Review

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CAR-T cell therapy for glioblastoma: advances, challenges, and future directions

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Background: Chimeric antigen receptor T (CAR-T) therapy for glioblastoma involves critically evaluating progress, effectiveness, and challenges. By examining current research, clinical trials, and emerging trends, the analysis highlights clinical outcomes and biological insights that demonstrate the therapeutic potential of CAR-T cells, along with technological innovations aimed at enhancing their efficacy and safety. However, significant obstacles such as overcoming the blood-brain barrier and managing severe side effects like cytokine release syndrome remain.
Methods: A systematic search using PubMed, Scopus, Web of Science, and Google Scholar from 2010 to 2024 has been conducted. Search terms included "CAR-T," "glioblastoma," "immunotherapy," and "clinical trials." Inclusion criteria were English-language studies focusing on CAR-T applications in glioblastoma. Exclusion criteria included non-peer-reviewed articles and preclinical-only studies.
Findings: The findings suggest promising prospects for integrating CAR-T cell therapy into existing glioblastoma treatment paradigms, emphasizing the need for continued research and innovation in genetic engineering and combination therapies to fully realize the potential of CAR-T cells in transforming glioblastoma treatment.
Conclusions: CAR-T cell therapy offers groundbreaking potential in transforming glioblastoma treatment by harnessing the immune system to target and destroy cancer cells.

Keywords: CART, GBM, glioblastoma, immunotherapy

Introduction

Overview of glioblastoma as an aggressive brain tumor with poor prognosis

Glioblastoma multiforme (GBM), also known simply as glioblastoma, is currently classified by the World Health Organization (WHO) as astrocytoma, IDH-wild type, WHO grade 4 [WHO Classification of Tumours Editorial Board, WHO Classification of Tumors of the Central Nervous System, 5th ed., Lyon: International Agency for Research on Cancer (IARC), 2021]. It represents the most prevalent, aggressive, and lethal form of primary brain tumors.

These tumors are characterized by rapid growth and extensive infiltration into surrounding brain tissue[1]. Histologically, GBMs exhibit a high degree of cellular polymorphism, necrosis, and microvascular proliferation[2].

The peak incidence occurs between the ages of 55 and 75 years, and its occurrence in children and young adults is relatively rare[3–5]. The standard treatment protocol for GBM involves surgical removal of the tumor, followed by radiotherapy combined with concurrent and subsequent chemotherapy using temozolomide[6]. Despite extensive research and advancements in medical science, there is still no definitive treatment for this devastating disease. Current research primarily focuses on prolonging survival and developing more effective strategies to treat glioblastoma.

Introduction to CAR-T cell therapy

Recent advances in cancer immunotherapy have enabled the development of CAR T-cell therapy[7]. During the process of leukophoresis, T cells collected from patient's blood are isolated, which are then genetically modified to express a chimeric antigen receptor (CAR) specific for a particular tumor antigen (TA)[8]. T cells are multiplied *ex vivo* then administered to the patient to target and eliminate cancer cells[9]. The advantage of CAR T cells is that they do not require MHC-mediated antigen presentation. Hence, this allows them to attack a broad set of TAs[7]. This opens the way for the discovery of an effective treatment for GBM. While surgical resection remains the

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cornerstone of GBM management, especially for tumor debulking and histopathological diagnosis, CAR-T therapy offers a nonoperative, immunological approach that targets residual infiltrative disease. Although not mutually exclusive, CAR-T is particularly valuable for non-resectable or recurrent tumors. Combining CAR-T with maximal safe resection may potentially enhance survival while minimizing systemic toxicity.

HIGHLIGHTS

- Chimeric antigen receptor T (CAR-T) cell therapy shows promising potential in treating glioblastoma.
- Advances in technology aim to improve CAR-T cell efficacy and safety.
- Key challenges include the blood-brain barrier and severe side effects.
- Integration with existing treatments offers new therapeutic directions.
- Continued innovation in genetic engineering is essential for clinical success.

Principles of CAR-T

Description of the CAR-T cell design and engineering specific to brain tumors

CAR-T therapy based on an antigen-specific receptor can also be modulated by designing signaling domains. CAR includes extracellular, trans-membrane and intracellular domains[8]. The extracellular domain consists of an antigen-binding domain and a hinge domain. The antigen-binding domain is most often a single-chain variable fragment (ScFv) although it can also be modified binding scaffolds, receptors, or ligands. The hinge domain connects the ectodomain to the transmembrane domain, which is made of alpha-helix. It stabilizes CARs in the cell membrane and enables proper signaling[10]. In contrast, the intracellular domain consists of a CD3 complex with a T-cell receptor[7]. In addition, the CAR endodomain consists of different activation and costimulation domains causing CAR to divide into five generations. By looking for new genetic modifications, we can improve T-CAR cells in carrying out the effector function of killing cancer cells[11]. Immunohistochemical (IHC) analysis of gliomas has revealed potential therapeutic targets, which are the antigens epidermal growth factor receptor variant III (EGFRvIII), human epidermal growth factor receptor 2 (HER2), interleukin alpha 2 receptor (IL-13Rα2), and ephrin type-A receptor 2 (EphA2). Clinical trials on the efficacy of specific interventions are currently underway[12].

Key components of CAR-T cells: antigen recognition, signaling domains, and co-stimulatory molecules.

Chimeric antigen receptor T cells (CAR-T cells) have emerged as a groundbreaking therapy in oncology, offering new hope for patients with certain types of cancers, particularly hematologic malignancies[13]. These genetically engineered T cells are designed to specifically target and eliminate cancer cells. The efficacy of CAR-T cell therapy hinges on three critical components (Fig. 1): antigen recognition, signaling domains, and co-stimulatory molecules. Furthermore, ongoing advancements have introduced a variety of innovative CAR-T cell designs and combination therapies[14].

Antigen recognition

The first step in the mechanism of CAR-T cells is antigen recognition, a process that determines the specificity and efficacy of the therapy[15]. CAR-T cells are equipped with a synthetic receptor, the CAR, which is designed to recognize and bind to specific antigens on the surface of cancer cells[16]. This receptor is a fusion of several components, including an extracellular antigen-binding domain, usually derived from a monoclonal antibody, that provides the specificity to recognize a tumor-associated antigen (TAA)[17].

The choice of antigen is crucial. Ideal target antigens are those that are abundantly expressed on cancer cells but have limited or no expression on normal cells to minimize off-tumor effects. For example, CD19 is a common target in B-cell malignancies because it is primarily expressed on B cells. The binding of the CAR to its target antigen is the initiating event that triggers the activation and cytotoxic functions of the CAR-T cells[18].

Signaling domains

Once the CAR binds to the antigen, the intracellular signaling domains of the receptor come into play. These domains are responsible for transmitting the activation signal from the extracellular domain to the T cell, prompting it to kill the cancer cell. The signaling domain is typically derived from the CD3ζ chain of the T cell receptor, which is a key player in T cell activation[19].

The CD3ζ domain contains immunoreceptor tyrosine-based activation motifs that initiate a cascade of intracellular signaling events leading to T cell activation, proliferation, and cytotoxic activity. However, the CD3ζ domain alone is often insufficient for robust and sustained T cell activation, which is where the co-stimulatory molecules come into play[20].

Co-stimulatory molecules

Co-stimulatory molecules are essential for enhancing the efficacy and persistence of CAR-T cells. These molecules provide additional signals required for full T cell activation and function. The most common co-stimulatory domains used in CAR constructs are derived from CD28 and 4-1BB (CD137)[21].

CD28: The inclusion of the CD28 co-stimulatory domain in CAR-T cells leads to rapid and robust T cell activation. CD28 enhances the production of interleukin-2 (IL-2), a cytokine critical for T cell proliferation and survival. CAR-T cells with CD28 domains tend to have a potent initial response but may have shorter persistence in the body[22].

4-1BB (CD137): The 4-1BB co-stimulatory domain promotes T cell survival and enhances their longevity and memory phenotype. CAR-T cells incorporating 4-1BB typically exhibit sustained activity over a more extended period, which can be beneficial for long-term cancer control[23].

The combination of CD3ζ with either CD28 or 4-1BB co-stimulatory domains has been shown to significantly improve the overall function of CAR-T cells[24]. Each co-stimulatory molecule imparts distinct properties to the CAR-T cells, and ongoing research aims to optimize these combinations for various therapeutic applications[25].

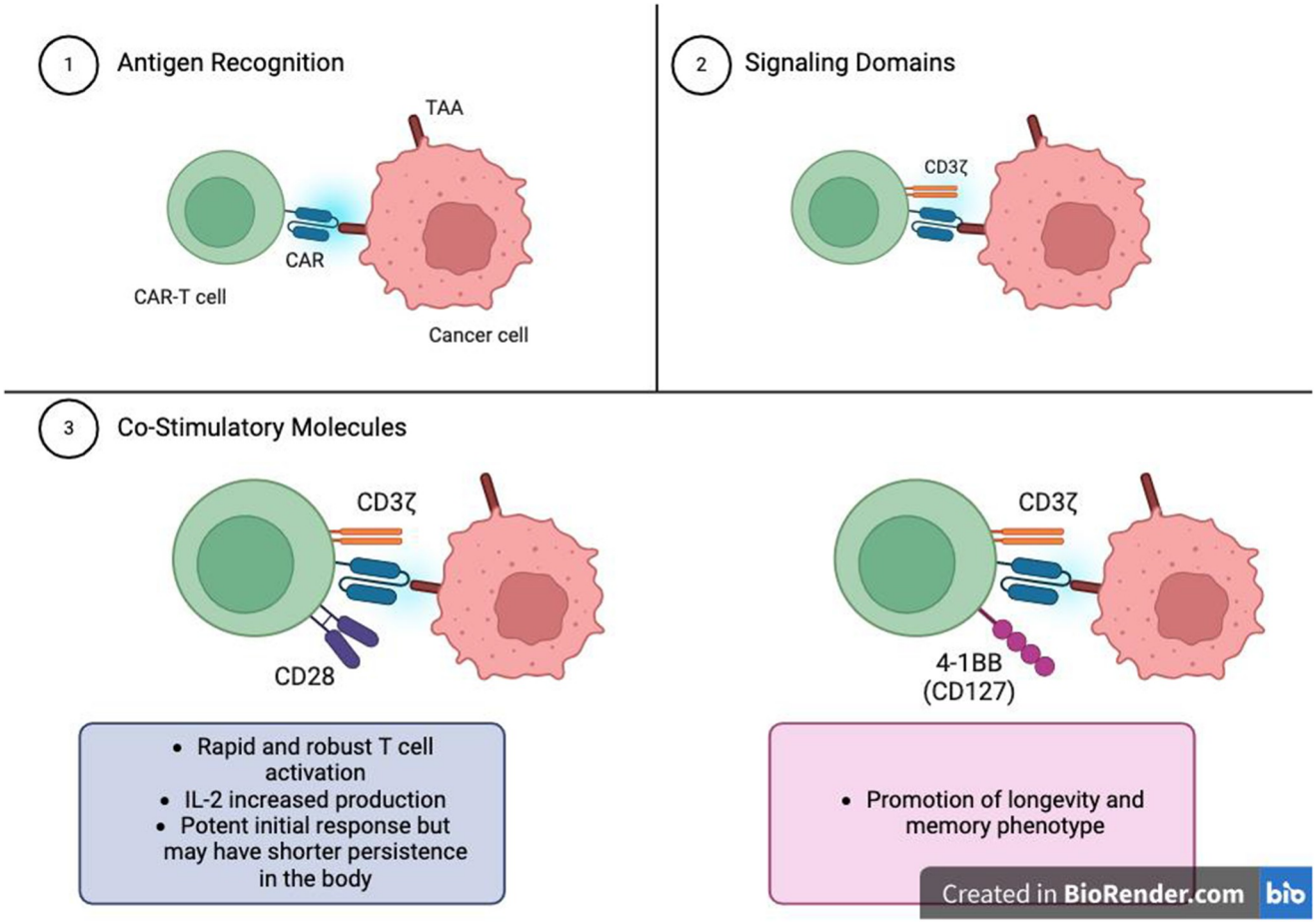

Figure 1. The efficacy of CAR-T cell therapy hinges on three critical components.

Overview of the production and administration process of CAR-T cells

Production of CAR-T cells occurs through viral vectors, which use reverse transcriptase to incorporate artificial genes into the host genome[26]. They are then proliferated and blood-preserved under good manufacturing practice conditions[27]. They are delivered locoregionally: intraventricularly and intratumorally (ICT)[28]. This is due to evidence that CAR-T cells do not work by intravenous infusion in solid tumors, due to immune escape of antigen[29,30]. In intratumoral injections, the inflammatory niche created enhances their anti-tumor efficacy[31]. The injection materials are hydrogels. They surround the CAR-T cells by acting as a support, where they accumulate proliferation to then migrate efficiently towards the tumor[32]. In addition, they provide an opportunity to co-encapsulate other therapeutic agents, such as cytokines, metformin or oxygen carriers. Currently in development are special hydrogel platforms for locoregional delivery and controlled release of CAR-T cells[33].

CAR-T cell therapy in glioblastoma

Through IHC analysis, various molecules have been identified as potential targets for CAR T cell therapy against GBMs (Table 1). Completed and ongoing clinical trials using CAR-T cells targeting glioblastoma-specific antigens including EGFRvIII, B7-H3, IL13Rα2, HER2, GD2, NKG2D, CARv3-TEAM-E, CD70, and EphA2 are summarized below.

EGFRvIII

In 2009, Bullain *et al* employed the sequence of the MR1 murine scFv targeting EGFRvIII to develop a first-generation CAR. Their findings demonstrated that T cells engineered with MR1-ζ were capable of specifically recognizing glioma cell lines expressing EGFRvIII and effectively inhibiting intracranial tumor growth in immunodeficient murine models[34].

The following year, Ohno *et al*[35] developed a different CAR targeting EGFRvIII, utilizing the mouse monoclonal antibody (mAb) 3C10. Their research revealed that T cells modified with this CAR could successfully infiltrate intracranial gliomas and, when administered systemically, suppress tumor growth in immunodeficient mouse models. Further advancements came in 2012 when Morgan *et al*[36] engineered an anti-EGFRvIII CAR based on the human monoclonal antibody mAb-139. This CAR specifically recognized glioma stem cells expressing EGFRvIII without targeting control or wild-type EGFR-engineered cell lines *in vitro*. With a T-cell signaling domain that included CD28, 41BB, and CD3ζ, the 139-based CAR T cells were expanded to large quantities while retaining their antitumor efficacy[36]. Progress in the field continued

Table 1

Summary of CAR-T cell therapy in glioblastoma

Type of CAR-T + others	Subject	Phase	Status	ID
SNC-109 CAR-T	The Safety and Efficacy of SNC-109 CAR-T Cells Therapy the Recurrent Glioblastoma	1	Recruiting	NCT05868083
NKG2D CAR-T	Pilot Study of NKG2D CAR-T in Treating Patients With Recurrent Glioblastoma	1	Unknown status	NCT04717999
B7-H3 CAR-T + Temozolomide	Pilot Study of B7-H3 CAR-T in Treating Patients With Recurrent and Refractory Glioblastoma	1	Recruiting	NCT04385173
	B7-H3 CAR-T for Recurrent or Refractory Glioblastoma	1/2	Recruiting	NCT04077866
EGFRvIII CAR T	EGFRvIII CAR T Cells for Newly-Diagnosed WHO Grade IV Malignant Glioma (ExCeL)	1	Terminated	NCT02664363
	Intracerebral EGFR-vIII CAR-T Cells for Recurrent GBM (INTERCEPT)	1	Terminated	NCT03283631
	The Efficacy and Safety of Brain-targeting Immune Cells (EGFRvIII-CAR T Cells) in Treating Patients With Leptomeningeal Disease From Glioblastoma. Administering Patients EGFRvIII -CAR T Cells May Help to Recognize and Destroy Brain Tumor Cells in Patients (CARTREMENDOUS)	1	Unknown status	NCT05063682
	A Study to Evaluate the Safety, Tolerance and Initial Efficacy of EGFRvIII CAR-T on Glioblastoma	1	Not yet recruiting	NCT05802693
	Autologous T Cells Redirected to EGFRVIII-With a Chimeric Antigen Receptor in Patients With EGFRVIII+ Glioblastoma	1	Terminated	NCT02209376
CHM-1101 CAR-T	CAR T Cells in Patients With MMP2+ Recurrent or Progressive Glioblastoma	1	Recruiting	NCT05627323
NKG2D-based CAR T	NKG2D-based CAR T-cells Immunotherapy for Patient With r/r NKG2DL+ Solid Tumors	1	Withdrawn	NCT04270461
	NKG2D-based CAR T-cells Immunotherapy for Patient With r/r NKG2DL+ Solid Tumors	1	Unknown status	NCT05131763
Truncated IL7Ra modified CAR T (Tris-CAR-T)	Tris-CAR-T Cell Therapy for Recurrent Glioblastoma	1	Recruiting	NCT05577091
Anti-EGFRvIII CAR T + cyclophosphamide + Fludarabine	Pilot Study of Autologous Anti-EGFRvIII CAR T Cells in Recurrent Glioblastoma Multiforme	1	Unknown status	NCT02844062
B7-H3 CAR-T	Safety and Efficacy Study of Anti-B7-H3 CAR-T Cell Therapy for Recurrent Glioblastoma	1	Recruiting	NCT05241392
	B7-H3 Chimeric Antigen Receptor T Cells (B7-H3CART) in Recurrent Glioblastoma Multiforme	1	Recruiting	NCT05474378
	Loc3CAR: Locoregional Delivery of B7-H3-CAR T Cells for Pediatric Patients With Primary CNS Tumors	1	Recruiting	NCT05835687
	Autologous CAR-T Cells Targeting B7-H3 in Recurrent or Refractory GBM CAR.B7-H3Tc	1	Recruiting	NCT05366179
IL-8 Receptor-modified CD70 CAR T	Phase I Study of IL-8 Receptor-modified CD70 CAR T Cell Therapy in CD70+ Adult Glioblastoma (IMPACT) (IMPACT)	1	Recruiting	NCT05353530
IL13Rα2 CAR-T + Ipilimumab + Nivolumab	IL13Ra2-CAR T Cells With or Without Nivolumab and Ipilimumab in Treating Patients With GBM	1	Recruiting	NCT04003649
Anti-EGFRvIII synNotch Receptor Induced Anti-EphA2/IL-13R alpha2 CAR (E-SYNC) T	Anti-EGFRvIII synNotch Receptor Induced Anti-EphA2/IL-13Ralpha2 CAR (E-SYNC) T Cells	1	Recruiting	NCT06186401
Chlorotoxin (EQ)-CD28-CD3zeta-CD19t-expressing CAR T	Chimeric Antigen Receptor (CAR) T Cells With a Chlorotoxin Tumor-Targeting Domain for the Treatment of MMP2+ Recurrent or Progressive Glioblastoma	1	Recruiting	NCT04214392
IL13Rα2 CAR-T	Brain Tumor-Specific Immune Cells (IL13Ralpha2-CAR T Cells) for the Treatment of Leptomeningeal Glioblastoma, Ependymoma, or Medulloblastoma	1	Recruiting	NCT04661384
	Genetically Modified T-cells in Treating Patients With Recurrent or Refractory Malignant Glioma	1	Active, not recruiting	NCT02208362
HER2-CAR CMV-T	CMV-specific Cytotoxic T Lymphocytes Expressing CAR Targeting HER2 in Patients With GBM (HERT-GBM)	1	Completed	NCT01109095
CD147-CAR T	CD147-CART Cells in Patients With Recurrent Malignant Glioma	1	Unknown status	NCT04045847
EGFRvIII CAR T transduced PBL + Aldesleukin + Fludarabine + Cyclophosphamide	CAR T Cell Receptor Immunotherapy Targeting EGFRvIII for Patients With Malignant Gliomas Expressing EGFRvIII	1/2	Completed	NCT01454596
Anti-PD-L1 CSR T + Cyclophosphamide + Fludarabine	Pilot Study of Autologous Chimeric Switch Receptor Modified T Cells in Recurrent Glioblastoma Multiforme	1	Unknown status	NCT02937844
EGFRvIII CAR T + Pembrolizumab	CART-EGFRvIII+ Pembrolizumab in GBM	1	Completed	NCT03726515
CARv3-TEAM-E T	CARv3-TEAM-E T Cells in Glioblastoma	1	Recruiting	NCT05660369

with a study published in 2014 by Sampson *et al*[37], which detailed the creation of an intracranial tumor model in immunocompetent VM/Dk mice using the murine glioma parental cell line SMA560. This work demonstrated that intracranial infusion of EGFRvIII-specific third-generation CARs was highly effective against brain gliomas, with success depending on lymphodepletive host conditioning. Additionally, the cured mice exhibited resistance to rechallenge with EGFRvIII-negative tumors[37].

Johnson *et al*[38] refined the EGFRvIII-specific CAR design, focusing on minimizing cross-reactivity with native EGFRwt protein. Using a low-affinity scFv 2173, they ensured specificity for EGFRvIII, reducing on-target, off-tumor effects. Their CAR, incorporating 41BB-CD3ζ, induced earlier tumor regression and was chosen for clinical translation. The antitumor efficacy, enhanced by temozolomide, was validated both *in vitro* and *in vivo*, with specificity confirmed

in models using EGFR-expressing keratinocytes and human skin grafts[38].

Singh *et al* discovered that lenalidomide, approved by the FDA in 2013 for mantle lymphoma, enhances the growth and sustained antitumor efficacy of 3C10 CAR-modified PBMCs targeting EGFRvIII *in vivo*[39,40].

A 2017 first-in-human study reported on the intravenous administration of autologous T-cells reprogrammed to target EGFRvIII via a CAR in 10 patients with recurrent glioblastoma. The study confirmed the feasibility and safety of EGFRvIII-targeted CAR T-cell production and infusion, with no off-target toxicity or cytokine release syndrome (CRS). One patient showed stable disease for over 18 months, though overall CAR T-cell expansion in peripheral blood was transient. The study highlighted the need to address tumor heterogeneity and the immunosuppressive tumor microenvironment (TME) to improve efficacy[41].

Dong *et al*[42] demonstrated that combining EGFRvIII-directed CAR T-cells with anti-VEGF therapy enhanced their antitumor effect, improving infiltration, distribution, and activation in murine GBM models. This approach also reprogrammed the TME, increasing endogenous effector T cells and improving antitumor efficacy, suggesting potential benefits for other cell-based therapies[42].

A phase 1 trial with six patients receiving intrathecal dual-target CAR T-cells for recurrent GBM showed promising early results. The main objectives – safety and establishing the maximum tolerated dose – were met, with early radiographic improvements seen in all patients. CAR T cells were detected in cerebrospinal fluid, along with cytokine release, supporting the initial safety and bioactivity of CAR T cells targeting EGFR and IL13Rα2 in GBM[43].

A CAR T-cell construct targeting the tumor-specific antigen EGFRvIII demonstrated durable responses in an immunocompetent, orthotopic GBM mouse model, enhanced by a single local dose of IL-12. IL-12 improved CAR T-cell cytotoxicity by remodeling the TME, increasing infiltration of proinflammatory CD4+ T cells, reducing regulatory T cells, and activating the myeloid compartment. These findings suggest that localized IL-12 is a promising adjuvant for CAR T-cell therapy in GBM[44].

Another study showed that the L8A4 antibody used in CAR T-cell therapy, when combined with tyrosine kinase inhibitors (TKIs), enhanced epitope presentation on EGFRvIII by stabilizing existing dimers without disrupting binding. Structural analysis indicated that the L8A4 antibody recognizes both monomeric and dimeric forms of EGFRvIII, independent of cysteine-bridging patterns. This suggests that combining L8A4-based CAR T-cell therapy with TKIs could improve treatment outcomes for glioblastoma[45].

Swan *et al*[46] engineered CAR T cells to secrete IL-7 and/or Flt3L in an orthotopic tumor model, achieving increased intratumoral CAR T-cell presence without lymphodepleting radiation. Co-expression of IL-7 and Flt3L led to higher dendritic cell infiltration and improved overall survival (OS), reaching 67% and 50% for IL-7 and IL-7/Flt3L CAR T cells, respectively, compared to 9% with standard treatment. The study concluded that IL-7 secretion by CAR T cells enhances their efficacy and survival in EGFRvIII-heterogeneous tumors[46].

B7-H3

The cell surface glycoprotein B7-H3 is overexpressed in various solid tumors with limited presence in normal tissues. Kramer *et al*[47] demonstrated that compartmental radioimmunotherapy using the anti-B7-H3 monoclonal antibody omburtamab, administered intraventricularly, effectively targeted CNS cancers. A phase I study of intraventricular 131I-omburtamab in 38 patients with metastatic neuroblastoma and B7-H3-positive tumors showed a median progression-free survival of 7.5 years, with minimal toxicity. Long-term survival was observed, particularly in patients with isolated CNS relapses[47].

Tang *et al*[48] reported a patient with recurrent glioblastoma, overexpressing B7-H3, who experienced temporary tumor reduction following intracerebral administration of B7-H3 CAR T cells. However, the tumor recurred after six infusion cycles[48].

Nehama *et al*[49] confirmed B7-H3 expression in glioblastoma (GBM) through TCGA data and immunohistochemistry. B7-H3-targeted CAR T-cells showed efficacy against GBM cell lines and patient-derived neurospheres, both *in vitro* and in xenograft models, demonstrating significant tumor inhibition. CD28 co-stimulation increased cytokine production, but both CD28 and 4-1BB co-stimulated CAR T cells were effective[49].

In a related study, B7-H3-targeted CAR T cells administered locally and systemically led to tumor regression and improved survival in glioma models[50].

Similarly, Tang *et al*[51] found that B7-H3 was broadly expressed in primary glioblastoma samples, correlating with higher malignancy grades. B7-H3-specific CAR T cells exhibited potent antitumor activity in vitro and *in vivo*, leading to significantly improved survival in treated GBM models[51].

IL13Rα2

A first-in-human trial by Brown *et al*[52] evaluated IL13Rα2-targeting CAR T cells for recurrent GBM. Three patients received IL13(E13Y)-zetakine CD8(+) cytotoxic T lymphocytes (CTLs) directly into the resection cavity. The treatment was well-tolerated, with manageable brain inflammation, and two patients exhibited transient anti-glioma responses. Tumor analysis indicated a reduction in IL13Rα2 expression, and MRI showed necrosis in one patient[52].

In a separate study, Brown *et al*[53] tested allogeneic GRm13Z40-2T cells with recombinant IL-2 in six patients with recurrent GBM. The treatment was well-tolerated, and four patients showed temporary tumor reduction and/or necrosis at the infusion site[53].

A recent trial involving 65 patients treated with IL13Rα2 CAR T cells showed promising efficacy, with 50% achieving stable or improved conditions. Median OS for the cohort was 7.7 months, with Arm 5 showing 10.2 months. Elevated CNS inflammation markers corresponded with CAR T-cell activity, and both intracranial and spinal lesions regressed following dual administration[54].

Gu *et al*[55] developed three IL13Rα2-specific CARs with different transmembrane domains. The IL13-CD28TM-28.BB.ζ CAR demonstrated the strongest antitumor activity in xenograft models, with improved infiltration and gene expression related to cell migration and adhesion[55]. Xu *et al*[56] engineered a humanized CAR targeting IL13Rα2, showing enhanced expansion and reduced cytokine production compared to murine-based CARs. This CAR exhibited potent antitumor activity and presents a promising option for clinical GBM treatment[56].

Starr *et al*[57] compared IL13(E12Y)-CARs with various signaling domains. Second-generation IL13-BBζ CARs demonstrated superior antitumor efficacy and proliferative capacity compared to first- and third-generation CARs, with selective recognition of IL13Rα2 over IL13Rα1[57].

HER2

Ahmed *et al*[58] assessed the activity of HER2-specific T cells derived from 10 GBM patients against autologous GBM cells, including CD133-positive stem cells, both *in vitro* and in an orthotopic mouse model. HER2-positive GBM cells stimulated T-cell proliferation and secretion of IFN-gamma and IL-2. HER2-specific T cells effectively killed CD133-positive and negative GBM cells but not HER2-negative cells. *In vivo*, HER2-specific T cells induced sustained regression of GBM xenografts. These findings suggest that HER2-redirected T cells may offer a promising immunotherapy strategy for GBM[58].

To address antigen escape, Hedge *et al*[59] engineered a bispecific CAR (TanCAR) targeting HER2 and IL13Rα2. TanCAR T cells could bind to either antigen and lyse autologous GBM cells, reducing antigen escape and enhancing antitumor efficacy. In a mouse model, TanCAR T cells extended survival and showed improved efficacy by simultaneously targeting both antigens[59].

In another study, HER2-CAR virus-specific T cells were administered to 17 patients with progressive HER2-positive GBM. The treatment was well tolerated, with one partial response lasting over 9 months, and seven patients achieving stable disease for up to 29 months. Median OS was 11.1 months, suggesting HER2-CAR T cell therapy may offer clinical benefits[60].

A first-in-human trial of HER2-targeted CAR-NK cells in nine patients with recurrent GBM showed that the treatment was safe, with no major toxicities observed. Five patients achieved stable disease, and the median OS was 31 weeks. The study indicated that intracranial HER2-targeted CAR-NK cell therapy is feasible and safe for GBM patients[60].

GD2

Liu *et al*[61] evaluated GD2-specific fourth-generation safety-engineered CAR (4SCAR) T-cell therapy in eight patients with GD2-positive GBM. Patients received autologous GD2-specific 4SCAR-T cells either intravenously or through a combination of intravenous and intracavitary administration. Four patients achieved a partial response lasting 3–24 months, three had progressive disease for 6–23 months, and one maintained stable disease for 4 months. The median OS was 10 months. Post-infusion tumor analysis revealed GD2 antigen loss and T cell infiltration. The treatment was well tolerated, with no significant adverse events[61].

NKG2D

In immunocompetent orthotopic glioblastoma mouse models, a CAR based on NKG2D (chNKG2D) was tested. chNKG2D T cells demonstrated strong IFNγ production and cytolytic activity *in vitro*. Systemic administration *in vivo* resulted in successful migration to brain tumors, extended survival, and cured some glioma-bearing mice. Surviving mice were protected against tumor rechallenge due to immune memory and local persistence of chNKG2D T cells. Combining chNKG2D T cells with subtherapeutic local radiotherapy enhanced T-cell migration and efficacy in two glioma models. The study demonstrated the potential of NKG2D CAR T-cell therapy, especially when combined with radiotherapy, for treating gliomas[62].

CARv3-TEAM-E

In this first-in-human trial, three patients with recurrent GBM were treated with CARv3-TEAM-E T cells, designed to target both the EGFR variant III (EGFRvIII) tumor-specific antigen and the wild-type EGFR protein through the secretion of a T-cell–engaging antibody molecule (TEAM). The therapy was well tolerated, with no adverse events above grade 3 and no dose-limiting toxicities. Notably, rapid and significant tumor regression was observed on imaging within days of a single intraventricular infusion. However, in two of the three patients, these responses were transient[63].

CD70

Jin *et al* identified CD70 as a promising target for CAR T-cell therapy in brain tumors, including GBM. A detailed analysis of 52 normal tissues and both low-grade gliomas (LGGs) and GBMs revealed that CD70 was absent in normal brain and peripheral tissues but consistently overexpressed in IDH wild-type LGGs and GBMs. Higher CD70 expression was linked to poorer survival, likely due to its role in chemokine production and CD8+ T-cell death. Both human and mouse CD70-specific CAR T cells effectively targeted CD70+ GBM cells *in vitro*, leading to tumor regression in xenograft and syngeneic models without adverse effects. These findings suggest CD70 as a viable target for CAR T-cell-based immunotherapy in gliomas.

EphA2

EphA2 is a TAA that is highly overexpressed in glioblastoma. The first human trial of EphA2-directed CAR T-cells in patients with recurrent, EphA2-positive glioblastoma was performed by Lin *et al* Each of the three enrolled patient received a single intravenous infusion of EphA2-directed CAR T-cells following a lymphodepletion. Two patients experienced grade 2 CRS accompanied by pulmonary edema, which was fully resolved with dexamethasone treatment. No other organ toxicities, including neurotoxicity, were observed. CAR T-cell expansion was detected in both the peripheral blood and cerebrospinal fluid, with persistence for over 4 weeks. One patient experienced temporary tumor reduction, while the overall outcomes among the three patients included one with stable disease and two with progressive disease, with survival times ranging from 86 to 181 days. The intravenous administration of EphA2-targeted CAR T-cells was generally well tolerated and showed temporary clinical efficacy[64].

Challenges and limitations

Blood-brain barrier

CAR-T cell therapy represents a significant advancement in cancer treatment, particularly for hematological malignancies. However, the application of CAR-T cells in treating brain

tumors faces a major challenge: crossing the BBB[65]. The BBB is a selective barrier that protects the brain from harmful substances but also limits the entry of therapeutic agents. Understanding how CAR-T cells can cross the BBB is crucial for developing effective brain tumor treatments[66]. The BBB consists of endothelial cells (ECs) tightly joined by tight junctions, creating a highly selective barrier. These cells are supported by astrocytes, pericytes, and the extracellular matrix, forming a complex neurovascular unit that regulates the movement of molecules between the bloodstream and the brain[67,68]. There are a number of preclinical studies that undertake the task of facilitating CAR-T Cell migration across the BBB[69–71]. The presence of brain tumors provides to disruption of BBB and presents as an incomplete pericyte coverage, malfunctioning astrocytes, and damaged basement membrane. It allows leukocytes to cross the barrier. This process explains the lower number of leukocytes in the immune microenvironment of LGGs, where the BBB remains relatively intact[72]. Despite the extent of BBB disruption in GBM, achieving consistent and effective drug concentrations within the tumor tissue is still a significant challenge, and T cells encounter persistent obstacles when attempting to penetrate the brain. There are several preclinical studies that undertake the task of facilitating CAR-T Cell migration across the BBB[73]. To confront the problem of BBB combination of intravenous and intraventricular administration of IL13Rα2-CART resulted in enhanced antitumor effects. Findings of Brown *et al*[74] suggested that intraventricular infusion is more effective than intravenous infusion alone. The limitation of the above strategy occurs in patients who have not undergone surgery, as it could elevate intracranial pressure and become a significant risk for patients. Moreover, CART cell-induced inflammation in the brain stem can lead to obstructive hydrocephalus, elevated intracranial pressure, seizures, sterile inflammation, and fever, along with more severe complications like CRS and immune effector cell-associated neurotoxicity syndrome (ICANS)[73,75].

Transcellular migration

CAR-T cells can potentially cross the EC layer through a process called transcellular migration. This involves the CAR-T cells interacting with the ECs and creating temporary openings in the cell membranes, allowing them to pass through. CAR-T cells may utilize receptor-mediated mechanisms to traverse the BBB[76]. Specific receptors on the surface of ECs can bind to ligands expressed by CAR-T cells, facilitating their transport across the barrier. Before leukocyte diapedesis, an ICAM-1 cluster forms on the EC membrane surrounding the leukocytic filopodia-like structures. Diapedesis can occur in two ways: across ECs (transcellular) or between ECs (paracellular). Transcellular diapedesis is thought to be preferable when cell junctions are very tight – for example, at the BBB[77]. This pathway requires the homophilic interaction of platelet EC adhesion molecule-1 (PECAM-1, CD31) and CD99 between leukocytes and ECs. Junctional adhesion molecule A (JAM-A, JAM-1) is also necessary for transcellular migration, but its role remains controversial. As for paracellular diapedesis, the IgSF–integrin interaction induces the loosening of the adherent junctions. In addition to the already mentioned PECAM-1, CD99, and JAM-A, other molecules, such as ICAM-2, VCAM-1, and JAM-C, are involved in paracellular transmigration[78].

It is hypothesized that CAR-T cells can utilize transcellular migration to cross the BBB. The molecular interactions between CAR-T cells and ECs play a crucial role in this process, where temporary openings in cell membranes are formed, allowing CAR-T cells to pass through[79]. Experimental studies demonstrate the feasibility of transcellular migration both *in vitro* and *in vivo*, highlighting the therapeutic potential of this mechanism for treating brain tumors[80].

Inflammatory signals, often present in the TME, can increase BBB permeability. CAR-T cells can exploit this increased permeability to migrate into the brain tissue[81]. A study has shown that severe CRS and ICANS limit the clinical applicability of CAR-T cell therapy. Vascular endothelial activation contributes to the development of CRS and ICANS, with tumor necrosis factor α (TNFα) from CAR-T cells and interleukin 1β (IL1β) from myeloid cells being the primary cytokines involved. The study investigated the effectiveness of blocking TNFα and IL1β signaling on endothelial activation in CAR-T therapy. The use of adalimumab and anti-IL1β antibodies, along with a focal adhesion kinase inhibitor, successfully reduced endothelial activation. Moreover, adalimumab and anti-IL1β antibody showed a synergistic effect in preventing endothelial activation, indicating their therapeutic potential for managing CAR-T therapy-associated CRS and neurotoxicity[82].

Heterogeneity and antigen loss in glioblastoma

A high mutational diversity in tumors leads to the generation of numerous neoantigens. GBM typically has a low mutational burden, limiting the effectiveness of therapies like CAR-T due to the few neoantigens that T cells can recognize. Additionally, GBM is characterized by considerable heterogeneity[83]. Common CAR-T cell targets in GBM, like IL-13Rα2 and EGFRvIII, show variable expression both between different patients and within the same patient, and these expressions can change over time and across different tumor regions. The heterogeneity can be observed also at the cellular level[84]. GBM tumors contain multiple subtypes and subclones driven by clonal evolution and the plasticity of cancer stem cells[12]. Hypoxia gradients are a key factor in spatial heterogeneity; within central necrotic areas, microglia and macrophages upregulate pro-inflammatory markers, while the tumor periphery expresses more anti-inflammatory and pro-angiogenic markers. Notably, there is also a difference between primary and recurrent tumors[85]. Local recurrences often maintain the mutational profile of the original tumor, while distant recurrences tend to lose many of the initial mutations, which leads to reduced drug sensitivity after recurrence. After the initial tumor reduction by CAR-T cells, antigen-negative cells can survive and evade therapy, leading to tumor relapse. This process, known as antigen escape, occurs when the tumor evades CAR targeting by downregulating or mutating the targeted antigen[86]. This phenomenon has been observed in clinical trials of CAR-T cell therapy in GBM: in one study targeting EGFRvIII, the tumor lost target expression and increased immunosuppressive molecule production. In another trial targeting IL-13Rα2, the patient developed a recurrent tumor with reduced antigen expression. The challenges of tumor heterogeneity and antigen escape highlight the need to target multiple antigens or to use combination approaches in treatment. However, combining therapies also raises the risks of increased toxicity, drug interactions, and adverse effects[87].

Suppression of CART therapy by the TME

The effectiveness of CAR-T cell therapy in treating solid tumors, such as glioblastoma, faces significant challenges due to the complex and suppressive TME. Unlike hematologic cancers, solid tumors exist within a complex microenvironment, where numerous factors – including inhibitory receptors, suppressive cells, cytokines, physical barriers, hypoxia, and tumor metabolism – dampen immune responses and support tumor growth[88] When CAR-T cells infiltrate these tumors, they encounter suppressive cells, cytokines, and chemokines that can impair their function. Specific mechanisms driving this dysfunction are still being studied, but research has shown that IFNγ signaling is crucial for CAR-T cell adhesion and cytotoxicity[89]. It was found that deleting IFNγR1 in glioblastoma cells reduces CAR-T cell effectiveness[90]. To further enhance CAR-T cell activity in such challenging environments, various strategies have been developed. Cytokine administration, such as IL-15, has been employed to boost CAR-T cell proliferation, persistence, and overall efficacy. Also enhancing IFN-γ release through oncolytic herpes simplex virus-1 has been observed[91]. Additionally, receptor-modified CAR-T cells have shown promise in overcoming the inhibitory TME. For instance, CXCR2-modified CAR-T cells have been found to improve *in vivo* trafficking and accumulation in tumors, while other modifications like CXCR4 and CX3CR1 have enhanced tumor infiltration and suppressed migration of suppressive cells like MDSCs[73].

The hypoxic and metabolically challenging conditions within glioblastoma further contribute to resistance against CAR-T therapy. Elevated PHGDH expression in glioblastoma ECs, for example, has been linked to increased tumor hypoxia and angiogenesis, making tumors more resistant to treatment. Anti-angiogenic therapies have shown potential in counteracting these effects and improving CAR-T cell efficacy[92].

Combining CAR-T cells with immune checkpoint inhibitors targeting PD-1, PD-L1, and CTLA-4 has also demonstrated improved tumor control in some cancer patients, although response rates vary due to differences in receptor expression. The discovery of additional inhibitory receptors, such as TIM-3, LAG-3, and B7-H3, suggests that using various combinations of checkpoint inhibitors alongside CAR-T therapy could further enhance treatment outcomes. Predictive biomarkers like tumor mutation load could help identify patients who are more likely to benefit from these combination therapies[93].

Overall, the solid TME is highly complex and heterogeneous, requiring a multifaceted approach to therapy. While CAR-T cells have the potential to directly target and eliminate tumor cells, combining them with other strategies – such as cytokine modifications, immune checkpoint inhibitors, anti-angiogenic treatments, and approaches targeting hypoxia and tumor metabolism – offers the best chance to overcome the numerous obstacles present in solid tumors like glioblastoma. These combined therapies significantly improve the effectiveness of CAR-T cell therapy in solid tumors[94].

Side effects and toxicities associated with CAR-T cell therapies

CAR-T cell therapy besides many advantages and promising results is also linked to serious toxicities, including CRS and ICANS[95]. Although these adverse effects are generally manageable and reversible with appropriate supportive care, they can be life-threatening, necessitating careful monitoring and rapid intervention. Early detection and swift management of these toxicities are crucial in minimizing both morbidity and mortality. The diagnosis and management of adverse events related to CAR-T cell therapy are particularly challenging due to the limited understanding of many underlying mechanisms. These side effects can vary widely in severity and presentation, potentially affecting multiple organ systems, much like immune-related adverse events observed with immune checkpoint inhibitors[96]. One common issue is the on-target off-tumor effect, where CAR-T cells attack healthy cells expressing the target antigen. Reports have also documented allergic reactions and metabolic disturbances, such as tumor lysis syndrome, following CAR-T cell infusion[8,94]. However, the most prominent toxicities associated with CAR-T cell therapy are CRS, ICANS, and hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS)[97,98].

The incidence of CRS ranged from 49% to 95%, with grade ≥ 3 CRS occurring in 1%–24% of cases. A consistent trend is that CRS when compared to ICANS typically has an earlier median onset, usually within the first week after CAR-T cell infusion, while ICANS tends to have a longer average duration. CRS impacts multiple organ systems and often begins with general symptoms such as high fever (above 40°C), fatigue, tachycardia, and muscle aches. In more severe cases, CRS may manifest with hypotension, hypoxia, capillary leak syndrome, multi-organ failure, disseminated intravascular coagulation (DIC), and even HLH/MAS. The rise in circulating inflammatory cytokines leads to increased vascular permeability and fluid third-spacing, creating a sepsis-like syndrome, typically accompanied by neutropenia. CRS can be classified based on severity: mild CRS includes constitutional symptoms and/or grade ≤ 2 organ toxicity, while severe CRS is marked by grade ≥ 3 organ toxicity, which may be life-threatening. Tocilizumab is currently the primary treatment for managing CRS following CAR-T cell infusion, with corticosteroids being added for cases that are severe or resistant. The use of corticosteroids as a preventive measure for CRS needs further research. Siltuximab is an alternative option to tocilizumab[99,100].

Endothelial activation and disruption of the BBB are key factors contributing to ICANS. Angioproteins 1 and 2 (Ang-1, Ang-2) serve as ligands for the Tie-2 receptor, playing opposite roles in endothelial regulation. Under normal conditions, Ang-1 is more prevalent, promoting EC stability and suppressing pro-inflammatory pathways. However, during inflammation, Ang-2 levels rise, leading to endothelial activation and increased microvascular permeability. Patients who experience severe neurotoxicity following CAR-T cell therapy often have a higher Ang-2 ratio, along with elevated von Willebrand Factor (VWF) levels, both of which are markers of endothelial activation[101]. The signs of ICANS are more varied than those of CRS, including mild symptoms like dysgraphia, attention deficits, apraxia, headaches, sleep disturbances, anxiety, myoclonus, motor impairments, and dyscalculia, ranging to more severe manifestations, including encephalopathy, aphasia, delirium, tremors, seizures, and cerebral edema. The Immune Effector Cell-Associated Encephalopathy score has been developed as an objective and reliable tool to screen cognitive function in adult patients. ICANS was reported in 12%–60% of patients, with severe cases (grade ≥ 3) occurring in 3%–50% of patients. Corticosteroids are the primary treatment for ICANS, while

seizures are typically managed with levetiracetam, with or without benzodiazepines[102]. For refractory CRS or ICANS treatment with anakinra proved to be safe and effective. The overall response rate remained high at 77% following CAR-T therapy, indicating that anakinra likely has a minimal effect on the efficacy of CAR-T cells. A higher dose of anakinra may lead to quicker resolution of CRS/ICANS and was independently linked to reduced treatment-related mortality[103].

Despite the frequent occurrence of these side effects, a meta-analysis estimated the rate of treatment-related death at only 1%. A preliminary survey reported an incidence of just 3.48% for HLH/MAS following CAR-T cell therapy between 2016 and 2018. However, more recent phase I trials involving anti-CD22 CAR-T cells indicated that 32.7% and 35.6% of patients developed HLH, respectively. These results, along with the significant morbidity and mortality linked to this syndrome, have heightened concern over CAR-T cell-associated HLH/MAS[102].

Innovations and improvements

Advances in genetic engineering of CAR-T cells for enhanced specificity and safety

CAR-T cells have been successful in treating hematological malignancies, but their application in treating GBM remains a challenge due to the difficulty in finding an ideal antigen on tumor cells. Very few antigens are specific only to glioblastoma cells. The use of lymphocytes that are not specific to cancer cells causes side effects, as CAR T cells destroy healthy cells in the body. On the other hand, even a highly specific TA is rarely homogeneous, meaning it is seldom present on all glioblastoma cells[104]. The use of CAR T cells targeted against heterogeneous antigens (not homogeneous) would result in not all tumor cells being killed. Those that survive would lead to glioblastoma regrowth. Therefore, it is necessary to find antigens that are both highly specific to glioblastoma tumor cells and homogeneous, meaning they are present on all glioblastoma cells. The use of CAR T cells targeted against heterogeneous antigens (not homogeneous) would result in not all tumor cells being killed. Those that survive would lead to glioblastoma regrowth. Thus, ideal targets must be both specific and uniformly expressed. So far, this has not been achieved A highly specific antigen for GBM cells has been found: the epidermal growth factor receptor splice variant III (EGFRvIII). Unfortunately, although it is highly specific to glioblastoma cells, it is not homogeneous, meaning not every glioblastoma cell possesses it. The use of conventional CAR T cells targeted against this antigen will cause that glioblastoma cells without this antigen will survive and the tumor will regrowth. On the other hand, scientists have found antigens such as ephrin type A receptor 2 (EphA2) and interleukin 13 receptor α2 (IL13Rα2), which are homogeneous on glioblastoma cells, but are not highly specific to them. It means, they are also present on healthy cells in the body, such as liver, kidney, and genital cells. The use of CAR T cells programmed against these antigens would result in the death of all glioblastoma cells, but also the death of healthy cells in the body, leading to very dangerous complications[105].

The solution to the problem of finding a specific and homogeneous antigen is the creation of SynNotch-CAR T cells. Their action is described as "prime-and-kill" circuits. This means that SynNotch-CAR T cells recognize the EGFRvIII antigen, which is highly specific to glioblastoma cells but is heterogeneous. The binding of SynNotch-CAR T cells to a highly specific antigen stimulates them to transcribe genes (prime), which encode proteins that detect EphA2 and IL13Rα2 receptors, which are homogeneous for glioblastoma cells but not highly specific. Recognition of EphA2 and IL13Rα2 receptors signals the killing of the cell that possesses these antigens. As a result, CAR T cells destroy only glioblastoma cells and are unable to kill healthy cells in the body that possess the EphA2 and IL13Rα2 antigens. Healthy organs, despite having the EphA2 and IL13Rα2 receptors, are not damaged by SynNotch-CAR T cells because they do not have the EGFRvIII antigen. This avoids side effects.

SynNotch-CAR T cells leverage the specificity of the EGFRvIII antigen and the homogeneity of EphA2 and IL13Rα2 antigens to target and kill cancer cells. Upon detection of the EGFRvIII antigen, SynNotch-CAR T cells are primed, and when they encounter EphA2 or IL13Rα2 antigens, they execute cancer cell killing. This occurs in two ways: cis-killing, where the same cancer cell presents both types of antigens, and trans-killing, where one cancer cell activates the T cell, and it then kills a different cell with the EphA2 or IL13Rα2 antigens. The study demonstrated that SynNotch-CAR T cells effectively target glioblastoma cells lacking specific antigens, provided they are first activated by cells with the EGFRvIII antigen. *In vivo* studies on mice with GBM showed significantly improved survival compared to those treated with conventional CAR T cells[106].

Combination therapies involving CAR-T cells and other treatments such as checkpoint inhibitors, tumor vaccines, or chemotherapy

To achieve improved outcomes in the treatment of GBM, two main strategies are being pursued for the development of CAR T-cells. The first strategy focuses on the continuous enhancement and modification of CAR T-cells to increase their effectiveness in targeting and killing cancer cells. The second strategy involves combining CAR T-cell therapy with other established cancer treatments, such as chemotherapy, cancer vaccines, and immune checkpoint inhibitors[107].

Temozolomide, a chemotherapeutic agent, has been successfully used in the treatment of GBM for many years. There have been attempts to combine temozolomide therapy with CAR T-cells, but the results have been disappointing. Temozolomide promotes hypermutation in GBM cells, which leads to the development of resistance and antigen escape, reducing the effectiveness of CAR T-cells. On the other hand, it is known that temozolomide induces lymphodepletion, increases the expression of pro-inflammatory factors, and promotes the proliferation of CAR T-cells. Therefore, there are some studies which comparing the efficacy of temozolomide alone versus temozolomide in combination with CAR T-cells in the treatment of GBM. While the results of these studies have not been published, it is known that positive outcomes have been observed in the treatment of acute lymphoblastic leukemia and lung cancer with this combined approach[108].

Cancer vaccines stimulate the proliferation of lymphocytes in lymph nodes, particularly T-cells. Theoretically, administering CAR T-cells followed by a cancer vaccine could induce massive proliferation of CAR T-cells, thereby enhancing their effectiveness in destroying cancer cells compared to CAR T-cell therapy alone. There are two types of cancer vaccines. The first type is

peptide vaccines that contain an amphipathic CAR-T cell ligand (amph-ligand) as a vaccine, which is transported to lymph nodes after injection and modified antigen-presenting cell surface. Thus, this can initiate CAR-T cells in the natural lymph node microenvironment and trigger massive CAR-T cells expansion, which can demonstrate stronger antitumor efficacy compared to the single CAR-T cell treatment[109]. The second type includes cell-based vaccines, which involve radiation-induced immunogenic cell death of glioma stem cells, which also stimulate CAR-T cells to proliferation. Currently, research on these vaccines is at the preclinical trial stage[110].

Emerging evidence highlights the synergy between CAR-T cells and other immunomodulatory strategies. Immune checkpoint inhibitors (e.g. anti-PD-1, anti-CTLA-4) can enhance CAR-T efficacy by counteracting the immune suppressive TME. Similarly, tumor vaccines can stimulate endogenous T-cell responses and expand CAR-T cell functionality, especially in antigen-heterogeneous tumors. CRISPR-engineered CAR-T cells offer precise genome editing to enhance T-cell persistence, reduce exhaustion, and prevent off-tumor toxicity. Collectively, these combination approaches represent a future direction for optimizing CAR-T-based glioblastoma therapy[111–113].

Immune checkpoints are natural components of the immune system, protecting healthy cells from destruction by immune cells. T-cells express PD-1 proteins on their surface, while healthy cells express PD-L1 receptors. The binding of these molecules signals T-cells not to kill healthy cells[114]. Unfortunately, cancer cells exploit this mechanism to avoid destruction by overexpressing PD-L1, thereby evading T-cell-mediated killing. The solution to this problem is the use of checkpoint inhibitors, which are molecules that block the PD-1/PD-L1 interaction, leading to T-cell activation and the subsequent destruction of cancer cells. The combination of this therapy with CAR T-cells could theoretically significantly enhance the effectiveness of GBM treatment[115,116]. Clinical trials investigating the combination of CAR T-cells and immune checkpoint inhibitors for GBM treatment are currently underway, but their results have not yet been published.

Role of biomarkers in predicting response to CAR-T cell therapies

Potential wider-scale application of CART therapy would require reliable tools for qualification and stratification of candidate patients and assessment of efficacy and safety of the ongoing therapy. As of now, list of targets available for CART in therapy of gliomas is limited to nine, therefore group of candidate patients is narrowed by presence of specific antigen in tumor cells[12]. Further, presence of a certain antigen may be related to distinct and rare subtype of glioma, that is, disialoganglioside GD2 specific in cells of H3K27M-mutated diffuse midline gliomas[117]. Despite its presence on GBM cells, trial on GD2-targeted therapy did not conclude with unambiguously favorable outcome[61]. GBM cells are additionally characterized by heterogeneity of surface antigens and over the time certain cells may present plasticity of their antigen profile. In contrast to acute B-cell lymphoblastic leukemia, these profiles do not have a common component (i.e. CD19) and are not easily translatable into biomarkers obtainable from peripheral blood[118–120]. In summary, no laboratory parameters are available yet to predict eligibility and expect efficacy of therapy and material acquired only by surgical resection or biopsy allows examination of antigen profile[121].

While the essence of CART therapy of glioblastoma is based on permeation of lymphocytes through endothelium and exerting its cytotoxic activity within brain parenchyma, the same mechanism is responsible for ICANS[122,123]. Occurrence of neurotoxicity was demonstrated to correlate with several laboratory parameters expressing endothelial activation and increased permeability. Among patients receiving CART therapy for acute lymphoblastic B-cell leukemia it was associated with release of VWF from Weibel-Palade bodies and activation of angiopoietin tyrosine kinase with immunoglobulin and epidermal growth factor homology domains (ANG/Tie) pathway. This association was followed by increased concentration and steep dynamics of change of inflammatory biomarkers, including IL-1 beta, IL-6, and IFN gamma. This reminds that apart from ICANS, infusion of CART cells is more commonly associated with CRS. Its clinical presentation may range from mild ailments to life-threatening conditions and multi-organ failure. Therefore CART infusion is required to be secured with anti-inflammatory agent, most commonly either tocilizumab (anti-IL-6) or anakinra (anti-IL-1), however in terms of GBM siltuximab may be favored over tocilizumab due to not crossing the BBB[41,103]. What is worth noting here, regular protocol for administration of CART includes co-administration of exogenic IL-2 to upsurge cytotoxic activity and may circuitously escalate inflammatory basis of neurotoxicity. Influx of lymphocytes, including CAR-T cells is confirmed by their presence in CSF and their longer retention is associated with more severe toxicity. Eventually, hyperferritinemia concomitant to signs of ICANS may manifest CART-induced hemophagocytic lymphohistiocytosis (HLH) which corresponds with activation of microglia in perivascular area in brain specimens from patients who experienced severe ICANS[124,125]. Fortunately, initial experiences with application of CART in GBM reported CRS to be not as frequent and severe as in treatment of acute B-cell lymphoblastic leukemia[41,126,127].

Limitations

While this review provides an extensive overview of CAR-T therapy for glioblastoma, several limitations must be acknowledged. First, the majority of cited clinical studies are early-phase trials with limited patient numbers, restricting generalizability. Second, variability in antigen expression across tumors introduces heterogeneity, which affects the reproducibility of treatment outcomes. Additionally, most data are derived from preclinical or murine models, and there remains a lack of large-scale, randomized controlled trials in human subjects. Finally, publication bias toward positive outcomes may skew the apparent efficacy of CAR-T therapies.

Conclusion

CAR-T cell therapy offers groundbreaking potential in transforming glioblastoma treatment by harnessing the immune system to target and destroy malignant cells. The ability to genetically engineer T cells to recognize tumor-specific antigens such as EGFRvIII, IL-13Rα2, and HER2 has already shown promise in early-phase clinical trials. Despite glioblastoma's status as one of the most aggressive primary brain tumors,

CAR-T therapy represents a hopeful avenue for extending survival and improving patient outcomes. Advances in CAR design – including multi-target CAR constructs and localized delivery strategies – further underscore its potential to address the complex challenges posed by central nervous system malignancies.

Nonetheless, significant barriers remain to making CAR-T therapy both effective and widely accessible. One of the primary biological obstacles is the BBB, which impedes therapeutic cell trafficking to the tumor site. Innovative delivery approaches such as local or intraventricular administration are under investigation, though they carry risks including CRS and neurotoxicity. Moreover, glioblastoma's intratumoral heterogeneity, including antigen escape and an immunosuppressive microenvironment, compromises sustained CAR-T efficacy. To counter these challenges, ongoing research is focused on next-generation CAR designs incorporating dual-targeting receptors and combination strategies with immune checkpoint inhibitors or anti-angiogenic agents. Managing the therapy's toxicities remains another priority for optimization.

In developing countries, the implementation of CAR-T therapy is further hindered by systemic limitations. High production costs, inadequate infrastructure for cell engineering, shortage of trained personnel, and regulatory hurdles significantly restrict access. Moreover, public healthcare systems frequently lack the capacity to support or subsidize high-cost, personalized therapies. To mitigate these issues, strategic government interventions are needed – such as funding for translational research, establishment of local manufacturing facilities, development of public-private partnerships, and promotion of international collaborations for technology transfer and workforce training.

The progress made in CAR-T therapy for glioblastoma underscores the critical need for sustained research and cross-sectoral collaboration. Future breakthroughs in genetic engineering, precision drug delivery, and immunotherapy could transform CAR-T into a safer, more scalable, and clinically viable treatment modality. Importantly, expanding global access will require parallel investment in infrastructure, education, and policy reform. Only through coordinated efforts among academia, clinical institutions, biotech industries, and governmental agencies can the promise of CAR-T therapy be fully realized – ensuring that its benefits extend beyond high-resource settings to patients worldwide.

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References

[1] D'Alessio A, Proietti G, Sica G, *et al*. Pathological and molecular features of glioblastoma and its peritumoral tissue. Cancers (Basel) 2019;11:469.
[2] Becker AP, Sells BE, Jaharul Haque S, *et al*. Tumor heterogeneity in glioblastomas: from light microscopy to molecular pathology. Cancers (Basel) 2021;13:761.
[3] Lah TT, Novak M, Breznik B. Brain malignancies: glioblastoma and brain metastases. Semin Cancer Biol. 2020;60:262–73.
[4] Colopi A, Fuda S, Santi S, *et al*. Impact of age and gender on glioblastoma onset, progression, and management. Mech Ageing Dev 2023;211:111801.
[5] Grochans S, Cybulska AM, Simińska D, *et al*. Epidemiology of glioblastoma multiforme–literature review. Cancers (Basel) 2022;14:2412.
[6] Rodgers LT, Villano JL, Hartz AMS, *et al*. Glioblastoma standard of care: effects on tumor evolution and reverse translation in preclinical models. Cancers (Basel). 2024;16:2638.
[7] Luksik AS, Yazigi E, Shah P, *et al*. CAR T cell therapy in glioblastoma: overcoming challenges related to antigen expression. Cancers (Basel) 2023;15:1414.
[8] Maggs L, Cattaneo G, Dal AE, *et al*. CAR T cell-based immunotherapy for the treatment of glioblastoma. Front Neurosci. 2021;15:662064.
[9] Li G, Zhang Z, Cai L, *et al*. Fn14-targeted BiTE and CAR-T cells demonstrate potent preclinical activity against glioblastoma. Oncoimmunology 2021;10. doi:10.1080/2162402X.2021.1983306.
[10] Asmamaw Dejenie T, Dessie Terefe G, Tadele Admasu F, *et al*,,. Current updates on generations, approvals, and clinical trials of CAR T-cell therapy. Hum Vaccin Immunother 2022;18. doi:10.1080/21645515.2022.2114254.
[11] Land CA, Musich PR, Haydar D, *et al*. Chimeric antigen receptor T-cell therapy in glioblastoma: charging the T cells to fight. J Transl Med 2020;18:1–13.
[12] Kringel R, Lamszus K, Mohme M. Chimeric antigen receptor T cells in glioblastoma—Current concepts and promising future. Cells. 2023;12:1770.
[13] Vitanza NA, Ronsley R, Choe M, *et al*. Locoregional CAR T cells for children with CNS tumors: clinical procedure and catheter safety. Neoplasia 2023;36:100870–100870.
[14] Guedan S, Calderon H, Posey AD, *et al*. Engineering and design of chimeric antigen receptors. Mol Ther Methods Clin Dev. 2019;12:145.
[15] Jaspers JE, Brentjens RJ. Development of CAR T cells designed to improve antitumor efficacy and safety. Pharmacol Ther. 2017;178:83.
[16] Qin VM, D'souza C, Neeson PJ, *et al*. Chimeric antigen receptor beyond CAR-T cells. Cancers (Basel). 2021;13:1–16.
[17] Sheykhhasan M, Ahmadieh-Yazdi A, Vicidomini R, *et al*. CAR T therapies in multiple myeloma: unleashing the future. Cancer Gene Ther 2024;31:667–86.

[18] Zhang Y, Li Y, Cao W, *et al*. Single-cell analysis of target antigens of CAR-T reveals a potential landscape of 'on-target, off-tumor toxicity. Front Immunol 2021;12. doi:10.3389/FIMMU.2021.799206/FULL.
[19] Mazinani M, Rahbarizadeh F. CAR-T cell potency: from structural elements to vector backbone components. Biomark Res. 2022;10:70.
[20] Shah K, Al-Haidari A, Sun J, *et al*. T cell receptor (TCR) signaling in health and disease. Signal Transduct Target Ther 2021;6. doi:10.1038/S41392-021-00823-W.
[21] Honikel MM, Olejniczak SH. Co-stimulatory receptor signaling in CAR-T cells. Biomolecules 2022;12:1303.
[22] Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J. 2021;11:1–11.
[23] Philipson BI, O'Connor RS, May MJ, *et al*. 4-1BB costimulation promotes CAR T cell survival through noncanonical NF-κB signaling. Sci Signal 2020;13. doi:10.1126/SCISIGNAL.AAY8248.
[24] Chu GJ, Bailey CG, Nagarajah R, *et al*. The 4-1BBζ costimulatory domain in chimeric antigen receptors enhances CD8+ T-cell functionality following T-cell receptor stimulation. Cancer Cell Int. 2023;23:1–18.
[25] Feucht J, Sadelain M. Function and evolution of the prototypic CD28ζ and 4-1BBζ chimeric antigen receptors. Immuno-Oncol Tech. 2020;8:2.
[26] Mohanty R, Chowdhury CR, Arega S, *et al*. CAR T cell therapy: a new era for cancer treatment (Review). Oncol Rep. 2019;42:2183–95.
[27] Blache U, Popp G, Dünkel A, *et al*. Potential solutions for manufacture of CAR T cells in cancer immunotherapy. Nat Commun. 2022;13:1–5.
[28] Brown CE, Hibbard JC, Alizadeh D, *et al*. Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. Nat Med 2024;30:1001.
[29] Prapa M, Chiavelli C, Golinelli G, *et al*. GD2 CAR T cells against human glioblastoma. NPJ Precis Oncol 2021;5. doi:10.1038/S41698-021-00233-9.
[30] Cherkassky L, Hou Z, Amador-Molina A, *et al*. Regional CAR T cell therapy: an ignition key for systemic immunity in solid tumors. Cancer Cell. 2022;40:569–74.
[31] Grosskopf AK, Labanieh L, Klysz DD, *et al*. Delivery of CAR-T cells in a transient injectable stimulatory hydrogel niche improves treatment of solid tumors. Sci Adv 2022;8:8264.
[32] Hu Q, Li H, Archibong E, *et al*. Inhibition of post-surgery tumour recurrence via a hydrogel releasing CAR-T cells and anti-PDL1-conjugated platelets. Nat Biomed Eng 2021;5:1038.
[33] Wu S-Y, Ji F, Xu B, *et al*. Delivering CAR-T cells into solid tumors via hydrogels. MedComm – Oncol. 2023;2:e40.
[34] Sahin A, Sanchez C, Bullain S, *et al*. Development of third generation anti-EGFRvIII chimeric T cells and EGFRvIII-expressing artificial antigen presenting cells for adoptive cell therapy for glioma. PLoS One. 2018;13:e0199414.
[35] Ohno M, Natsume A, Ichiro Iwami K, *et al*. Retrovirally engineered T-cell-based immunotherapy targeting type III variant epidermal growth factor receptor, a glioma-associated antigen. Cancer Sci 2010;101:2518–24.
[36] Morgan RA, Johnson LA, Davis JL, *et al*. Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. Hum Gene Ther 2012;23:1043.
[37] Sampson JH, Choi BD, Sanchez-Perez L, *et al*. EGFRvIII mCAR-modified T-cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. Clin Cancer Res 2014;20:972–84.
[38] Johnson LA, Scholler J, Ohkuri T, *et al*. Rational development and characterization of humanized anti–EGFR variant III chimeric antigen receptor T cells for glioblastoma. Sci Transl Med 2015;7:275ra22.
[39] Yang J, Yan J, Liu B. Targeting EGFRvIII for glioblastoma multiforme. Cancer Lett. 2017;403:224–30.
[40] Singh N, Lee YG, Shestova O, *et al*. Impaired death receptor signaling in leukemia causes antigen-independent resistance by inducing CAR T-cell dysfunction. Cancer Discov 2020;10:552–67.
[41] O'Rourke DM, Nasrallah MP, Desai A, *et al*. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 2017;9. doi:10.1126/SCITRANSLMED.AAA0984.
[42] Dong X, Ren J, Amoozgar Z, *et al*. Anti-VEGF therapy improves EGFR-vIII-CAR-T cell delivery and efficacy in syngeneic glioblastoma models in mice. J Immunother Cancer 2023;11:e005583.
[43] Bagley SJ, Logun M, Fraietta JA, *et al*. Intrathecal bivalent CAR T cells targeting EGFR and IL13Rα2 in recurrent glioblastoma: phase 1 trial interim results. Nat Med 2024;30:1320–29.
[44] Agliardi G, Liuzzi AR, Hotblack A, *et al*. Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. Nat Commun 2021;12:1–11.
[45] Tręda C, Włodarczyk A, Pacholczyk M, *et al*. Increased EGFRvIII epitope accessibility after tyrosine kinase inhibitor treatment of glioblastoma cells creates more opportunities for immunotherapy. Int J Mol Sci 2023;24:4350.
[46] Swan SL, Mehta N, Ilich E, *et al*. IL7 and IL7 Flt3L co-expressing CAR T cells improve therapeutic efficacy in mouse EGFRvIII heterogeneous glioblastoma. Front Immunol 2023;14. doi:10.3389/FIMMU.2023.1085547.
[47] Kramer K, Pandit-Taskar N, Kushner BH, *et al*. Phase 1 study of intraventricular 131I-omburtamab targeting B7H3 (CD276)-expressing CNS malignancies. J Hematol Oncol 2022;15. doi:10.1186/S13045-022-01383-4.
[48] Tang X, Wang Y, Huang J, *et al*. Administration of B7-H3 targeted chimeric antigen receptor-T cells induce regression of glioblastoma. Signal Transduct Target Ther 2021;6. doi:10.1038/S41392-021-00505-7.
[49] Nehama D, Di Ianni N, Musio S, *et al*. B7-H3-redirected chimeric antigen receptor T cells target glioblastoma and neurospheres. EBioMedicine 2019;47:33–43.
[50] Haydar D, Houke H, Chiang J, *et al*. Cell-surface antigen profiling of pediatric brain tumors: B7-H3 is consistently expressed and can be targeted via local or systemic CAR T-cell delivery. Neuro Oncol 2021;23:999.
[51] Tang X, Zhao S, Zhang Y, *et al*. B7-H3 as a novel CAR-T therapeutic target for glioblastoma. Mol Ther Oncolytics 2019;14:279–87.
[52] Brown CE, Badie B, Barish ME, *et al*. Bioactivity and safety of IL13Rα2-redirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. Clin Cancer Res 2015;21:4062–72.
[53] Brown CE, Rodriguez A, Palmer J, *et al*. Off-the-shelf, steroid-resistant, IL13Rα2-specific CAR T cells for treatment of glioblastoma. Neuro Oncol 2022;24:1318.
[54] Brown CE, Hibbard JC, Alizadeh D, *et al*. Author Correction: locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. Nat Med 2024;30:1501.
[55] Gu A, Bai Y, Zhang C, *et al*. IL13Rα2-targeted third-generation CAR-T cells with CD28 transmembrane domain mediate the best anti-glioblastoma efficacy. Cancer Immunol Immunother 2023;72:2393–403.
[56] Xu C, Bai Y, An Z, *et al*. IL-13Rα2 humanized scFv-based CAR-T cells exhibit therapeutic activity against glioblastoma. Mol Ther Oncolytics. 2022;24:443–51.
[57] Starr R, Aguilar B, Gumber D, *et al*. Inclusion of 4-1BB costimulation enhances selectivity and functionality of IL13Rα2-targeted chimeric antigen receptor T cells. Cancer Res Commun 2023;3:66–79.
[58] Ahmed N, Salsman VS, Kew Y, *et al*. HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. Clin Cancer Res 2010;16:474–85.
[59] Hegde M, Mukherjee M, Grada Z, *et al*. Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. J Clin Invest 2016;126:3036–52.
[60] Ahmed N, Brawley V, Hegde M, *et al*. HER2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma: a phase 1 dose-escalation trial. JAMA Oncol 2017;3:1094–101.
[61] Liu Z, Zhou J, Yang X, *et al*. Safety and antitumor activity of GD2-Specific 4SCAR-T cells in patients with glioblastoma. Mol Cancer 2023;22:1–15.
[62] Weiss T, Weller M, Guckenberger M, *et al*. NKG2D-based CAR T cells and radiotherapy exert synergistic efficacy in glioblastoma. Cancer Res. 2018;78:1031–43.
[63] Choi BD, Gerstner ER, Frigault MJ, *et al*. Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. N Engl J Med 2024;390:1290–98.
[64] Lin Q, Ba T, Ho J, *et al*. First-in-human trial of EphA2-Redirected CAR T-cells in patients with recurrent glioblastoma: a preliminary report of three cases at the starting dose. Front Oncol 2021;11. doi:10.3389/FONC.2021.694941/FULL.
[65] Akhavan D, Alizadeh D, Wang D, *et al*. CAR T cells for brain tumors: lessons learned and road ahead. Immunol Rev. 2019;290:60.
[66] Mitusova K, Peltek OO, Karpov TE, *et al*. Overcoming the blood–brain barrier for the therapy of malignant brain tumor: current status and prospects of drug delivery approaches. J Nanobiotechnology. 2022;20:412.

[67] Alahmari A. Blood-Brain Barrier Overview: structural and Functional Correlation. Neural Plast 2021;2021. doi:10.1155/2021/6564585.
[68] Czyżewski W, Mazurek M, Sakwa L, *et al*. Astroglial cells: emerging therapeutic targets in the management of traumatic brain injury. Cells 2024;13:148.
[69] Daei Sorkhabi A, Mohamed Khosroshahi L, Sarkesh A, *et al*. The current landscape of CAR T-cell therapy for solid tumors: mechanisms, research progress, challenges, and counterstrategies. Front Immunol 2023;14. doi:10.3389/FIMMU.2023.1113882.
[70] Akbari P, Katsarou A, Daghighian R, *et al*. Directing CAR T cells towards the tumor vasculature for the treatment of solid tumors. Biochimica Et Biophysica Acta (BBA) - Rev Cancer 2022;1877:188701.
[71] Si X, Xiao L, Brown CE, *et al*. Preclinical evaluation of CAR T cell function: in vitro and in vivo models. Int J Mol Sci 2022;23:3154.
[72] Mo F, Pellerino A, Soffietti R, *et al*. Blood–Brain barrier in brain tumors: biology and clinical relevance. Int J Mol Sci 2021;22:12654.
[73] Lu J, Huo W, Ma Y, *et al*. Suppressive immune microenvironment and CART therapy for glioblastoma: future prospects and challenges. Cancer Lett. 2024;600:217185.
[74] Brown CE, Alizadeh D, Starr R, *et al*. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med 2016;375: 2561–69.
[75] Chang Y, Cai X, Syahirah R, *et al*. CAR-neutrophil mediated delivery of tumor-microenvironment responsive nanodrugs for glioblastoma chemo-immunotherapy. Nat Commun 2023;14. doi:10.1038/S41467-023-37872-4.
[76] Zhang Y, Qin D, Shou AC, *et al*. Exploring CAR-T cell therapy side effects: mechanisms and management strategies. J Clin Med 2023;12:6124.
[77] Bui TM, Wiesolek HL, Sumagin R. ICAM-1: a master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. J Leukoc Biol. 2020;108:787.
[78] Achón Buil B, Tackenberg C, Rust R. Editing a gateway for cell therapy across the blood–brain barrier. Brain. 2023;146:823.
[79] Del Baldo G, Del Bufalo F, Pinacchio C, *et al*. The peculiar challenge of bringing CAR-T cells into the brain: perspectives in the clinical application to the treatment of pediatric central nervous system tumors. Front Immunol 2023;14. doi:10.3389/FIMMU.2023.1142597.
[80] Wimmer I, Tietz S, Nishihara H, *et al*. PECAM-1 stabilizes blood-brain barrier integrity and favors paracellular T-cell diapedesis across the blood-brain barrier during neuroinflammation. Front Immunol 2019;10:447986.
[81] Guzman G, Pellot K, Reed MR, *et al*. CAR T-cells to treat brain tumors. Brain Res Bull. 2023;196:76–98.
[82] Chen Y, Li R, Shang S, *et al*. Therapeutic potential of TNFα and IL1β blockade for CRS/ICANS in CAR-T therapy via ameliorating endothelial activation. Front Immunol 2021;12:623610.
[83] Merchant M, Ranjan A, Pang Y, *et al*. Tumor mutational burden and immunotherapy in gliomas. Trends Cancer 2021;7:1054.
[84] Schmidts A, Srivastava AA, Ramapriyan R, *et al*. Tandem chimeric antigen receptor (CAR) T cells targeting EGFRvIII and IL-13Rα2 are effective against heterogeneous glioblastoma. Neurooncol Adv 2023;5. doi:10.1093/NOAJNL/VDAC185.
[85] Haley MJ, Bere L, Minshull J, *et al*. Hypoxia coordinates the spatial landscape of myeloid cells within glioblastoma to affect survival. Sci Adv 2024;10:3301.
[86] Li D, Jiang L, Zhou W, *et al*. Chimeric antigen receptor-T cell therapy decreases distant metastasis and inhibits local recurrence post-surgery in mice. Hum Gene Ther 2023;34:1248–56.
[87] Arnesen VS, Navarro AG, Chekenya M. Challenges and prospects for designer T and NK cells in glioblastoma immunotherapy. Cancers (Basel) 2021;13:4986.
[88] Rojas-Quintero J, Díaz MP, Palmar J, *et al*. Car T cells in solid tumors: overcoming obstacles. Int J Mol Sci 2024;25:4170.
[89] Johnson A, Townsend M, O'Neill K. Tumor microenvironment immunosuppression: a roadblock to CAR T-cell advancement in solid tumors. Cells 2022;11. doi:10.3390/CELLS11223626.
[90] Larson RC, Kann MC, Bailey SR, *et al*. CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours. Nature 2022;604: 563–70.
[91] McGrath K, Dotti G. Combining oncolytic viruses with chimeric antigen receptor T cell therapy. Hum Gene Ther. 2021;32:150.
[92] Zhang D, Li AM, Hu G, *et al*. PHGDH-mediated endothelial metabolism drives glioblastoma resistance to chimeric antigen receptor T cell immunotherapy. Cell Metab 2023;35:517–534.e8.
[93] Liu Z, Zhou Z, Dang Q, *et al*. Immunosuppression in tumor immune microenvironment and its optimization from CAR-T cell therapy. Theranostics 2022;12:6273–90.
[94] Liu G, Rui W, Zhao X, *et al*. Enhancing CAR-T cell efficacy in solid tumors by targeting the tumor microenvironment. Cell Mol Immunol. 2021;18:1085–95.
[95] Sievers S, Watson G, Johncy S, *et al*. Recognizing and grading CAR T-cell toxicities: an advanced practitioner perspective. Front Oncol. 2020;10:885.
[96] Adkins S, RN, MSN, ANP-C. CAR T-cell therapy: adverse events and management. J Adv Pract Oncol 2019;10:21.
[97] Zu C, Wu S, Zhang M, *et al*. A distinct cytokine network distinguishes chimeric antigen receptor T cell (CAR-T)-associated hemophagocytic lymphohistiocytosis-like toxicity (carHLH) from severe cytokine release syndrome following CAR-T therapy. Cytotherapy 2023;25:1167–75.
[98] Hughes AD, Teachey DT, Diorio C. Riding the storm: managing cytokine-related toxicities in CAR-T cell therapy. Semin Immunopathol. 2024;46:1–19.
[99] Jain MD, Smith M, Shah NN. How I treat refractory CRS and ICANS after CAR T-cell therapy. Blood. 2023;141:2430.
[100] Miao L, Zhang Z, Ren Z, *et al*. Reactions Related to CAR-T Cell Therapy. Front Immunol 2021;12. doi:10.3389/FIMMU.2021.663201.
[101] Gurnik S, Devraj K, Macas J, *et al*. Angiopoietin-2-induced blood–brain barrier compromise and increased stroke size are rescued by VE-PTP-dependent restoration of Tie2 signaling. Acta Neuropathol 2016;131:753.
[102] Yang C, Nguyen J, Yen Y. Complete spectrum of adverse events associated with chimeric antigen receptor (CAR)-T cell therapies. J Biomed Sci. 2023;30:1–18.
[103] Gazeau N, Liang EC, Wu Q, *et al*. Anakinra for refractory cytokine release syndrome or immune effector cell-associated neurotoxicity syndrome after chimeric antigen receptor T cell therapy. Transplant Cell Ther 2023;29:430–37.
[104] Agosti E, Garaba A, Antonietti S, *et al*. CAR-T cells therapy in glioblastoma: a systematic review on molecular targets and treatment strategies. Int J Mol Sci 2024;25:7174.
[105] Garima G, Thanvi S, Singh A, *et al*. Epidermal growth factor receptor variant III mutation, an emerging molecular marker in glioblastoma multiforme patients: a single institution study on the indian population. Cureus 2022;14. doi:10.7759/CUREUS.26412.
[106] Choe JH, Watchmaker PB, Simic MS, *et al*. SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. Sci Transl Med 2021;13. doi:10.1126/SCITRANSLMED.ABE7378.
[107] Lertsumitkul L, Iliopoulos M, Wang SS, *et al*. EphA3-targeted chimeric antigen receptor T cells are effective in glioma and generate curative memory T cell responses. J Immunother Cancer 2024;12:e009486.
[108] Jezierzá Nski M, Nafalska N, Stopyra M, *et al*. Temozolomide (TMZ) in the treatment of glioblastoma multiforme—A literature review and clinical outcomes. Curr Oncol 2024;31:3994–4002.
[109] Liang T, Song Y, Gu L, *et al*. Insight into the progress in CAR-T cell therapy and combination with other therapies for glioblastoma. Int J Gen Med. 2023;16:4121.
[110] Sun T, Li Y, Yang Y, *et al*. Enhanced radiation-induced immunogenic cell death activates chimeric antigen receptor T cells by targeting CD39 against glioblastoma. Cell Death Dis 2022;13. doi:10.1038/S41419-022-05319-1.
[111] Babamohamadi M, Mohammadi N, Faryadi E, *et al*. Anti-CTLA-4 nanobody as a promising approach in cancer immunotherapy. Cell Death Dis 2024;15:1–16.
[112] Zhou X, Cao H, Fang S-Y, *et al*. CTLA-4 tail fusion enhances CAR-T antitumor immunity. Nat Immunol 2023;24:1499–510.
[113] Agarwal S, Aznar MA, Rech AJ, *et al*. Deletion of the inhibitory co-receptor CTLA-4 enhances and invigorates chimeric antigen receptor T cells. Immunity 2023;56:2388–2407.e9.
[114] Filippone A, Lanza M, Mannino D, *et al*. PD1/PD-L1 immune checkpoint as a potential target for preventing brain tumor progression. Cancer Immunol Immunother 2022;71:2067.
[115] Sun Q, Hong Z, Zhang C, *et al*. Immune checkpoint therapy for solid tumours: clinical dilemmas and future trends. Signal Transduction Targeted Ther. 2023;8:1–26.
[116] Najafi S, Mortezaee K. Modifying CAR-T cells with anti-checkpoints in cancer immunotherapy: a focus on anti PD-1/PD-L1 antibodies. Life Sci. 2024;338:122387.

[117] Majzner RG, Ramakrishna S, Yeom KW, *et al*. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. Nature 2022;603:934–41.
[118] Ronvaux L, Riva M, Coosemans A, *et al*. Liquid Biopsy in Glioblastoma. Cancers (Basel) 2022;14:3394.
[119] Pant A, Lim M. CAR-T therapy in GBM: current challenges and avenues for improvement. Cancers (Basel). 2023;15:1249.
[120] Hara T, Chanoch-Myers R, Mathewson ND, *et al*. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. Cancer Cell 2021;39:779–792.e11.
[121] Yang K, Li J, Hölting TLB, *et al*. Glioma targeted therapy: insight into future of molecular approaches. Mol Cancer 2022;21:1–32.
[122] Ma W, Wang Y, Zhang R, *et al*. Targeting PAK4 to reprogram the vascular microenvironment and improve CAR-T immunotherapy for glioblastoma. Nat Cancer 2020;2:83–97.
[123] Chohan KL, Siegler EL, Kenderian SS. CAR-T cell therapy: the efficacy and toxicity balance. Curr Hematol Malig Rep. 2023;18:9–18.
[124] Santomasso BD, Park JH, Salloum D, *et al*. Clinical and biological correlates of neurotoxicity associated with CAR T-cell therapy in patients with B-cell acute lymphoblastic leukemia. Cancer Discov 2018;8:958–71.
[125] Gust J, Hay KA, Hanafi L-A, *et al*. Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. Cancer Discov 2017;7:1404–19.
[126] Freyer CW, Porter DL. Cytokine release syndrome and neurotoxicity following CAR T-cell therapy for hematologic malignancies. J Allergy Clin Immunol. 2020;146:940–48.
[127] Gargett T, Ebert LM, Truong NTH, *et al*. GD2-targeting CAR-T cells enhanced by transgenic IL-15 expression are an effective and clinically feasible therapy for glioblastoma. J Immunother Cancer 2022;10:e005187.