Review

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Recent Development of Immunotherapy for Treating Glioblastoma With a Focus on CAR-T Cells

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Glioblastoma remains one of the most aggressive and treatment-resistant brain tumors, with current standard-of-care therapies offering limited survival benefits. Immunotherapy has emerged as a promising avenue to enhance anti-tumor immunity, with chimeric antigen receptor T (CAR-T) cell therapy gaining significant attention. While CAR-T therapies have demonstrated remarkable success in hematologic malignancies, their application in glioblastoma faces several challenges, including tumor heterogeneity, an immunosuppressive tumor microenvironment, limited blood-brain barrier penetration, T-cell exhaustion, and potential neurotoxicity. This review explores recent advancements in CAR-T therapy for glioblastoma, focusing on key tumor-associated antigens and innovative strategies to overcome treatment barriers. These include multi-antigen targeting, checkpoint inhibition, locoregional CAR-T delivery, synNotch, personalized CAR-T systems, and combination approaches with oncolytic viruses. We also discuss future directions for optimizing efficacy and safety. As the field advances, integrating synthetic biology and personalized antigen selection may further refine CAR-T therapy, offering renewed hope for glioblastoma patients.

Keywords: glioblastoma; CAR-T; tumor antigen; synNotch; immunotherapy

Introduction

Glioblastoma is the most aggressive and lethal primary brain tumor, characterized by rapid proliferation, diffuse infiltration, and resistance to conventional therapies [1]. Being the most common malignant primary brain tumors in adults, incidences of glioblastomas increase after the age of 40 with risk steadily growing with age [2]. Originating from the neuroglial stem cells, glioblastoma exhibits extensive invasiveness, spreading to the brain parenchyma and developing microscopic branches that infiltrate multiple regions of the brain [3]. The diffused nature of glioblastoma makes complete surgical resection virtually impossible. Current treatment plans, including maximal safe surgical resection followed by radiation therapy and chemotherapy, provide only limited survival benefits, with median survival remaining around 12–18 months post-diagnosis [3–5]. Given these challenges, there is an urgent need for innovative therapeutic strategies to address the complex and aggressive nature of glioblastoma and to improve patient outcomes. Immunotherapy has emerged as a promising alternative, leveraging the body's immune system to target and eliminate tumor cells. Various immunotherapeutic strategies have been explored, including checkpoint inhibitors, cancer vaccines, oncolytic viruses, cytokine therapy, and adoptive cell therapy [6,7]. Among these, checkpoint inhibitors and adoptive cell therapy, particularly chimeric antigen receptor T (CAR-T) cell therapy, have gained considerable attention for their potential applications in treating a variety of cancers [7–9]. Several checkpoint inhibitors have been Food and Drug Administration (FDA)-approved for treating malignancies such as melanoma [10]. However, their efficacy as monotherapy for glioblastoma has been limited due to the highly immunosuppressive tumor microenvironment of glioblastoma, combined with the challenges of antibody delivery across the blood-brain barrier [11].

Adoptive cell therapy involves isolation, modification, and reinfusion of a patient's immune cells to enhance their anti-tumor activity. One of the most successful forms of adoptive cell therapy is CAR-T cell [8,12]. CAR-T cells are engineered by introducing a gene encoding a synthetic receptor that recognizes specific tumor-associated antigens. These modified T cells are expanded *ex vivo* and reinfused into the patient to target and destroy cancer cells (Fig. 1). CAR-T cell therapy has shown remarkable success in hematological malignancies, including B-cell acute lymphoblastic leukemia, some B-cell lymphomas, and multiple myeloma [13–15]. In some cases, patients with advanced hematologic cancers have achieved complete and durable remissions [16]. CAR-T cell therapy has also demonstrated promise in solid tumors, with a notable case of a neuroblastoma patient who remained disease-free for over 18 years following Disialoganglioside 2 (GD2)-targeted CAR-T treatment [17]. Encouraged by the success of CAR-T

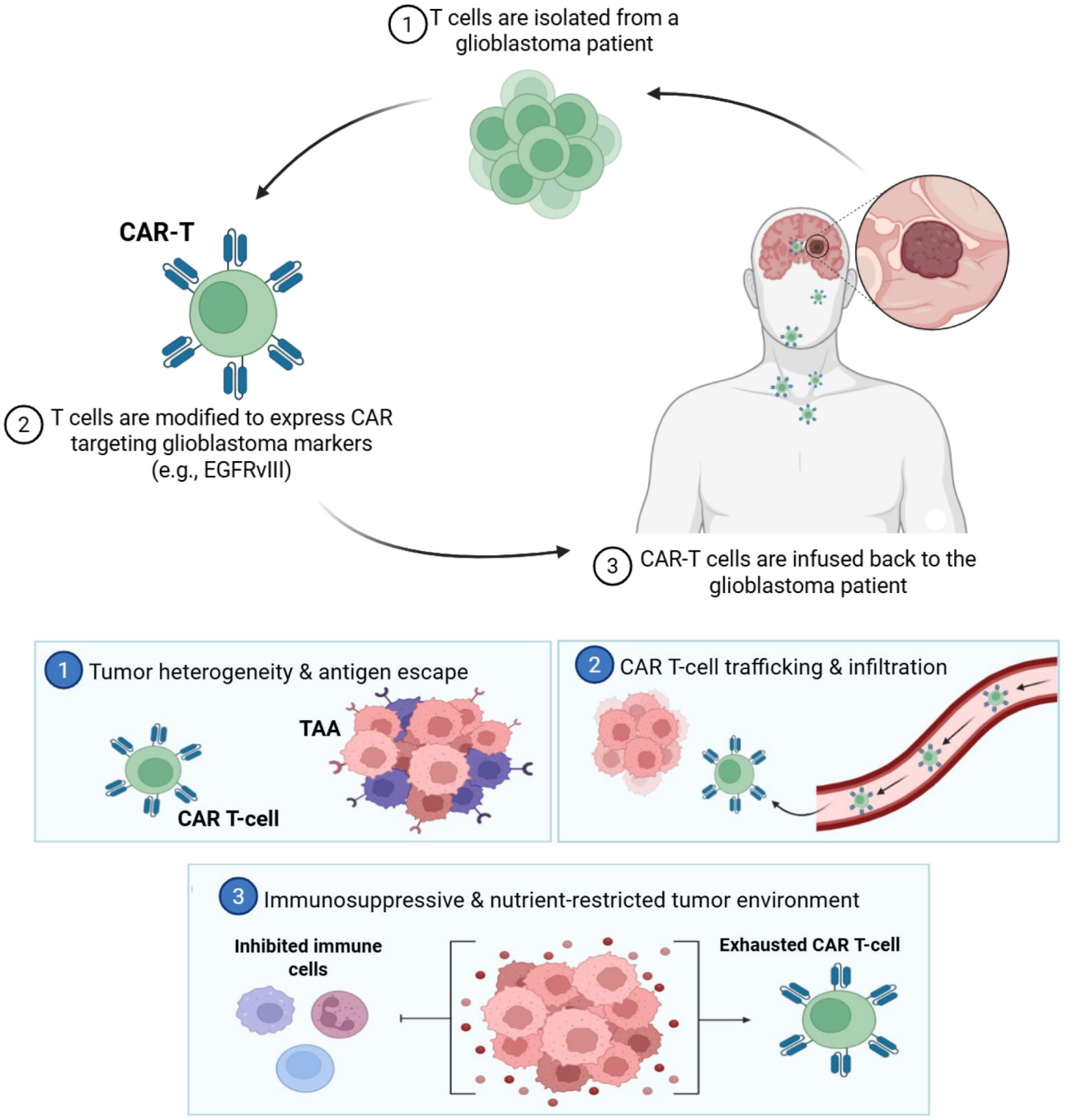

Fig. 1. CAR-T cell therapy for glioblastoma. T cells are isolated from the patient's blood and transfected with a virus that carries a man-made gene for chimeric antigen receptor (CAR). The resulting CAR-T cells are expanded and infused back to the patient. In the body, CAR-T cells are trafficked to the brain, recognize glioblastoma cells, and destroy them. Bottom panels illustrate key obstacles of CAR-T therapy for glioblastoma (see text for more explanation). TAA, tumor-associated antigen; EGFRvIII, epidermal growth factor receptor variant III. The schemes were made by BioRender (https://www.biorender.com/).

cell therapy in other solid tumors, researchers are actively investigating its application in glioblastoma. Compared to checkpoint inhibitors, CAR-T cells may offer advantages in glioblastoma treatment, including better blood-brain barrier penetration and sustained anti-tumor effects [18].

Tumor-Associated Antigens for CAR-T Applications in Treating Glioblastoma

A critical requirement for effective CAR-T cell therapy is the identification of tumor-specific antigens that allow selective targeting of cancer cells while sparing normal tissues. Proteomic and transcriptomic analyses have identi-

Table 1. Markers for CAR-T cell therapy against glioblastoma and related clinical trials.

Marker	Suitability	Clinical trials	Phase	Status	Outcome/Notes
EGFRvIII	Highly expressed in tumor cells	NCT01454596	Phase I/II	Completed	Safe; transient and limited antitumor response
		NCT02664363	Phase I	Terminated	Terminated early due to the end of funding
		NCT02844062	Phase I	Unknown	In patients with recurrent glioblastoma
		NCT05802693	Phase I	NY recruit	No results posted
		NCT06186401	Phase I	Recruiting	synNotch is used
		NCT03283631	Phase I	Terminated	Shift toward the next iteration of a related trial
		NCT03726515	Phase I	Completed	Safe but not strong clinical efficacy
		NCT05063682	Phase I	Active	No results
		NCT02209376	Phase I	Terminated	Safe; promising efficacy in some patients
IL-13Rα2	Expressed in tumor cells only	NCT02208362	Phase I	Active	Safe and promising clinical activity
		NCT04003649	Phase I	Recruiting	Possible combination of ICI
		NCT05168423	Phase I	Recruiting	Dual-targeting (IL-13Rα2 & EGFR); rGBM
		NCT04661384	Phase I	Recruiting	Likely to complete in November, 2025
HER2	Highly expressed in tumor cells	NCT01109095	Phase I	Completed	HER2-CAR CMV is safe & clinically beneficial
		NCT05868083	Phase I	Recruiting	No results
		NCT06616727	Phase I	Enrolling	No results
B7-H3	Highly expressed in tumor cells	NCT05366179	Phase I	Recruiting	No results
		NCT04077866	Phase I/II	Recruiting	CAR-T given between Temozolomide cycles
		NCT06482905	Phase I	NY recruit	No results
		NCT04385173	Phase I	Recruiting	Combination with temozolomide
		NCT05241392	Phase I	Active	Treatment deemed safe and tolerated
		NCT05835687	Phase I	Recruiting	For participants younger than 21 years old
		NCT05474378	Phase I	Active	Intracranial administration is feasible and safe
NKG2D	Highly expressed in tumor cells	NCT04717999	Phase I	NY recruit	No results
		NCT04270461	Phase I	Withdrawn	No results
		NCT05131763	Phase I	Unknown	No results
CD147	Involved in tumor metastasis	NCT04045847	Phase I	Recruiting	Unknown
CD70	Involved in immune homeostasis	NCT05353530	Phase I	Enrolling	4 patients have been enrolled to date
EphA2	Highly expressed	NCT03423992	Phase I	Recruiting	Personalized immunotherapy for patients with rGBM based on the expression of EphA2, EGFRVIII, IL-13Rα2, HER2, CD133, GD2.

NY, not-yet-recruiting; ICI, check point inhibitor; rGBM, recurrent glioblastoma; EGFRvIII, epidermal growth factor receptor variant III; IL-13Rα2, interleukin-13 receptor subunit alpha-2; HER2, human epidermal growth factor receptor 2; B7-H3, B7 homolog 3; NKG2D, natural killer group 2 member D; CD147, cluster of differentiation 147; CD70, cluster of differentiation 70; EphA2, EPH receptor 2; GD2, Disialoganglioside 2.

fied several tumor-associated markers uniquely or highly expressed in glioblastoma, making them potential candidates for CAR-T cell therapy (Table 1). Key glioblastoma-associated antigens under investigation include epidermal growth factor receptor variant III (EGFRvIII), interleukin-13 receptor subunit alpha-2 (IL-13Rα2), human epidermal growth factor receptor 2 (HER2), B7 homolog 3 (B7-H3), natural killer group 2 member D (NKG2D), cluster of differentiation 147 (CD147), cluster of differentiation 70 (CD70), and EPH receptor 2 (EphA2) [19,20].

Among these markers, EGFRvIII is widely used and investigated in at least nine clinical trials (Table 1). EGFRvIII is a mutant variant of EGFR found in approximately 25–30% of glioblastoma cases [21]. Single-chain variable fragment (scFv) that can specifically recognize EGFRvIII have been developed and used in the design of chimeric antigen receptors [22]. IL-13Rα2 is overexpressed in glioblastoma with minimal expression in normal brain tissue, making it a popular choice for CAR-T design [23]. Currently, there are five clinical trials focusing on IL-13R α2 as a marker. A phase I trial evaluating IL-13Rα2-targeted CAR-T cells in patients with recurrent high-grade glioma demonstrated the feasibility and safety of this approach [24]. HER2 is upregulated in a subset of glioblastoma and is associated with the development and progression of the disease [25]. Preclinical studies have shown that HER2-targeting CAR-T cells exhibit effective anti-tumor activity both *in vitro* and *in vivo* [26]. B7-H3 is

broadly expressed in glioblastoma in about 70% of patients and has limited expression in normal tissue. Its expression correlates with tumor malignancy and poor survival [27]. B7-H3 is another widely used marker and there are seven ongoing clinical trials associated with it. NKG2D ligands are widely expressed on several cancer types, including gliomas, but are mostly absent in healthy tissues [28,29]. To enhance the effectiveness of NKG2D CAR-T therapy, combining it with sodium valproate has been shown to increase surface NKG2D ligand expression on glioblastoma cells [30]. CD147, also known as basigin, is highly expressed in glioblastoma tissues compared to normal brain tissue, and its expression is associated with poor overall survival and a higher risk of recurrence [31]. Similarly, CD70 is expressed in malignant tissue but has limited expression in healthy tissue, while EphA2 is highly expressed in glioblastoma and is expressed at low levels in normal brain tissue [32]. A first-in-human trial of EphA2-redirected CAR-T cells in patients with recurrent glioblastoma has been conducted, demonstrating the potential of this target [33].

Challenges of Applying CAR-T in Glioblastoma

Despite its promise, the application of CAR-T cell therapy to glioblastoma faces several formidable biological and technical obstacles (Fig. 1). A major barrier is its extensive intra- and inter-tumoral antigenic heterogeneity, which leads to rapid immune escape [34]. Glioblastoma tumors display remarkable cellular diversity, driven by genetic instability, clonal evolution, and the presence of distinct subpopulations such as glioma stem-like cells, each expressing different antigen profiles [35]. This heterogeneity severely undermines the efficacy of CAR-T therapies targeting a single antigen. For instance, EFGRvIII was among the first targets of CAR-T therapy in glioblastoma. In a landmark phase I trial (NCT02209376), EGFRvIII-directed CAR-T cells were administered to patients with recurrent glioblastoma. While the T cells trafficked to the tumor and induced a reduction in EGFRvIII-positive cells, this did not translate to durable clinical responses. Post-treatment biopsies revealed antigen loss and the emergence of EGFRvIII-negative tumor clones [36], highlighting antigen escape under selective pressure from therapy as a major mechanism of resistance. This phenomenon is not unique to EGFRvIII. Preclinical models targeting other glioblastoma-associated antigens including IL-13Rα2 have similarly shown that residual tumor cells lacking the target antigen can survive and lead to recurrence [37]. Mechanistically, this is facilitated by the plasticity of glioblastoma cells and epigenetic regulation of surface antigens, allowing subclones to transiently or permanently lose expression of targeted molecules [38].

Another challenge is that glioblastoma fosters a highly immunosuppressive microenvironment that undermines CAR-T cell activation, proliferation, and persistence. Unlike hematologic malignancies, where the immune milieu is often more permissive to T cell activity, glioblastoma creates a hostile immunological landscape dominated by immunosuppressive cells and cytokines. Several cell types within the glioblastoma microenvironment actively suppress T cell function. Regulatory T cells (Tregs), which are elevated in both tumor core and periphery, inhibit CAR-T activity via cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), interleukin 10 (IL-10), and transforming growth factor beta (TGF-β) secretion [39]. Myeloid-derived suppressor cells and tumor-associated macrophages further exacerbate immune suppression by depleting nutrients essential for T cell metabolism [40]. Cytokines within the glioblastoma microenvironment also contribute significantly to the immunosuppression. For example, TGF-β is highly expressed in glioblastoma and has been shown to directly suppress the cytotoxic function and proliferation of both native and CAR-T cells [41]. Moreover, chronic antigen exposure in this immunosuppressive context can lead to T cell exhaustion, marked by upregulation of inhibitory receptors such as programmed cell death protein 1 (PD-1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), and lymphocyte-activation gene 3 (LAG-3), and a decline in effector cytokine production [42].

In addition, penetrating the blood-brain barrier poses a significant hurdle. While T cells can cross the blood-brain barrier via mechanisms involving immune cell trafficking pathway [18], their migration is often insufficient. In preclinical models, only a fraction of infused CAR-T cells reaches intracranial tumors, significantly limiting therapeutic impact [43]. Furthermore, targeting brain-specific antigens carries the risk of on-target/off-tumor toxicity, which poses serious safety concerns when targeting antigens that are also expressed on normal brain tissue. For example, CAR-T cells targeting the HER2, which has low-level expression in normal brain, led to a fatal cytokine release syndrome in a glioblastoma patient in early clinical testing [44]. Given these key challenges of applying CAR-T therapy in glioblastoma, it is no surprising that there is no FDA-approved CAR-T therapies for glioblastoma at the present time.

Strategies and Innovations to Overcome Major Barriers of CAR-T Therapy for Glioblastoma

A main strategy in overcoming the problem of tumor heterogeneity of glioblastoma is via multi-antigen targeting. Researchers have tried dual-redirected CAR T-cell therapy, in which two pooled single-specific CAR T-cell products were administered to patients [45]. However, stronger CAR-T cells outcompete weaker ones in circulation, reducing overall targeting capacity. An alternative

approach incorporates recognition of two antigens within a single gene-modified T-cell, allowing either antigen to trigger activation [45]. More advanced dual-antigen CAR-T cells, known as tandem CARs, combine two antigen-recognition exodomains to engage both antigens simultaneously in a bivalent immune system. These tandem CARs enhance T-cell activation and cytokine secretion when encountering both targets, but face challenges related to compromised protein folding [46]. One potential solution is using single-domain antibody mimics instead of single-chain variable fragments (scFvs) to improve protein stability. Interestingly, it has been shown that two scFvs assembled in a single gene expression cassette can effectively target multiple antigens, such as EGFR and HER2 that are commonly found in solid tumors [26,47].

Multi-antigen targeting can have additional advantages when the antigens selected co-exist on the same cell and interact to promote tumor growth [48]. For example, EGFRvIII and IL-13Rα2 can coexist in some glioblastoma cells and simultaneous targeting both appeared to be more effective, as shown by the performance of a tandem CAR T-cell (TanCART) with dual specificity for both EGFRvIII and IL-13Rα2 [49,50]. In preclinical models, TanCART-treated mice exhibited complete and durable treatment responses, whereas those receiving monospecific CAR-T cells showed incomplete responses and shorter survival times [51]. *In vivo* studies further demonstrated that TanCART-treated mice with BT74 glioma cells achieved complete tumor regression, with no detectable tumors upon histopathological examination [51].

The immunosuppressive tumor microenvironment presents another major obstacle in applying CAR-T for glioblastoma treatment, which limits CAR-T cell expansion, persistence, and function [11]. Several strategies have been explored to overcome this barrier. One strategy is to engineer CAR-T cells to secrete immune stimulatory cytokines like IL-12 or IL-15 [52]. IL-12 can help activate innate immune cells and counteract immunosuppressive T regulatory cells (Tregs), while IL-15 can enhance CAR-T survival and memory phenotype [53]. TGF-β is a key factor causing immunosuppression in the tumor microenvironment. One promising approach to overcome this is the integration of a dominant-negative TGF-β receptor II (dnTGFβRII) into CAR constructs, which mitigates the immunosuppressive effects of TGF-β in the tumor microenvironment [54]. This modification has been shown to enhance CAR-T cell proliferation and prolong cytokine activation, ensuring a sustained antitumor response. Like many cancers, glioblastoma upregulates immune checkpoints that inhibit T cell activity [11]. This can be counteracted via co-administration of checkpoint inhibitors alongside CAR-T cells [55]. Anti-CTLA-4 antibodies (lpilimumab) and anti-PD-1 antibodies (nivolumab and pembrolizumab) have been used as a co-administration agent in at least two clinical trials of using CAR-T cell therapy for glioblastoma [56].

In addition, the glioblastoma microenvironment is rich in suppressive cells that include tumor-associated macrophages (TAMs) and Tregs. The colony-stimulating factor 1 receptor (CSF-1R) is a tyrosine kinase receptor that regulates glioblastoma-associated macrophage survival, differentiation, and polarization [57]. Activation of CSF-1R by its ligands IL-34 and CSF1 supports tumor-promoting macrophages, while inhibition of CSF-1R reduces tumor-associated macrophage activity, limits tumor development, prevents recurrence, and promotes cytotoxic T-cell activation [58,59]. Thus, combining CAR-T with CSF-1R inhibitors could deplete TAMs and boost the efficacy of CAR-T therapy. Tregs also contribute to tumor immune evasion by suppressing anti-tumor immune responses. High Treg infiltration in tumors correlates with poor prognosis [53]. Tregs express forkhead box protein P3 (FOXP3), a transcription factor responsible for producing suppressive cytokines and inhibitory surface molecules. Strategies targeting FOXP3 could modulate Treg activity, enhancing anti-tumor immunity of CAR-T.

Another significant barrier to glioblastoma treatment is the blood-brain barrier, which limits CAR-T cell penetration [60]. Several delivery strategies that help CAR-T cells to reach the brain are being explored. Intravenous (IV) infusion of CD19-directed CAR-T cells has demonstrated BBB penetration, with CAR-T cells detected in cerebrospinal fluid [61,62]. However, more localized and targeted delivery methods are needed to enhance efficacy while minimizing toxicity. These delivery strategies include spinal intrathecal infusion, intraventricular infusion, intra-tumoral injection, and blood-brain barrier disruption via focal ultrasound [63]. Spinal intrathecal infusion, commonly used in pediatric oncology, is well-tolerated and allows intermittent injections through subarachnoid catheters. Intratumoral infusion, particularly convection-enhanced delivery (CED), enables precise distribution of therapeutics, with computational modeling aiding delivery optimization [64]. However, this method requires surgical implantation of catheters, which carries risks of infection, cerebrospinal fluid leakage, and bleeding. Localized blood-brain barrier modulation with focal ultrasound (FUS) is another potential strategy, as it temporarily increases blood-brain barrier permeability, though clinical efficacy remains under investigation [65]. Regardless of the approach, localized CAR-T cell delivery has demonstrated superior efficacy over systemic administration in preclinical glioblastoma models [66].

Oncolytic viruses represent a potential adjunct therapy for CAR-T cells [67]. Oncolytic viruses selectively infect and replicate in tumor cells, triggering immune responses and reversing tumor-induced T-cell suppression [68]. Studies using vesicular stomatitis virus (VSV) and reovirus in mice have produced mixed outcomes. While oncolytic viruses induced T-cell-attracting chemokines, type I interferon signaling led to CAR-T cell apoptosis. Stimulating native T-cell receptors (TCRs) improved CAR-T cell pro-

liferation, function, and memory formation [69]. Additionally, *in vivo* expansion of dual-specific (DS) CAR-T cells preloaded with VSV or reovirus enhanced tumor infiltration and reactivation via homologous boosting [67]. CD8 T-cells carrying VSV effectively delivered the virus to tumors, improving survival in murine subcutaneous melanoma models. Further research is needed to optimize oncolytic virus-CAR-T combination therapies and overcome potential drawbacks.

Clearly, addressing antigen heterogeneity, overcoming tumor microenvironment immunosuppression, enhancing blood-brain barrier penetration, and integrating innovative therapeutic combinations are critical to improving CAR-T therapy outcomes for glioblastoma. Continued advancements in multi-antigen targeting, localized delivery methods, and adjunct therapies hold promise for overcoming current limitations and achieving durable treatment responses.

New Innovations in CAR-T Therapy for Glioblastoma

Personalized CAR-T approaches, driven by patient-specific tumor-antigen profiling, offer a promising strategy to enhance therapeutic efficacy [70]. By identifying an individual patient's tumor-specific antigenic landscape, clinicians can optimize CAR-T antigen selection, improving tumor targeting while minimizing off-target effects and treatment resistance. This is especially beneficial for glioblastoma, given the heterogeneity of tumor antigens and variations among patients [71]. Recent advances in high-throughput sequencing, single-cell transcriptomics, and proteomics have enabled more precise identification of tumor-associated antigens (TAAs) and neoantigens, paving the way for the next generation of personalized CAR-T therapies [70–72]. Personalized antigen selection can account for the specific characteristics of a patient's tumor microenvironment, allowing for the design of CAR-T cells that are better equipped to function in that specific environment.

Applying synthetic biology to CAR-T cell engineering is another important area of innovation. Among the emerging tools in this exciting field, synthetic Notch (synNotch) receptors show exceptional promise [73]. These engineered receptors enable context-dependent activation of CAR-T cells, ensuring they function only within specific tissue environments (Fig. 2, Ref. [45,74–76]). The synNotch system allows T cells to detect extracellular antigens and, in response, initiate a controlled, programmable transcriptional response [74]. Unlike conventional CARs, which are constitutively expressed and can trigger off-target effects, synNotch receptors activate T cells only upon encountering defined environmental cues. In a very exciting recent development, synNotch receptors have been engineered to recognize brain-specific extracellular matrix proteins, such as brevican (BCAN), which are highly expressed in glioblastoma and other brain tissues but absent in peripheral tissues [74]. In their preclinical study, mice implanted with cancer stem cell line in the brain and treated with synNotch-CAR T cells exhibited complete tumor control and durable remission. In contrast, untreated mice experienced rapid disease progression and died within 51 days of tumor inoculation [74]. These results underscore the ability of the synNotch system to sense BCAN and selectively prime T cells for tumor eradication within the brain.

The synNotch platform represents a paradigm shift in CAR-T cell design, offering several advantages over traditional approaches. Most notably, it enhances safety by tightly regulating CAR expression, thereby minimizing off-tumor activity in healthy tissues [77]. Its ability to confer spatial precision also improves tumor specificity, limiting collateral damage. Furthermore, because CAR expression is inducible rather than constant, synNotch-based systems help mitigate T cell exhaustion, persevering antitumor activity over time. These receptors can also be customized to detect different tissue-specific markers, making the system broadly adaptable for targeting various solid tumors and even immune-related neurological disorders [74].

Another major challenge in CAR-T therapy is the risk of on-target, off-tumor toxicity, where CAR-T cells attack healthy tissues expressing the same antigen as tumor cells. To address this, researchers have developed next-generation CAR constructs with switchable and inducible designs that offer precise control over T cell activation [78]. These CAR systems include small molecule-responsive CARs to make ON/OFF switchable CARs that allow for fine-tuning of T cell responses (Fig. 2). For example, switchable CARs use a soluble adaptor to remotely control CAR activity, enabling clinicians to turn T cells on or off as needed [54,79]. This not only improves safety and reduces side effects but also prevents premature T cell exhaustion, thereby enhancing therapeutic efficacy. Tandem CAR-T cell therapy is another important innovation, in which a single intracellular signaling protein is linked to two scFvs [45]. Thus, one CAR-T cell is able to recognize two distinct tumor-associated antigens.

Future Directions and Considerations

One major direction for further innovations in CAR-T cell therapy for glioblastoma is the continued application of synthetic biology. Advancements in CAR design now include inducible constructs and molecular "switches" that enable dynamic, externally controlled T cell activation. These synthetic biology tools reduce systemic toxicity while sustaining potent anti-tumor responses, thereby offering a safer therapeutic window. Additionally, alternative cell platforms—such as $\gamma\delta$ T cells—offer new promises. Due to their innate ability to recognize stress-induced ligands independently of major histocompatibility complex (MHC) presentation, $\gamma\delta$ T cells may circumvent

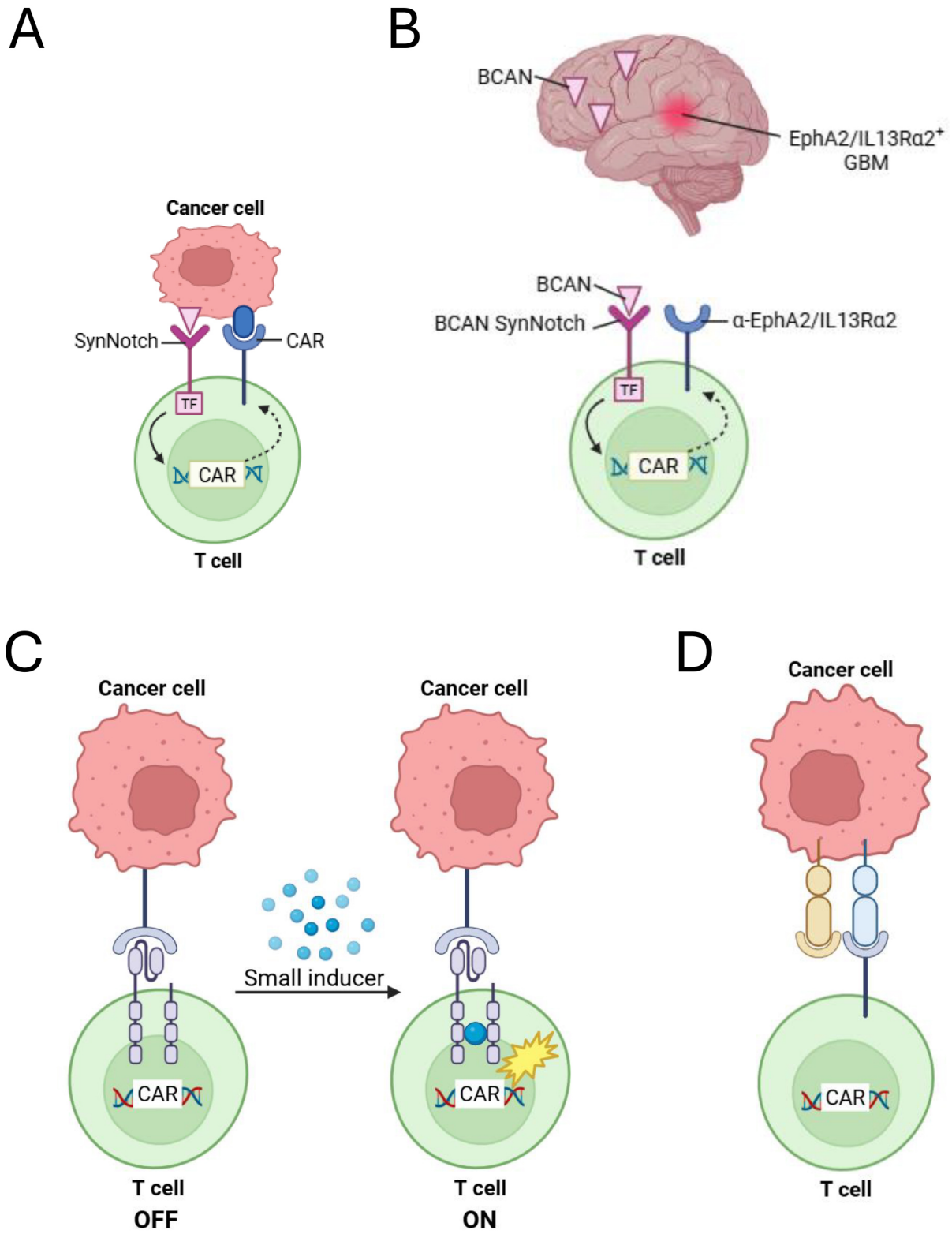

Fig. 2. Innovative CAR-T cell therapies. (A) SynNotch is an engineered receptor. Upon interaction with its ligand, it releases a transcription factor (TF), which induces the expression of CAR [75]. (B) SynNotch system for glioblastoma. SynNotch senses brevican (BCAN), a brain-specific extracellular matrix material, and drives the expression of single-chain variable fragment (scFv) that binds EphA2 and IL-13Rα2 in glioblastoma [74]. (C) ON/OFF switchable CAR-T cells. Recognition and signaling modules are split into distinct polypeptides that can be dimerized upon the addition of inducer [76]. (D) Tandem CAR-T cell [45]. In this innovation, CAR incorporates two scFvs that uses the same intracellular signaling domain, allowing it to recognize two different antigens in a cancer cell. The schemes in the figure were made by BioRender (https://www.biorender.com/).

some mechanisms of tumor immune evasion and broaden the applicability of CAR-T based therapies [80,81]. Ongoing clinical trials continue to shed light on strategies to optimize CAR-T cell trafficking, tumor infiltration, persistence, and resistance to exhaustion. Future directions are expected to involve multi-modal strategies that integrate CAR-T therapy with immune checkpoint inhibitors, oncolytic viruses, and small molecules targeting the tumor stroma and vasculature. Furthermore, advances in spatial transcriptomics and single-cell profiling may guide personalized target selection, minimizing off-tumor effects and enhancing precision.

However, the clinical translation of CAR-T cell therapies for glioblastoma presents ethical and regulatory challenges. Personalized gene therapies require robust oversight to ensure safety, particularly when synthetic biology is employed to reprogram immune cell behavior. The potential for off-target effects, insertional mutagenesis, and unanticipated immune responses demands rigorous preclinical validation and careful regulatory scrutiny. Moreover, ethical questions arise around the equitable distribution of experimental therapies, informed consent for first-in-human trials, and long-term patient monitoring in the context of novel gene edits.

Real-world implementation also hinges on addressing practical and economic barriers. The production of patient-specific CAR-T cells is labor-intensive, costly, and time-sensitive, with a typical price of more than $300,000. This presents economic burden and logistical hurdles, especially in resource-limited settings or for patients with rapidly progressing disease. Healthcare systems will need to develop infrastructure for timely delivery, post-treatment monitoring, and management of potential toxicities, including cytokine release syndrome and neurotoxicity.

Conclusion

Glioblastoma remains one of the most difficult cancers to treat due to its aggressive growth, profound intratumoral heterogeneity, and highly immunosuppressive microenvironment. Although CAR-T cell therapy has revolutionized the treatment of hematologic malignancies, its application in glioblastoma has been limited by several major obstacles, including antigen heterogeneity across the tumor, highly suppressive tumor microenvironment, poor penetration of the blood-brain barrier, and on-target/off-tumor toxicity. Nonetheless, recent advances—such as multi-antigen targeting strategies, locoregional delivery methods, and combination therapies involving checkpoint inhibitors or oncolytic viruses—are beginning to overcome these challenges. Innovations in technologies such as single-cell sequencing and antigen discovery are accelerating the development of personalized, next-generation CAR-T therapies. Breakthroughs in synthetic biology—particularly the development of synNotch-based CAR-T systems—are enabling more refined control over T cell activation, minimizing off-tumor toxicity while preserving T cell function over time. Together, these innovations would hopefully make CAR-T therapy for glioblastoma patients a reality soon.

Availability of Data and Materials

Not applicable.

Author Contributions

GS, MM, and PP collected and analyzed references and wrote the manuscript. YW conceived the concept of the review and critically analyzed the references. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Fig. 1 and Fig. 2 were created using BioRender. The authors have no financial or personal relationship with BioRender, and the use of this tool does not imply any endorsement.

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