are under active investigation and may complement current editing strategies [53, 54].

In practice, optimized CAR-T cell production frequently combines both viral and non-viral technologies, balancing efficiency, stability, cost, and safety. Current development is directed toward generating highly functional CAR-T cells with improved persistence, controllability, and reduced toxicity. The integration of CRISPR, transposons, and switchable CAR designs illustrates the rapid technological evolution in the field, with the ultimate aim of refining CAR-T therapy for broader and safer clinical use [55].

Therapeutic potential for GBM

CAR-T as a therapeutic strategy in GBM

CAR-T cell therapy represents a promising immunotherapeutic strategy for GBM, developed to overcome the limitations of conventional modalities such as surgery, radiotherapy, and temozolomide chemotherapy. CAR-T cells are genetically engineered to recognize tumor-specific antigens such as EGFRvIII and IL13Rα2, enabling selective cytotoxicity against GBM cells [56–58]. Because of their large restriction to tumor tissue, combined with high-level expression on the surface of GBM cells, they are regarded as highly promising targets. Moreover, simultaneous targeting of both antigens expands patient coverage and mitigates the risk of antigen escape. Targeting these molecules is of particular importance, as EGFRvIII represents a constitutively active oncogenic receptor variant, while IL13Rα2 is a tumor-restricted receptor implicated in glioma progression and poor prognosis. [56, 58–60]. Preclinical studies and early phase clinical trials have demonstrated promising anti-tumor activity, although efficacy remains constrained by issues such as antigen escape, limited persistence, and tumor immunosuppression [56, 58, 59]. To overcome these obstacles, innovative designs are being developed, including dual-target CAR-T constructs, "armored" CAR-T cells capable of secreting cytokines such as IL-12 or IL-18, and synthetic Notch (synNotch) circuits that enhance tumor specificity through conditional activation. Initial clinical evidence suggests these approaches are biologically active and clinically feasible, with acceptable toxicity profiles, underscoring their potential as complementary strategies to standard-of-care treatments and as avenues toward durable tumor control in GBM patients [56, 58, 61, 62].

Molecular and genetic features of GBM

The aggressive biology of GBM is largely driven by its extensive genetic and epigenetic heterogeneity. Molecular profiling has become essential for both prognostication and therapeutic decision-making. Key alterations include IDH1/2 mutations, EGFR amplification, and variant expression such as EGFRvIII, PDGFRA mutations, NF1 loss, and disruptions in chromatin remodeling genes, including SMARCB1 [9–11]. The Cancer Genome Atlas (TCGA) has further stratified GBM into four molecular subtypes—classical, neural, proneural, and mesenchymal—each associated with distinct gene expression signatures, anatomical distribution, and therapeutic responsiveness [12, 13, 63]. These molecular characteristics not only define disease progression but also critically influence the success of CAR-T therapy by shaping antigen availability, tumor susceptibility to immune recognition, and mechanisms of adaptive resistance. An overview of the molecular determinants most relevant to CAR-T efficacy is provided in (Table 1) [4, 8, 12, 13, 60, 63].

Tumor microenvironment

The tumor microenvironment of GBM poses one of the greatest challenges for CAR-T efficacy. GBM establishes a profoundly immunosuppressive milieu characterized by infiltration of Myeloid-derived suppressor cells (MDSCs), Glioma-Associated Macrophages (GAMs), and microglia often skewed toward a pro-tumoral phenotype, and Tregs. These populations inhibit cytotoxic responses via checkpoint signaling through Cytotoxic T-Lymphocyte–Associated Protein 4 (CTLA-4) and PD-1, as well as the release of suppressive cytokines and metabolites. Additional factors such as hypoxia, nutrient depletion, and altered lipid metabolism further exacerbate tumor aggressiveness, foster cancer stem-like phenotypes, and impair CAR-T persistence and function. When combined with the high degree of genetic heterogeneity and antigenic diversity within GBM, these features create a formidable barrier to immune eradication. Understanding

Table 1 Molecular, genetic, and microenvironmental features of GBM relevant to CAR-T therapy

Feature	Relevance to CAR-T therapy	Implications
Tumor Location	CNS localization limits immune surveillance and CAR-T access	BBB restricts cell trafficking; intratumoral delivery may be required
Genetic Heterogeneity	Antigen expression is variable	EGFRvIII, IL13Rα2, HER2, GD2 may not be uniformly present; risk of an antigen escape
Molecular Subtype	Influences CAR-T efficacy	Classical, mesenchymal, proneural, and neural subtypes differ in antigen expression and microenvironment
TME	Immunosuppressive niche hinders CAR-T function	MDSCs, GAMs, Tregs, cytokines (IL-10, TNF-α) reduce CAR-T persistence and cytotoxicity
Hypoxia / Metabolic Stress	Reduces CAR-T activity	Hypoxic areas promote stem-like tumor cells resistant to immune attack
Antigen Heterogeneity	Limits targeting specificity	Dual-target or armored CAR-T designs are considered to overcome escape
Immune Checkpoints	TME expresses inhibitory receptors	PD-1 and CTLA-4 can be co-targeted to enhance CAR-T function
Cancer Stem-like Cells	Contribute to recurrence	CAR-T strategies may require targeting stem cell-associated antigens

Table 2 Key components of the GBM tumor microenvironment and their impact on CAR-T therapy [4, 7, 8]

Feature	Relevance to CAR-T therapy	Implications
BBB	Limits CAR-T infiltration across CNS	Physical barrier that restricts immune cell trafficking into the tumor
MDSCs	Suppress immune activation & promote glioma immune evasion	Reduce CAR-T proliferation and cytotoxicity
GAMs and Tumor-Associated Macrophages (TAMs)	Secrete immunosuppressive cytokines and promote angiogenesis	Impair CAR-T persistence and immune response
Tregs	Suppress anti-tumor immune responses via inhibitory signaling	Decrease CAR-T cell activity and expansion
Tumor-derived cytokines (IL-6, IL-1β, TNF-α)	Drive neuroinflammation and tumor progression	Promote CAR-T dysfunction and exhaustion
Hypoxia / Metabolic Stress	Supports stem-like phenotypes and immune evasion	Limits CAR-T efficacy and contributes to resistance
Antigen Heterogeneity	Limits targeting specificity of CAR-T cells	Encourages antigen escape; necessitates dual-target strategies

the interplay between tumor genetics and the microenvironment is therefore crucial for designing CAR-T strategies capable of overcoming immune evasion and producing durable therapeutic benefit [4, 7, 8, 64]. More detailed information is provided in (Table 2).

Tumor-associated antigens relevant for CAR-T therapy

The heterogeneity of GBM complicates the identification of reliable surface targets for CAR-T cell therapy. Candidate antigens are generally classified as tumor-specific antigens (TSAs) or TAAs. While TSAs such as EGFRvIII are more restricted to tumor cells, their prevalence is limited to a subset of patients. In contrast, TAAs—including HER2, IL13Rα2, cluster of differentiation 70 (CD70), B7-H3, GD2, and (prominin-1) CD133—are more widely expressed but may also occur at low levels in normal tissues, raising concerns about off-tumor toxicity. Moreover, recent studies indicate that AVIL has recently attracted attention as a novel therapeutic target. It is expressed in the majority of GBMs, while showing only limited expression in normal tissues, thereby providing a rationale for its exploration in CAR-T cell therapy. CAR-T therapies directed against these targets have demonstrated encouraging results in preclinical models and early phase clinical trials, although challenges remain in achieving durable efficacy without unacceptable adverse effects. A detailed summary of current antigenic targets under investigation in GBM, together with their expression patterns, is

Table 3 Overview of potential CAR-T cell target antigens in GBM

Antigen	Expression and function in GBM	Clinical/preclinical highlights
EGFRvIII	Tumor-specific EGFR mutation found in approximately 30% of GBM cases; absent in normal tissues	CAR-T cells targeting EGFRvIII showed tumor infiltration and immune activity; combination with IL-12 or PD-1 knockout improves efficacy
HER2	Overexpressed in GBM with low in normal tissue and other cancers; linked to poor prognosis	Early trials showed immune activation; one fatal case due to off-tumor toxicity highlighted the need for targeted delivery
IL13Rα2	Highly expressed in GBM, rarely in healthy tissue; expression increases with tumor grade	IL13Rα2 CAR-T cells induced cytokine release and tumor regression; explored as a prognostic marker and therapeutic target
CD70	Found in gliomas and activated immune cells; contributes to immunosuppression and tumor progression	Preclinical studies show tumor reduction and reshaping of the TME; ongoing early clinical trials
B7-H3 (CD276)	Highly expressed in GBM, minimal in normal tissues	CAR-T therapy combined with CXCL11-armed adenovirus enhanced tumor penetration and immune cell infiltration
EphA2	Overactive receptor tyrosine kinase involved in resistance and proliferation	Dual-epitope targeting Ephrin type-A receptor 2 (EphA2) CAR-T improved survival in mice; benefits from CXCR1/2 co-expression and Interferon alpha-2 (IFN-γ) modulation
CD133	Marker of glioma stem cells; linked to tumor growth, recurrence, and resistance	CD133 CAR-T showed selectivity without affecting normal hematopoietic stem cells; more studies are needed due to expression variability
GD2	Ganglioside antigen is common in neural tissues and GBM; it is uniformly expressed in some gliomas	Phase I trials showed improved neurological function without off-target toxicity; synergistic with radiotherapy
NKG2D ligands (NKG2DL)	Stress-induced ligands recognized by the NKG2D receptor; widely expressed on cancer cells	CAR-T co-engineered with IL-12 or IFN-α2 enhanced tumor killing and prolonged T cell activity; early human trials are ongoing

provided in (Table 3) and (Fig. 2). Ultimately, the optimization of CAR-T therapy in GBM depends on strategies that integrate antigen selection with approaches to counteract TME–mediated immunosuppression, enhance CAR-T persistence, and improve trafficking into the CNS [16, 65–70].

Routes of CAR-T cells delivery in GBM

Challenges of delivery

A major obstacle in GBM therapy is the BBB, which protects the CNS from pathogens and toxins but simultaneously prevents most therapeutic agents and biologics from reaching the brain. Although GBM can locally disrupt the BBB, this disruption is incomplete and insufficient for effective treatment. As a result, systemic drug exposure often leads to limited intracranial delivery alongside increased off-target toxicity. In addition to this anatomical barrier, the TME of GBM represents a dynamic immunosuppressive niche composed of glioma cells, immune populations such as Tregs, macrophages, and MDSCs, and non-immune components including stromal and endothelial cells. Hypoxia, metabolic stress, and cytokine networks further reinforce immunosuppression, limiting the activity and persistence of systemically delivered CAR-T cells. Together, the BBB and TME significantly reduce CAR-T efficacy in GBM, underscoring the need for optimized delivery routes that improve tumor infiltration and therapeutic concentration at the site of disease [14, 87, 88].

Intravenous (IV) delivery

Intravenous administration is the most common and least invasive route for CAR-T cell therapy and has demonstrated robust efficacy in hematological malignancies. In GBM, however, systemic infusion faces unique challenges. CAR-T cells must traverse the BBB and successfully home to intracranial tumors, yet their trafficking remains inefficient, and those that reach the brain encounter a profoundly immunosuppressive TME. Consequently, IV delivery has shown limited clinical activity in GBM, though it remains relevant as a platform for combination strategies that aim to enhance homing, increase BBB permeability, or precondition the CNS for immune infiltration [89, 90].

Local (locoregional) delivery methods

Locoregional administration directly bypasses the BBB, enabling CAR-T cells to accumulate at the tumor site with higher efficiency. Techniques include intratumoral, intracavitary (ICT), and intraventricular (ICV) delivery, each

Fig. 2 Heatmap of potential CAR-T target antigens in glioblastoma. The heatmap illustrates antigen expression profiles across GBM and healthy tissue samples and represents patient-level averaged data. Several antigens (EGFRvIII, HER2, IL13Ra2, CD70, B7-H3, EphA2, GD2, and NKG2DL) demonstrated increased expression in GBM relative to healthy controls. These findings highlight a subset of tumor-associated antigens with preferential expression in GBM, supporting their potential as immunotherapeutic targets. NKG2DL ligands are highly expressed (2.5) in ~ 68–72% of GBM patients and low (1) in normal tissue. IL13Rα2 is highly expressed (3) in ~ 78% of GBM and very low (1) in normal tissue. HER2 is moderately expressed (2) in ~ 42% of GBM and low (1) in normal tissue. GD2 is moderately expressed (2) in ~ 58% of primary GBM-derived cell lines and low (1) in normal tissue. EphA2 is moderately expressed (2) in ~ 60% of GBM and low (1) in normal tissue. EGFRvIII is moderately expressed (2) in ~ 30% of GBM patients and absent (0) in normal tissue. CD70 shows low expression (1) in ~ 18% of IDH-wild-type GBM and low (0.5) in normal tissue. CD133 (PROM1) shows low expression (1) in both GBM and normal tissue, marking glioma stem cell subpopulations. B7-H3 (CD276) is highly expressed (2.5) in ~ 77% of GBM, and low (1) in normal tissue. Data for CD133 and healthy tissue expression across all antigens were obtained from the Human Protein Atlas (HPA) database. According to published data, expression of these antigens in GBM is significantly higher than in healthy tissue ($p < 0.0001$) [71]. Expression was measured using bulk RNA-seq, single-cell RNA-seq, immunohistochemistry, flow cytometry, and immunofluorescence. Numeric values correspond to the intensity scale in the heatmap [16, 56, 57, 65–68, 72–86]. Semi-quantitative antigen expression data were obtained for GBM and compared to healthy brain tissue. Expression scores were assigned on a discrete scale: 0 (none), 1 (low), 2 (moderate), 2.5 (high), and 3 (very high). Data were organized into a matrix format and processed in R (version 4.4.1, R Foundation for Statistical Computing, Vienna, Austria) using RStudio (version 2025.05.1 Build 513, Posit Software, Boston, MA, USA). Color gradients were scaled from white (0) to purple (3) to represent increasing antigen expression levels. More detailed quantitative data for each antigen are provided in the Supplementary Table (Table S5)

adapted to the anatomical and biological features of GBM. Intratumoral strategy parallels methods already established in oncology, such as intravesical instillation in bladder cancer and intratumoral injection of oncolytic viruses [90, 91]. Preclinical evidence indicates superior tumor control with intratumoral delivery compared to IV infusion, and early phase clinical studies confirm safety, feasibility, and manageable toxicity profiles. It avoids damaging healthy tissue compared to surgery or radiation and is particularly suitable for accessible tumors.

Dual-route approaches (combining ICT and ICV) are also being explored. Sequential ICT and ICV administration can target both residual tumor tissue at the resection site and disseminated disease within cerebrospinal fluid (CSF). Similarly, combined IV and ICV infusion aims to leverage systemic reach alongside local CNS targeting [92]. Although repeated local infusions carry procedural risks, these strategies hold promise for improving CAR-T distribution, persistence, and tumor clearance. A comparative summary of available delivery routes and their respective advantages

and limitations is presented in the Supplementary Table (Table S6).

Strategies to enhance CAR-T cell penetration into the glioma microenvironment

Beyond route selection, several techniques are under investigation to enhance CAR-T penetration into the TME. Low-intensity pulsed focused ultrasound (LIPU) combined with microbubbles (MB) offers a minimally invasive approach to temporarily disrupt the BBB, enabling the entry of immune cells or therapeutic agents into the brain. The method uses a concave transducer to focus sound waves on a specific brain region while intravenously delivered lipid-coated gas MB (1–5 μm) oscillate in response to the ultrasound. This oscillation produces localized fluid movement that exerts mechanical stress on the endothelium, briefly loosening tight junctions and increasing BBB permeability. Early studies in malignant gliomas demonstrated enhanced drug delivery and immune infiltration with this method. Sonabend et al. further showed that an implantable ultrasound device could repeatedly and safely facilitate BBB opening in patients, increasing brain penetration of paclitaxel and carboplatin. Beyond drug delivery, ultrasound-mediated BBB modulation may itself exert immunomodulatory effects, creating transient inflammation that promotes immune cell recruitment. These findings suggest a potential role for LIPU + MB as an adjunct to improve CAR-T homing and persistence [93–96].

Emerging biomaterial platforms for CAR-T therapy: hydrogels, implants, and microneedles

Emerging biomaterial-based strategies provide new opportunities to improve CAR-T delivery and activity within GBM. Injectable hydrogels, composed of biodegradable and biocompatible polymers, can act as localized scaffolds to protect CAR-T cells, control their release, and co-deliver stimulatory molecules. Grosskopf et al. (2022) demonstrated that transient hydrogel scaffolds enhanced CAR-T infiltration, persistence, and anti-tumor efficacy in solid tumor models while reducing systemic toxicity. Similarly, Suraiya et al. (2022) reported micro-hydrogel systems that supported CAR-T viability, sustained tumor contact, and improved cytotoxicity in 3D tumor spheroids [97–99].

Other platforms, including implantable scaffolds and microneedle arrays, are also being investigated. Scaffolds mimic the extracellular matrix, supporting CAR-T proliferation and infiltration, while microneedles allow minimally invasive, localized delivery to surgical cavities or superficial tumors. Lin et al. (2024) highlighted "smart" hydrogels responsive to tumor-specific stimuli such as pH or enzymatic activity, enabling spatiotemporal control of CAR-T release. Multifunctional biomaterials that co-deliver CAR-T cells alongside checkpoint inhibitors or cytokines further offer a means to remodel the TME and enhance efficacy [100, 101]. Collectively, these platforms represent a promising frontier for improving CAR-T persistence, localization, and anti-tumor function in GBM.

Strategies to enhance CAR-T efficacy in GBM

Dual-targeting and multi-antigen CARs

One of the major limitations of CAR-T therapy in GBM is antigen heterogeneity. Glioblastoma cells display marked intratumoral and interpatient variability in target expression, allowing tumor subpopulations to escape single-antigen CAR-T surveillance. Dual- or multi-target CARs aim to overcome this obstacle by recognizing two or more antigens simultaneously. Constructs include tandem CARs, bicistronic CARs, and pooled CAR-T cell products targeting distinct epitopes. For instance, bispecific CARs directed against HER2 and IL13Rα2 or EGFRvIII and EphA2 have shown enhanced tumor recognition and reduced antigen loss–driven relapse in preclinical GBM models [1]. Early phase trials suggest that multi-target approaches may improve both response rates and durability of control compared with single-antigen designs, although at the expense of more complex engineering and manufacturing (Fig. 3) [102, 103]. A phase I trial (NCT05168423), sponsored by the University of Pennsylvania, evaluated intrathecal administration of bivalent CAR-T cells targeting EGFR and IL13Rα2 in six patients with recurrent, multifocal GBM. Both tested doses (1×10^7 and 2.5×10^7 cells) were generally manageable from a safety perspective, although all patients developed early onset neurotoxicity consistent with immune effector cell-associated neurotoxicity syndrome (ICANS). Symptoms were treated with corticosteroids and anakinra, with one case of dose-limiting toxicity. Early MRI assessments revealed reductions in contrast enhancement and tumor size in all patients, although none achieved objective radiographic response. High CAR-T cell levels and cytokine release in CSF confirmed bioactivity, suggesting preliminary efficacy warranting further investigation [103, 104].

SynNotch CAR-T

The synNotch "prime-and-kill" approach addresses these obstacles by using a tumor-restricted antigen (e.g., EGFRvIII) to trigger CAR expression against IL13Rα2 and EphA2, thereby achieving potent tumor clearance while sparing healthy tissue. The EGFRvIII-synNotch–primed CAR-T configuration (E-SYNC) demonstrated strong anti-tumor activity in preclinical models (Choe et al. 2021). For

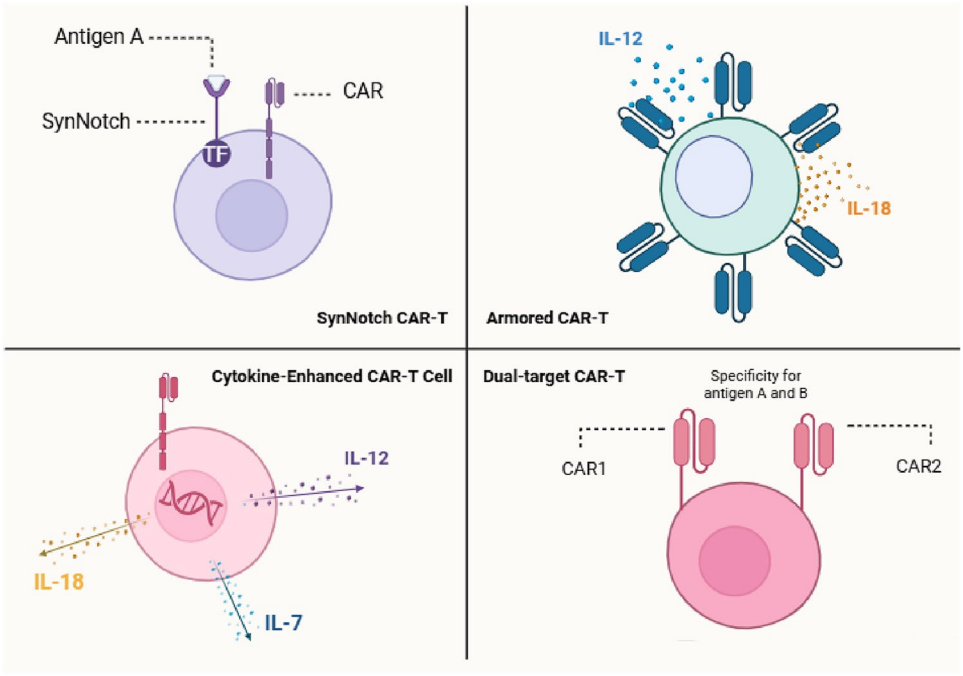

Fig. 3 Graphical representation of advanced CAR-T designs such as SynNotch CAR-T, Armored CAR-T, Cytokine-Enhanced CAR-T Cell, and Dual-target CAR-T. SynNotch cells contain a synthetic Notch receptor; upon binding to antigen A, they induce expression of a CAR receptor. Armored cells are equipped with CAR receptors and engineered to secrete immunostimulatory cytokines such as IL-12 or IL-18. Cytokine-enhanced CAR-T cells are genetically modified to produce survival and proliferation supporting cytokines (e.g., IL-7, IL-12, and IL-18). Dual-target cells express two distinct CAR receptors, enabling simultaneous or sequential recognition of two tumor-associated antigens [107–111]. Created with BioRender.com

EGFRvIII-negative tumors, Brevican (BCAN) has been identified as an alternative CNS-specific priming antigen, enabling BCAN-synNotch → IL13Rα2/EphA2 CAR (B-SYNC) cells to induce complete remission in preclinical GBM models with improved persistence and CNS homing (Simic et al., in press) [61]. These promising synNotch-based strategies have now advanced into clinical evaluation. The E-SYNC platform, an anti-EGFRvIII synNotch receptor inducing anti-EphA2/IL13Rα2 CAR-T construct, is currently being tested in a phase I clinical trial (NCT06186401), sponsored by Dr. Hideho Okada at UCSF [105]. According to the latest update, this trial represents a critical step toward assessing the feasibility, safety, and preliminary efficacy of synNotch-based CAR-T therapy in patients with GBM [106, 107].

Armored CAR-T

Recent approaches to enhance CAR-T efficacy in glioblastoma have explored the use of "armored" designs to counteract the profoundly immunosuppressive TME. In one preclinical study, CAR-T cells targeting IL13Rα2 were engineered to secrete IL-12 and IL-18 upon activation. This modification promoted robust anti-tumor activity in orthotopic glioma models and triggered significant remodeling of the immune landscape. By day 9 post-treatment, mice receiving IL-12/IL-18-armored CAR-T cells showed expansion of both exogenous CD8 + and endogenous CD4 + T cell populations, enriched for the resident memory marker CXCR6, alongside increased infiltration of NK cells and monocytes. These changes suggested a coordinated, multilineage immune response capable of sustaining long-term tumor surveillance [62, 104].

Recent advances in CAR-T cell therapy for GBM have focused on cytokine engineering to improve persistence and anti-tumor efficacy within the TME. Pawlowski et al. (2023) comprehensively reviewed cytokine modifications that enable CAR-T cells to secrete pro-inflammatory cytokines such as IL-12 and IL-18 upon activation. These cytokine-armed CAR-T cells improve infiltration, survival, and persistence by promoting a pro-inflammatory milieu that counteracts glioblastoma-associated immune suppression. This strategy stimulated both adaptive and innate immunity, including expansion of endogenous T cells, NK cells, and monocytes, thereby fostering a multifaceted immune response. Such cytokine-driven remodeling of the TME enhances tumor clearance and supports long-term immunosurveillance, addressing key challenges faced by conventional CAR-T therapies in solid CNS tumors [108, 109].

Recent clinical trials of CAR-T cell therapy in GBM

Recent advances in CAR-T cell therapy for GBM have been highlighted by early phase clinical trials conducted in 2025, demonstrating innovative delivery approaches and encouraging preliminary efficacy. These studies aim to translate preclinical advances into meaningful patient outcomes by investigating innovative CAR designs, locoregional delivery methods, and strategies to overcome the highly immunosuppressive TME. Most trials focus primarily on targeting key tumor-associated antigens, including IL13Rα2, HER2, EGFR, EGFRvIII, EphA2, GD2, and B7-H3. These antigens are frequently overexpressed in select solid tumors, offering potential targets for therapeutic intervention [92, 108].

An ongoing Phase I study (NCT05660369) led by Dr. Marcela V. Maus at Massachusetts General Hospital, the CARv3-TEAM-E T cell therapy is being evaluated in adults with recurrent or newly diagnosed EGFRvIII-positive glioblastoma. According to the trial registry, up to 21 patients are expected to be enrolled, receiving weekly intraventricular infusions over six doses, with safety and persistence being key endpoints. Preliminary results from the initial three patients confirmed the absence of dose-limiting toxicities, though some grade 3 adverse events, such as encephalopathy or fatigue occurred. All three developed fevers within two days of infusion. Remarkable anti-tumor activity was observed: One patient displayed immediate but transient radiographic regression; another achieved a sustained 150-day response; and a third experienced near-complete regression within five days but later relapsed. Liquid biopsy analyses demonstrated reductions in both EGFRvIII and EGFR copy numbers, particularly in cerebrospinal fluid. Updated safety data confirmed that multiple intraventricular infusions remain well tolerated, with no new safety concerns emerging [112–114]. A Phase I trial in the US (NCT02208362), sponsored by the City of Hope Medical Center, investigated IL13Rα2-targeted CAR-T cells in recurrent or refractory malignant glioma. Among 65 participants, only one had recurrent multifocal GBM. This patient received 16 intracranial infusions over 220 days (10 intraventricular, 6 intracavitary), resulting in 77–100% tumor reduction after intraventricular doses and no ≥ grade 3 toxicities. Clinical benefit lasted about 7.5 months. The most recent trial update (February 2025) confirmed ongoing evaluation of safety, feasibility, and CAR-T persistence in the CNS, with findings highlighting both the promise of locoregional delivery and the need for broader studies in this patient subgroup [56, 113, 115, 116]. Also, subsequent to these locoregional CAR-T trials, the Phase I/II SL-701 vaccine study (NCT02078648) explored a peptide-based immunotherapy in combination with poly-ICLC and bevacizumab for recurrent glioblastoma. The trial achieved a 12-month overall survival rate of approximately 50%, notably higher than that typically observed with standard therapies. A detailed immunophenotyping initiative revealed that specific cytotoxic memory T cell subsets, particularly $CD8^+$ $CD57^+$ $CD107a^+$ $PD\text{-}1^-$ cells, were enriched in patients with survival beyond one year, suggesting these T cell characteristics may serve as biomarkers for a favorable response [56, 117]. A summary of recent early phase CAR-T cell therapy trials in glioblastoma is presented in the Supplementary Table (Table S7), providing a comparative overview of each approach [114, 117–122].

Future perspectives

The future of CAR-T cell therapy in glioblastoma is likely to depend on combining these innovative approaches to maximize efficacy and safety. SynNotch circuits offer precision targeting, reducing the risk of off-tumor effects, while armored CAR-T designs enhance potency by actively remodeling the immunosuppressive microenvironment [34]. Dual-targeting strategies address tumor heterogeneity but carry a risk of increased neurotoxicity, requiring careful mitigation. Incorporating cytokine engineering may further promote immune activation and persistence, but this approach demands tight control to prevent systemic toxicity [123, 124]. In addition, novel delivery systems, such as biomaterial-based depots and locoregional infusion devices, together with combination regimens involving checkpoint inhibitors or molecularly targeted agents, are expected to further expand therapeutic options. Continued clinical evaluation and refinement of these approaches will be essential to unlock the full potential of CAR-T therapy against GBM [99].

Summary

Emerging CAR-T cell therapies for GBM, including synNotch prime and kill systems, armored cytokine-secreting cells, and dual-targeted constructs, demonstrate promising advances in overcoming challenges such as antigen heterogeneity, immunosuppressive TME, and the risk of on-target off-tumor toxicity. Each approach offers distinct advantages but also faces unique limitations, particularly regarding safety and clinical efficacy. Ongoing and future clinical trials will be essential to validate these innovative strategies. Ultimately, combining precision targeting with enhanced immune modulation holds significant promise for improving CAR-T therapeutic outcomes in glioblastoma.

Conclusions

CAR-T therapy represents a rapidly evolving and promising perspective for GBM treatment. Preclinical and clinical data demonstrate that novel CAR designs, such as armored CAR-T, synNotch, and dual-target approaches, which can increase intratumoral activity, overcome antigen heterogeneity and the immunosuppressive TME to an extent. Furthermore, locoregional delivery strategies and adjunctive technologies, e.g., BBB modulation, biomaterial scaffolds, further improve CNS bioavailability and reduce systemic toxicity, developing the potential of CAR-T approaches.

Nevertheless, durable clinical advantage in GBM remains constrained by antigen escape, limited CAR-T persistence, and the complexity of the GBM microenvironment. Future success will depend on antigen selection and multi-target strategies, improved manufacturing and cell engineering to sustain persistence and limit neurotoxicity, and carefully designed combination trials integrating CAR-T with local modulators of the tumor niche and immunomodulatory agents. Standardization of delivery routes, safety management (including ICANS mitigation), and robust biomarkers for patients are essential for progression to later-phase studies.

In conclusion, CAR-T therapy for GBM has advanced to promising clinical activity; however, further engineering and early phase trials are required before CAR-T can become a standard therapeutic option for GBM patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00262-025-04222-w.

Author Contributions All authors contributed to the study conception and design. Manuscript preparation, literature review and analysis were performed by AK and MT. The first draft of the manuscript was written by AK and MT. All authors read and approved the final manuscript.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability No data were generated during preparation of the manuscript.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Not applicable.

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