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REVIEW ARTICLE

Recent Locoregional CAR T-Cell Trials for the Treatment of Recurrent Glioblastoma: Implications for Neuro-Oncology Practice

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Abstract

Glioblastoma remains one of the most aggressive primary brain tumors in adults, with a survival rarely exceeding 15 months despite multimodal therapy. Novel immunotherapeutic strategies, particularly chimeric antigen receptor T-cell therapy, have emerged as promising approaches to overcome the limitations of conventional treatments. This review summarizes recent early-phase clinical trials investigating locoregional chimeric antigen receptor T-cell delivery in recurrent glioblastoma and highlights key considerations for multidisciplinary neuro-oncology teams involved in this evolving therapeutic paradigm. Phase I studies of intratumoral, intracavitary, intraventricular, or combined delivery routes have demonstrated technical feasibility and safety, with most adverse events being manageable. Dual-route delivery may enhance chimeric antigen receptor T-cell distribution and produce early radiographic and clinical responses in selected patients. However, therapeutic durability remains limited by tumor heterogeneity, antigen loss, and the immunosuppressive tumor microenvironment. Multidisciplinary care teams play a critical role in catheter and reservoir placement, infusion planning, and management of neuroinflammatory toxicities. Although current findings are preliminary, ongoing optimization of target selection, dosing strategies, and combination therapies may expand treatment options for recurrent glioblastoma and further integrate immunotherapy into contemporary neuro-oncology care.

1 Introduction

Effective treatment of malignant brain tumors, mainly glioblastoma (GBM), remains a major challenge in neurosurgery and neuro-oncology. The Stupp protocol [1], published in 2005, represents the last standard-of-care breakthrough. Immune-based strategies, particularly chimeric antigen receptor (CAR) T-cell therapy, have recently emerged as promising options for recurrent GBM (rGBM) [2–9], but systemic delivery is limited by poor blood–brain barrier (BBB) penetration and systemic toxicity [5]. To overcome these limitations, early-phase trials [4, 6–8, 10] have explored locoregional CAR T-cell delivery via intratumoral/intracavitary (ICT), intraventricular (ICV), or dual routes. This review summarizes key trials in adult rGBM,

Key Points

Delivering chimeric antigen receptor T-cells directly into the tumor cavity or the cerebrospinal fluid compartment is feasible and can induce a robust immune response close to the tumor.

Early trials have shown promising signs of tumor control in selected patients, but a lasting benefit has not yet been established.

Treatment requires close collaboration between several disciplines to plan delivery and the management of treatment-related (neuro)toxicity.

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focusing on target antigens, delivery strategies, safety, early efficacy signals, and the multidisciplinary integration of CAR T-cell therapy into current neuro-oncological practice.

2 Major Challenges of Treating Glioblastoma (GBM)

Glioblastoma (GBM) remains one of the most difficult-to-treat brain tumors because of biological and logistical challenges. The tumor microenvironment (TME), including tumor-associated macrophages and microglia, can compromise up to 50% of the total tumor mass and is highly immunosuppressive, substantially limiting the effectiveness of immunotherapies including CAR T-cells [11, 12]. Immunosuppressive cells such as M2-polarized tumor-associated macrophages and microglia, myeloid-derived suppressor cells, and regulatory T-cells inhibit T-cell activation through cytokines such as interleukin (IL)-10 and transforming growth factor-β, and upregulation of checkpoint molecules, including CTLA-4 and PD-1 [11, 13]. Even resident glial cells can be co-opted to support immune evasion [14].

GBM cells produce soluble inhibitory factors, a dense extracellular matrix, and hypoxic conditions that further impair immune cell function [12–14]. Tumor heterogeneity, both intratumoral and intertumoral, enables an antigen-negative escape after CAR T-cell therapy and complicates target standardization [15, 16]. Additional challenges include antigen loss [17], adaptive immune resistance [11], limited CAR T-cell persistence [12], risk of neurotoxicity [18], and the need for precise neurosurgical delivery and monitoring [17, 18].

3 How Chimeric Antigen Receptor (CAR) T-Cells Work

3.1 What Actually is CAR?

A CAR is a synthetic chimeric receptor that is genetically engineered into a T cell to redirect its activity. A typical CAR has an extracellular binding domain derived from a monoclonal antibody (usually a single-chain variable fragment) joined via a short hinge to a transmembrane linker with intracellular signaling regions [19, 20]. The single-chain variable fragment externally binds a specific protein on a cancer cell (antigen), while the intracellular portion contains one or more signaling motifs plus a co-stimulatory domain that activates the T cell when the antigen is engaged. Importantly, the antibody-derived fragments of CAR can directly recognize surface antigens on cancer cells and therefore do not require antigens to be presented by human leukocyte antigen/major histocompatibility complex. In other words, CARs mediate major histocompatibility complex-independent antigen recognition, allowing direct targeting of cancer cells even if the tumor has downregulated the human leukocyte antigen as an escape mechanism [21].

3.2 Life Cycle of a CAR T-Cell

Figure 1 outlines the CAR T-cell manufacturing process, which is traditionally autologous. Patient T cells are collected via leukapheresis, processed in a Good Manufacturing Practice facility, activated and genetically modified, most commonly using lentiviral or retroviral vectors, though non-viral methods exist [22].

The resulting CAR T-cells are expanded, quality controlled, cryopreserved, and returned for infusion, a process taking approximately 2–3 weeks [22, 23]. While several academic centers operate on point-of-care Good Manufacturing Practice facilities for rGBM, US Food and Drug Administration-approved commercial products rely on centralized manufacturing and complex logistics. Following locoregional infusion into tumor parenchyma, the resection cavity, or ventricular system, CAR T-cells traffic to the target site, recognize tumor antigens via their CAR, form an immune synapse, and initiate T-cell activation, cytokine release, and targeted as well as bystander cytotoxicity [21].

4 Why Administration Routes Matter

The route of CAR T-cell application is critical for therapeutic efficacy in GBM. As illustrated in Fig. 2, locoregional delivery offers distinct advantages over systemic infusion.

The route of CAR T-cell administration is a central determinant of therapeutic exposure, immune activation, toxicity, and possibly efficacy in GBM [5, 24, 25]. Unlike hematological malignancies, GBM is protected by anatomical and immunological barriers, most notably the BBB and blood–tumor barrier, which restrict lymphocyte trafficking and may limit effective tumor exposure after systemic infusion [12, 17, 26]. Although these barriers can be partially disrupted by tumor growth, surgery, radiation, or inflammation, systemic CAR T-cell therapy still depends on peripheral expansion, transvascular homing, and successful infiltration of an immunosuppressive TME [4, 6–9]. Locoregional administration was therefore developed to shift the key pharmacologic variable from peripheral circulation to direct central nervous system (CNS) access [5, 24, 25]. Intracavitary delivery aims to maximize CAR T-cell exposure at the resection cavity and residual tumor margin, whereas ICV delivery targets the CSF compartment and may

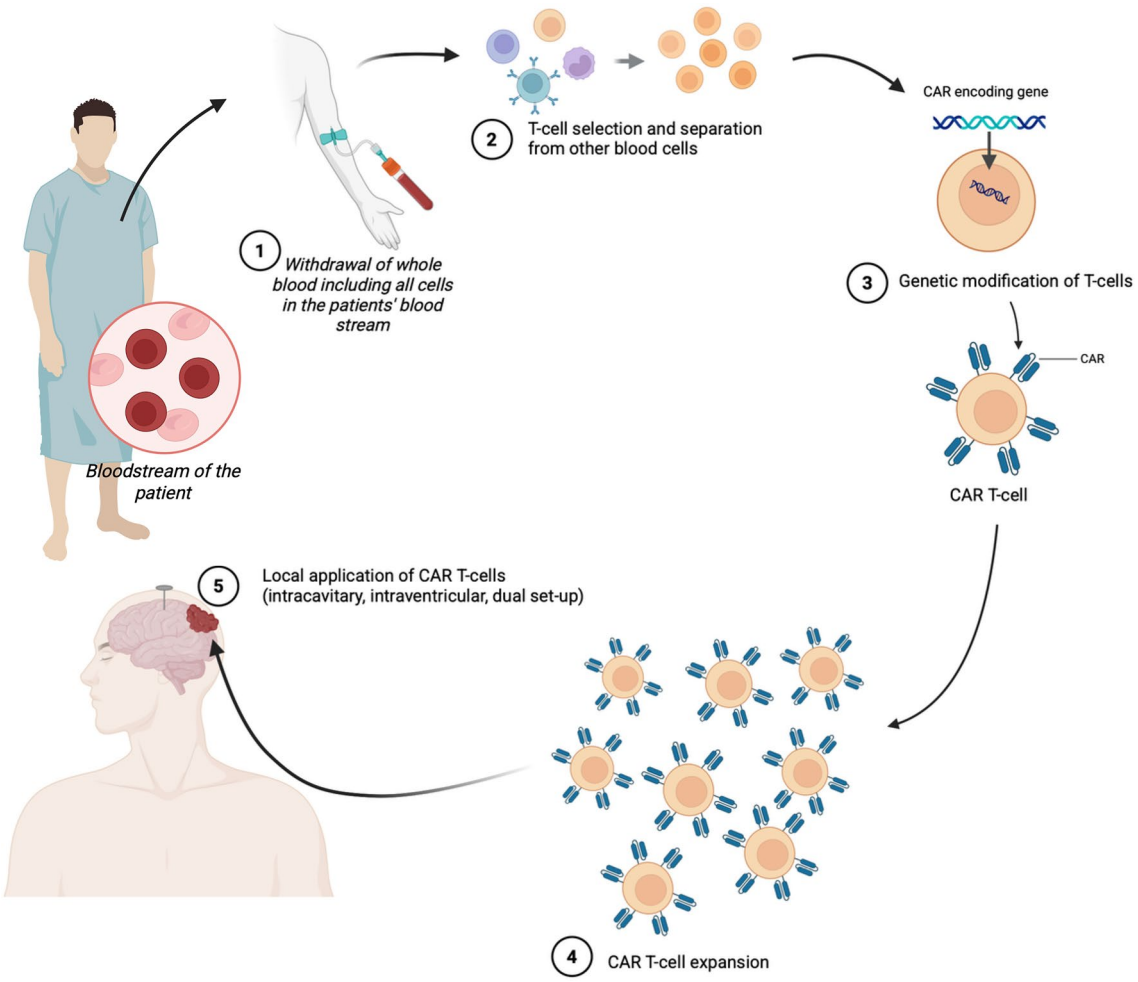
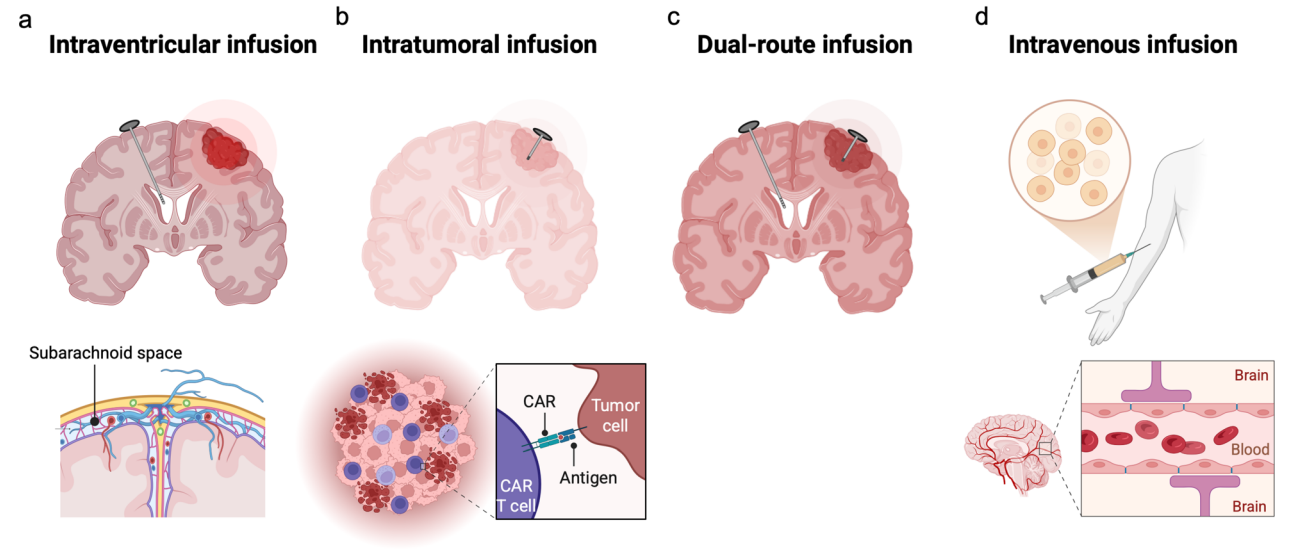


Fig. 1 Principle of chimeric antigen receptor (CAR) T-cell manufacturing and locoregional administration in recurrent glioblastoma. (**1**) Patient peripheral blood is collected via leukapheresis, and (**2**) T cells are isolated/selected ex vivo. (**3**) The CAR construct (encoding a tumor-specific antigen-recognizing receptor) is introduced into the T cells. (**4**) Genetically modified T cells (CAR T- cells) are expanded to achieve therapeutic doses and (**5**) delivered directly into the tumor, the tumor resection cavity, the ventricles, or in a dual set-up approach (here we show intratumoral delivery). This strategy bypasses the blood–brain barrier, achieves high local CAR T-cell concentrations, and minimizes systemic toxicity. Created in https://BioRender.com

be particularly relevant for multifocal, disseminated, or leptomeningeal disease. Dual-route strategies seek to combine focal tumor-bed exposure with broader CSF distribution [5, 24, 25].

Comparative correlative data from early clinical studies support the biological plausibility of this approach, although they do not yet establish a clearly superior route of administration [4, 6–9, 27, 28]. Across locoregional trials, CAR T-cell DNA and inflammatory cytokines are more consistently detected in CNS compartments than in peripheral blood, suggesting that local delivery can increase compartment-specific exposure while limiting systemic spillover [4, 6–9, 27, 28]. For example, Brown et al. [8] reported CAR T-cell signals and cytokine induction in the CSF shortly after infusion, whereas systemic GBM CAR T-cell trials have generally shown limited blood persistence and less robust evidence of sustained intracranial activity and therefore at tumor sites. Similarly, ICV approaches have produced marked CSF cytokines responses, including induction of interferon (IFN)-γ-associated chemokines, IL-6, and other inflammatory mediators, often with relatively modest corresponding serum changes [4, 8, 9]. These findings indicate that locoregional delivery not only alters CAR T-cell biodistribution but also compartmentalizes immune activation within the CNS.

The available imaging, tissue, and biomarker correlates further suggest important route-dependent differences



CSF-wide distribution Optimal for multifocal disease Repeated dosing via reservoir Lower surgical morbidity	High local concentration Direct targeting of tumor cells Catether placement during surgery	Local and CSF coverage Sequential/alternatig infusions May optimize exposure	Technically simple Repeated dosing Systemic inflammation monitoring No device related complications
Dilution/reduced local exposure Potential systemic cytokine leak Requires pressure management	Limited diffusion beyond tumor Possible cavity collapse Suboptimal for multifocal disease	Increased risk of infection Complex infusion management Precise neurosurgical planning	Limited by BBB Lower local exposure Antigen escape Adaptive immunosuppression Greater systemic toxicity risk

Fig. 2 Comparative routes of chimeric antigen receptor (CAR) T-cell administration for recurrent glioblastoma and their potential advantages and limitations Schematic of clinically investigated CAR T-cell delivery routes in glioblastoma. **a** Intraventricular infusion delivers CAR T-cells into the cerebrospinal fluid (CSF) compartment through a ventricular catheter connected to a reservoir, potentially enabling broader CSF-wide distribution, repeated dosing, and improved coverage of multifocal or disseminated disease. Potential limitations include dilution with reduced local tumor exposure, systemic cytokine leakage, and the need for careful infusion because of intracranial pressure management. **b** Intratumoral infusion enables direct delivery of CAR T-cells into the tumor, providing high local effector-cell concentrations and direct tumor-cell targeting, with the disadvantage of this approach being less suitable for multifocal disease. Catheter placement may be performed during surgery, with the limitation of limited coverage beyond the injected lesion and possible cavity collapse. **c** Dual-route infusion combines tumor-directed administration with interventricular delivery, aiming to integrate high local exposure with broader CSF-compartment coverage. Sequential or alternating infusions may further optimize spatial exposure. This strategy is associated with increased procedural complexity, greater device-related infection risk, and the need for precise neurosurgical planning and infusion management. **d** Intravenous infusion is technically straightforward, avoids intracranial catheter complications, and facilitates repeated dosing and systemic monitoring of inflammatory responses. Its principal limitations are dependence on CAR T-cell trafficking across the blood–brain barrier (BBB), potentially lower and less predictable intratumoral exposure, antigen escape, adaptive immunosuppression, and a potentially greater risk of systemic toxicity. Advantages and disadvantages of each approach are summarized below each schematic. Created in https://BioRender.com

[4, 6–9, 27, 28]. Recent CSF-directed or ICV studies have reported rapid radiographic tumor regressions in selected patients, supporting the concept that direct compartmental access can improve short-term tumor exposure in a way that systemic infusion has not consistently achieved in GBM [4, 8, 9]. By contrast, systemic epidermal growth factor receptor variant III (EGFRvIII)-directed CAR T-cell therapy demonstrated that intravenously infused CAR T cells can traffic into tumor tissue, but radiographic responses were limited and CAR T-cell persistence was short-lived [2, 10]. Importantly, however, locoregional delivery has not solved the major biological limitations of CAR T-cell therapy in GBM. Antigen loss, declining target expression in residual tumor, T-cell exhaustion, regulatory T-cell enrichment, and myeloid-dominated adaptive resistance have been observed after both systemic and locoregional approaches [2, 4, 6–10, 27, 28]. Thus, while locoregional administration appears to improve immediate CNS exposure and local immune activation, durable disease control remains constrained by persistence, antigen heterogeneity, and adaptive immune escape.

These correlative findings also help explain the distinct toxicity profiles observed across routes. Systemic administration in GBM has generally not been associated with severe systemic cytokine release syndrome (CRS) seen in some hematologic malignancies, but it has also produced limited evidence of sustained CNS pharmacodynamic activity [2, 10]. Locoregional delivery, in contrast, tends to shift toxicity toward compartment-specific neuroinflammation, including transient inflammatory, edema-related, or CSF-mediated events after CNS-directed dosing [4, 6–9, 27, 28]. Repeated dosing has therefore become a recurring feature of contemporary locoregional protocols, reflecting the observation that direct delivery improves initial tumor access more clearly than it ensures long-term CAR T-cell persistence [8, 28]. Taken together, the administration route should be understood not merely as a technical detail, but as a therapeutic design variable that shapes CAR T-cell distribution, CNS pharmacodynamics, local cytokine activation, systemic exposure, toxicity, dosing strategy, and mechanisms of resistance. The following sections review the rationale, clinical evidence, and practical advantages and limitations of locoregional and peripheral administration.

4.1 Clinical Evidence for Route-Dependent Exposure and Disease Coverage

Early GBM CAR T-cell trials show that intracranial infusion leads to strong CNS cytokine responses and CAR T-cell persistence that exceed systemic exposure. For example, Brown et al. [8] administered IL-13Rα2-targeted CAR T cells directly into the tumor or resection cavity (with/without ICV) and observed marked spikes of IFNγ-related cytokines CXCL9/10 in the CSF and tumor-cavity fluid, whereas systemic serum cytokines changed only little. Likewise, Bagley et al. [9] delivered bispecific CAR T cells intrathecally into patients with multifocal rGBM and detected a substantial CAR T-cell abundance and cytokine release in the CSF of all six patients. These locoregional administrations yielded high CAR T-cell concentrations in CNS compartments and evidence of local immune activation. By contrast, intravenous CAR T-cell infusions in GBM have generally produced much lower CNS CAR T-cell penetration. In O'Rourke et al.'s [2] trial of peripherally infused EGFRvIII CAR T-cells, only modest CAR accumulation was detected in the resected tumor tissue with most T cells remaining in the periphery. In sum, ICV/ICT delivery demonstrably concentrates CAR T cells and inflammatory cytokines at the tumor site and in the CNS compartments, whereas systemic delivery yields less consistent CNS trafficking, although no trial has directly compared these routes in the same study.

Intraventricular routes are used to address multifocal or disseminated disease by leveraging CSF flow. Consistent with this rationale, Bagley et al. [10] enrolled only patients with diffuse multifocal rGBM and reported early magnetic resonance imaging shrinkage at multiple sites after injection. Choi et al. [4] similarly observed rapid regression in several lesions following a single ICV dose of bispecific CAR T-cells (though this study is pending full publication). By contrast, ICT delivery is postulated to mainly treat non-disseminated/non-multifocal tumors and has therefore been chosen as the primary approach in these cases [8, 28]. However, comparative studies of ICT versus ICV do not exist so far.

4.2 Neurotoxicity According to Route of Administration

Published phase I studies have generally found acceptable safety for both locoregional and systemic CAR T-cell therapies in gliomas, with neurotoxic events occurring in each setting. In Brown et al.'s [8] locoregional IL-13Rα2-targeted CAR T trial (ICT/ICV), only two grade 3 neurotoxicities were attributed to CAR T-cell therapy among 58 evaluable patients, and two transient grade 4 cerebral edema events occurred in patients with bulky disease. Bagley et al. [9] reported early immune-effector cell-associated neurotoxicity (ICANS) in most of their six ICT-treated patients, managed with high-dose steroids and IL-1 blockade. Choi et al. [4] noted one brief grade 3 encephalopathy. By contrast, O'Rourke et al.'s [2] trial reported no CRS and only one non-convulsive seizure. Thus, systemic CAR T-cell therapy in GBM has so far shown very low neurotoxicity, whereas CNS-directed CAR T cells can induce more neurologic inflammation, although both approaches require vigilant monitoring. Crucially, these comparisons are indirect. Differences in patient selection, CAR design, dosing, and criteria make cross-trial comparison difficult. No randomized data exist to prove that locoregional delivery reduces toxicity (mainly peripheral toxicity) related to intravenous infusion. In summary, CNS-directed CAR T-cell administration elicits strong local immune activation, as measured by CSF cytokines and CAR T-cell counts, and is preferably chosen as the adequate treatment approach, but it has not been shown in a direct trial to confer superior safety or coverage compared to systemic infusion.

4.3 Why Systemic CAR T-Cell Therapy for GBM has Largely Disappointed

A concise explanation is that peripheral CAR T-cell therapy in GBM must solve several problems simultaneously and has, to date, solved only part of them. In the first-in-human EGFRvIII-directed study, only a small fraction

of intravenously administered CAR T-cells reached the tumor tissue and exerted biologic pressure on the target, but this translated into limited clinical benefit [2]. The full report in ten patients showed tumor trafficking, a decline of circulating CAR T-cells to undetectable levels by about 1 month, no dramatic radiographic regressions, reduced EGFRvIII expression in resected tumors, and evidence that treatment was followed by a more immunosuppressive TME, consistent with antigen escape and adaptive resistance rather than durable tumor control [2]. In other words, the trial demonstrated feasibility and on-target biologic activity of CAR T-cells but also exposed the central liabilities of systemic delivery in GBM: inefficient access relative to tumor burden, rapid loss of detectable persistence, marked intratumoral heterogeneity, and microenvironmental suppression. This framework also fits the later peripheral EGFRvIII program, whose phase I publication [10] explicitly reports that repeated peripheral anti-EGFRvIII CAR T-cells combined with pembrolizumab showed no efficacy in GBM.

5 Locoregional CAR T-Cell Delivery Strategies

5.1 Intratumoral (Intracavitary) CAR T-Cell Delivery

With this approach, CAR T-cells are infused directly into the tumor or the resection cavity. Infusion can be performed either at the time of surgery by stereotactic injection or by stereotactic catheter placement into an unresected tumor or when only a biopsy is performed. The aim is to achieve high concentrations of CAR T-cells directly at the tumor site while limiting systemic exposure and systemic toxicity. As expected, early-phase trials report robust local immune activation. Brown et al. [8] demonstrated that ICT (and combined ICT + ICV) delivery of IL-13Rα2-targeted CAR T-cells achieved disease control (stable disease or better) in approximately 50% of heavily pretreated patients with recurrent glioma. The regimen induced significant CSF inflammatory cytokine reactions with IFNγ and CXCL9/10 spikes after infusion. Chimeric antigen receptor T-cell DNA was readily detectable in the CSF in most patients within 1–7 days post-infusion. Notably, Brown et al. [8] found that high pretreatment $CD3^+$ T-cell infiltration in the tumor ("hot" tumors) correlated with longer survival, consistent with local immune engagement. Overall, ICT delivery allows CAR T-cells to function in the surgical cavity where (residual) tumor cells remain. The rationale is that locoregional administration avoids the BBB and may induce strong local effects in the immunosuppressive tumor microenvironment [17]. However, a disadvantage of intratumoral CAR T-cell delivery alone is that tumor cells distant from the injection site may not be reached effectively.

5.2 Intraventricular (ICV) [Intrathecal] CAR T-Cell Delivery

CAR T-cells are typically delivered into the CSF via a ventricle catheter connected to a subcutaneous reservoir or via a lumbar intrathecal catheter. These T cells can circulate throughout the craniospinal axis to target multifocal or disseminated disease, where tumor spread cannot be addresses by a single local injection. This route of administration is proposed to treat multifocal or leptomeningeal disease [4, 6–9, 27]. In the trial of Brown et al. [8], serial ICV infusions induced a relevant regression of leptomeningeal and multifocal GBM. Choi et al. [4] (intracerebroventricular EGFRvIII/EGFR CARs) and Bagley et al. [10] (intrathecal EGFR/IL-13Rα2 bispecific CARs) both observed rapid radiographic reductions in tumor enhancement within days of infusion. CAR T-cells expanded in the CSF after ICV/ICT delivery, but typically transiently. In the series of Choi et al. [4] CAR DNA in the CSF peaked early then declined by day 28, even as one patient maintained a 5-month response. Bagley et al. [9] detected very high CAR copy numbers in CSF (mean of approximately 10^5 copies/μg DNA) within the first week, which is several-fold higher than reported for ICV infusions alone. Cerebrospinal fluid cytokines also rose, indicating focused CNS immune activation. The potential downside of this approach is that CAR T-cells may have less contact with solid tumor mass and could be diluted or cleared more rapidly. Nevertheless, the approach has been proven to be feasible and well tolerated with frequent dosing; moreover, some early efficacy has been reported [4, 8, 9].

5.3 Dual Delivery (Combined Intratumoral and ICV)

Some trials have employed a dual-route strategy to maximize CAR T-cell distribution. The idea is to treat the primary tumor or tumor cavity and the CSF space at the same time. In the recent trial of Brown et al. [8], the final trial arm (arm 5) utilized dual delivery of CAR T-cells. These patients with dual treatment routes showed the longest median overall survival (OS) time. Further, Li et al. [7] placed two catheters (one into the resection cavity, one into the ventricle) and separated each dose. Dual delivery aims to combine the advantage of both routes by achieving high CAR T-cell concentrations in the tumor bed and broad CSF coverage for migratory

tumor cells. Dual-route CAR T-cell administration thus represents a promising strategy to overcome the infiltrative nature of GBM by enhancing CNS coverage.

6 Target Antigens and Key Locoregional CAR T-Cell Trials

Table 1 gives an overview of recent key locoregional CAR T-cell trials for rGBM. The target antigen of CAR T-cells should be abundantly and homogeneously expressed on tumor cells across patients with minimal or no expression in normal tissues. This strict tumor specificity maximizes efficacy and minimizes on-target/off-tumor toxicity [29, 30]. Further key features include rapid immunologic synapse formation, low spontaneous internalization, and resistance to downregulation, so that the antigen remains available for CAR binding as long as possible. Ideally, the antigen is essential for tumor cell survival or proliferation to prevent immune escape by antigen loss [29, 31].

6.1 Interleukin (IL)-13Rα2

Interluekin-13Rα2 represents an attractive CAR T-cell target owing to its high expression in GBM and minimal presence in normal brain tissue [8]. One of the earliest and largest clinical experiences was reported by Brown et al. [8], who conducted a phase I trial of IL-13Rα2-directed CAR T-cells in 65 patients with recurrent high-grade glioma, predominantly rGBM. The study [8] evaluated multiple delivery routes, including intratumoral, intracavitary, ICV, and dual-route administration, without lymphodepleting chemotherapy. Treatment was feasible and well tolerated, with no dose-limiting toxicities (DLTs) or maximum tolerated dose reached, even at the highest dose of 200×10^6 CAR T-cells per cycle. However, treatment-related neurotoxicity was clinically relevant: grade ≥ 3 toxicities with possible or higher attribution to CAR T-cell therapy occurred in 35% of treated patients, including one grade 3 encephalopathy and one grade 3 ataxia with probable attribution, as well as two episodes of transient grade 4 cerebral edema with possible attribution in patients with extensive residual or recurrent tumors [8]. These events improved with increased corticosteroid treatment. Accordingly, the study supports feasibility but also underscores the need for careful monitoring and management of treatment-associated neuroinflammation.

Although primarily a safety study, early efficacy signals were observed [8]. Among evaluable patients, 50% achieved stable disease or better, including partial and complete responses [8]. Median OS for patients with rGBM was 7.7 months, increasing to 10.2 months in the optimized dual-delivery arm [8]. Correlative analyses showed increased inflammatory cytokines in the CSF, supporting on-target immune activation [8]. Overall, this trial demonstrated that IL-13Rα2 CAR T-cell therapy can be safely administered within the CNS and may provide clinical benefit in selected patients.

6.2 Epidermal Growth Factor Receptor Variant III (EGFRvIII)/Wild-Type EGFR

The *EGFR* gene is the most frequently amplified and overexpressed proto-oncogene in GBM, which makes it a common mutational target. *EGFR* gene amplification is present in approximately 40% of GBMs [32]. Roughly 50% of EGFR-amplified GBMs do not amplify or overexpress the wild-type *EGFR* gene [32]. In these cases, they carry a tumor-specific deletion variant (EGFRvIII), which is not expressed in normal brain tissue and is characterized by an in-frame deletion of exon 2–7 [32].

Choi et al. [4] tested a novel "CARv3-TEAM-E" strategy that combines an EGFRvIII-specific CAR with secreted bispecific antibodies (T-cell-engaging antibody molecules) against wild-type EGFR. Intraventricular infusion of these CARv3-TEAM-E T cells in three patients with rGBM resulted in dramatic early tumor regressions within days of a single dose [4]. However, all three tumors eventually recurred after several months, a pattern attributed to limited CAR T-cell persistence. This trial [4] highlights the feasibility of dual targeting of EGFR variants and underscores the challenge of durability in the treatment of GBM.

6.3 EGFR and IL-13Rα2 (Bivalent CAR)

Bagley et al. [9] reported interim results from a trial assessing intrathecal CAR T-cells engineered to target both wild-type EGFR and IL-13Rα2. In this trial [9], six patients with multifocal rGBM received escalating doses of the bivalent CAR T-cell product. All patients showed early reductions in tumor size and contrast enhancing components on MRI, although none met formal criteria for objective response. Chimeric antigen T-cell infusions were associated with early-onset neurotoxicity (ICANS) at both dose levels, which was managed with high-dose corticosteroids and anti-IL-1 therapy. Overall, these first-in-human results demonstrate preliminary safety and bioactivity of dual-targeted CAR T cells in GBM [9]. However, the findings also underscore the need for further optimization to achieve durable responses through improved CAR T-cell durability.

Table 1 Summary of key locoregional CAR T-cell trials in rGBM, including author and publishing year, NCT ID, target antigens and CAR construct, number of participants, delivery routes and delivery regimen, and the main toxicities, clinical response, and CAR T-cell persistence

Study trial ID	Target and CAR design	Route and regimen	*N*	Key toxicity	Clinical activity	CAR T-cell persistence
Brown et al. [8], 2024 NCT2208362	IL-13(EQ)BBζ/CD19t+ CAR T c ells	ICT, ICV, or dual ICT/ICV; repeated weekly infusions; up to 200×10^6/cycle	65 treated; 58 evaluable	No DLT; grade 3 encephalopathy and ataxia; transient grade 4 cerebral edema in 2 patients	SD/better in 29/58; PR $n = 2$, CR $n = 1$ on-protocol; median OS in rGBM 7.7 months	CAR T cells detected in CSF in most sampled patients early after infusion; peripheral detection increased with dual delivery
Bagley et al. [9], 2024 NCT05168423	Bivalent EGFR806/IL-13Rα2 CAR T cells	ICV; dose escalation; MTD 2×10^7 cells	18; 6 at each DL; 13 with measurable disease on pre CAR T-cell MRI scan	One DLT (grade 3 anorexia, generalized muscle weakness, fatigue over 7 days) at MTD; Neurotoxicity in all patients (grade 3 in 10/18)	Tumor regression in 8/13 measurable patients; confirmed PR $n = 1$, SD $n = 8$, PD $n = 4$; ORR = 6%; OS data not mature yet	Dose-dependent CSF expansion by qPCR; CAR transgene detected in blood at all DLs; persistence up to 12 months in CSF/blood and 5 months in CSF in responding/stable patients
Choi et al. [4], 2024 NCT05660369	EGFRvIII CAR secreting EGFR-directed TEAM molecule	ICV; 10×10^6 cells; one patient received a second infusion	3	No DLT; grade 3 encephalopathy $n = 1$; grade 3 fatigue $n = 1$	Rapid radiographic regression $n = 3$; durable response ongoing in 1 patient at reporting	CAR T cells persistence in CSF up to week 4, in peripheral blood between 2 and 3 weeks after infusion
Barish et al. [28], 2025 NCT04214392	CLTX-CAR T cells	ICT; three weekly infusions of 4, 20 and 20×10^6 cells; additional infusions (4+) with up to 20×10^6 cells allowed after DLT monitoring after third infusion	4	No DLT; no CNS events of grade ≥3 attributed as probable/definite to CLTX CAR T cells; no CRS	Transient SD after 3 cycles $n = 3$, overall DCR of 75%; survival 6 months $n = 3$; survival >20 months $n = 1$	CAR T cells detected in TCF and peripheral blood in all patients

CAR chimeric antigen receptor, *CLTX* chlorotoxin, *CNS* central nervous system, *CR* complete response, *CRS* cytokine release syndrome, *CSF* cerebrospinal fluid, *DCR* disease control rate, *DL* dose level, *DLT* dose-limiting toxicity, *EGFRvIII* epidermal growth factor receptor variant III, *ICT* intracavitary, *ICV* intraventricular, *IL* interleukin, *MRI* magnetic resonance imaging, *MTD* maximum tolerated dose, *ORR* overall response rate, *OS* overall survival, *PD* progressive disease, *PR* partial response, *qPCR* quantitative polymerase chain reaction, *rGBM* recurrent glioblastoma, *TEAM* T-cell-engaging antibody molecule, *SD* stable disease, *TCF* tumor cavity fluid

6.4 B7-H3

B7-H3 (CD276) is an immune checkpoint molecule highly expressed on GBM cells with minimal expression in normal brain tissue [33], making it a compelling CAR T-cell target. A first-in-human phase I trial [7] evaluated intracranial B7-H3 CAR T-cell therapy in adults with rGBM following tumor resection. This study employed a dual-delivery approach using an intratumoral catheter in the resection cavity and in the ICV Ommaya reservoir, with monthly split infusions for up to six cycles [7]. Treatment was technically feasible and generally well tolerated [7]. One DLT occurred at 25×10^6 CAR T-cells, which subsequently defined the recommended phase II dose per route [7]. The main toxicity was tumor inflammation-associated neurotoxicity (TIAN) [34], which was reversible and manageable with anti-inflammatory treatment [7]. No grade 4 toxicities were reported [7]. Although objective radiographic responses were limited, disease stabilization was observed, and median OS reached 14.6 months, suggesting early efficacy [7].

B7-H3 CAR T-cell therapy has also been explored in pediatric populations [6]. A phase I trial as Seattle Children's Hospital (BrainChild-03) investigated repetitive ICV B7-H3 CAR T-cell infusions in children and young adults with diffuse intrinsic pontine glioma [6]. The study demonstrated the feasibility of prolonged multi-dose administration, with acceptable safety and only one DLT [6]. While median OS was similar to historical controls, several patients experienced prolonged disease control, including rare long-term survivors [6]. Together, these data support the safety of repeated CSF-based B7-H3 CAR T-cell delivery and inform future adult GBM trials.

6.5 Chlorotoxin (CLTX)-Directed CAR T Cells

Barish et al. [28] report the first-in-human trial of chlorotoxin (CLTX)-targeted CAR T-cells delivered directly into the tumor cavity of patients with rGBM. They demonstrate the feasibility and safety of ICT CAR T-cell infusions with evidence of CAR T-cell persistence and disease stabilization in most of their patients. [28]

The novel CAR construct was designed to use the scorpion-toxin peptide CLTX to target GBM heterogeneity via surface MMP-2 [28]. The construct comprises CLTX fused to an immunoglobulin G4 (EQ) hinge and transmembrane domain, CD28 co-stimulatory and CD3ζ signaling domains, delivered by lentiviral transduction [28]. In this phase I dose-escalation study, four adults with recurrent, MMP-2-positive GBM received locoregional infusions (ICT) of CLTX-CAR T cells over up to 3-weekly cycles via implanted catheters [28]. Treatment was well tolerated with no DLT and no grade ≥3 grade CRS or neurotoxicity [28]. In terms of efficacy, three of four patients (75%) achieved stable disease as the best response. No partial or complete responses were observed [28]. Translational assays detected CLTX-CAR T-cells in the tumor resection cavity fluid (and at lower levels in the peripheral blood), and no anti-CAR antibodies were found. Cytokine levels and post-treatment antigen expression were not reported [28].

7 Lessons Learned from Early Locoregional CAR T-Cell Trials in Recurrent GBM (rGBM)

Early locoregional CAR T-cell trials in rGBM have provided practical lessons, even though the current evidence base remains predominantly early phase and hypothesis generating [4, 6–9, 27]. Taken together, these studies showed that regional CNS delivery is feasible across multiple platforms and can produce rapid unequivocal biologic activity within the tumor-bearing compartment [4, 6–9, 27]. However, these trials also show that the feasibility and biological activity of CAR T-cells should not be conflated with established efficacy [4, 6–9, 27]. The evidence base is still very heterogeneous: Choi et al. [4] reported results of a three-patient safety study in recurrent EGFRvIII-positive GBM, Bagley et al. [10] reported interim data from the first six patients with multifocal rGBM, Brown et al. [8] studied recurrent high-grade glioma more broadly rather than rGBM alone, whereas Vitanza et al. [6, 27] studied pediatric and young adult diffuse intrinsic pontine glioma rather than adult rGBM.

A first major lesson is that delivery route should be aligned with tumor anatomy and the intended field of exposure. Brown et al. [8] effectively turned route selection into a biological question, progressing from intratumoral administration to ICV delivery and then to dual ICT/ICV administration to capture the complementary strengths of each route. Their rationale was explicit: ICV delivery appeared to be advantageous for multifocal disease, whereas ICT delivery remained valuable for dominant unifocal tumors. Notably, the dual route arm with Tn/mem-derived CAR T products became the preferred platform for subsequent development. The same principle is visible in other studies. Choi et al. [4] used an ICV infusion through an Ommaya reservoir, Bagley et al. [9] delivered bivalent CAR T-cells into the CSF compartment through a ventricular reservoir in patients with multifocal disease, and Li et al. [7] combined ICT and ICV Ommaya delivery after repeat resection. Collectively, data derived from these studies argue that "locoregional" therapy should no longer be treated as a single design category in rGBM, but route of delivery should be regarded as a mechanistic variable with likely efficacy implications.

A second lesson is that repeated regional dosing appears central to durability. Choi et al. [4] showed that a single

ICV infusion can induce relevant tumor regression within days, but responses in two of three patients were transient, prompting the authors themselves to suggest that future durability might require chemotherapy preconditioning or scheduled repeat infusions. Similarly, Bagley et al. [9] observed early radiographic response in all six patients, yet none met criteria for objective response in the interim analysis. By contrast, Brown et al. [8] delivered repetitive weekly cycles with additional treatment allowed and Li et al. [7] designed monthly dosing for up to 6 months. The emerging pattern is therefore consistent: single infusions can demonstrate proof of principle, but repeated exposure may be necessary if locoregional CAR T-cell therapy should move from a biologic signal to clinically durable tumor control in rGBM.

Overall, the safety experience across these trials suggests that the dominant acute hazard of locoregional CAR T-cell therapy is not classic high-grade systemic CRS, but rather local inflammatory and neuroinflammatory toxicity. [4, 6–9, 27] Choi et al. [4] reported no DLTs and no toxicity above grade 3, although grade 3 encephalopathy and grade 3 fatigue occurred. Brown et al. [8] similarly reported no DLTs across five trial arms, with only isolated probable grade 3 encephalopathy and ataxia. In contrast, Bagley et al. [9] observed early-onset neurotoxicity most consistent with ICANS in both tested dose levels, and one patient with a DLT in dose level 2 (grade 3 anorexia, generalized muscle weakness and fatigue). Moreover, Li et al. [7] described TIAN as common but manageable and reversible. Taken together, these findings support proactive neuroinflammation-specific management algorithms, including corticosteroids and anakinra where appropriate, as a core part of future CAR T-cell trial designs in rGBM.

A fourth lesson concerns how efficacy should be interpreted. These studies demonstrated antitumor activity, but they also caution against overreliance on headline radiographic impressions. [4, 6–9, 27] Choi et al. [4] demonstrated dramatic rapid regressions, but limited persistence in two patients. Bagley et al. [9] documented universal early imaging improvement without a confirmed objective response rate. Brown et al. [8] observed at least stable disease in half of evaluable patients and in 22% of evaluable patients for at least 90 days, but importantly the best objective responses occurred in IDH-mutant grade 3 tumors, which limits direct translation to typical adult IDH-wild-type rGBM. Long-term results of the Li et al. [7] trial are awaited. Accordingly, the current literature supports cautious wording. Locoregional CAR T-cell therapy has demonstrated biological activity, disease control in selected patients, and encouraging signals that warrant further investigation, but its clinical efficacy in rGBM remains to be established.

Finally, these trials highlight the importance of product design, manufacturing phenotype, and a compartment-specific biomarker analysis [4, 6–9, 27]. Choi et al. [4] addressed antigen heterogeneity with a platform that combined EGFRvIII-directed CAR with secretion of a wild-type EGFR-targeting T-cell-engaging antibody molecule, and they documented post-treatment EGFRvIII loss despite retained EGFR amplification, which is a vivid example of why serial molecular monitoring matters. Brown et al. [8] showed that a Tn/mem-enriched manufacturing platform improved feasibility and the product phenotype relative to Tcm-derived products, and that CSF IFNγ/CXCL9/CXCL10 induction as well as pretreatment intratumoral CD3 levels were associated with better outcomes. Bagley et al. [9] and Vitanza et al. [6] further reinforce that the CSF compartment is highly informative for detecting CAR exposure and cytokine dynamics, while peripheral blood may underrepresent intrathecal/ICV activity. The role of lymphodepletion remains unresolved in this literature. Vitanza et al. [6] explicitly delivered repeated ICV therapy without prior lymphodepletion, whereas the other cited reports do not clearly define lymphodepletion in the published source, and Choi et al. [4] specifically identify chemotherapy preconditioning as a future durability strategy rather than an established element of treatment. Overall, future rGBM studies should prioritize disease-matched regional delivery, repeated dosing, multiparametric biofluid and biomaterial correlatives, and product optimization aimed at persistence and resistance migration.

8 Surgical Aspects of Locoregional CAR T-Cell Therapy

The direct delivery of CAR T-cells into the CNS is usually performed by, or in close collaboration with, neurosurgeons. Key considerations include the catheter route of delivery, target sites, placement techniques, choice of reservoir systems, and management of device-related complications.

8.1 Catheter Placement and Reservoir Systems

In most locoregional CAR T-cell trials, the neurosurgeon implants one or more catheters connected to a reservoir for percutaneous access. Two main systems are used: the Ommaya and Rickham reservoirs. These dome-shaped ports are placed under the scalp and typically attached to a ventricular catheter. Ommaya reservoirs have been used for ICV and intratumoral CAR T-cell infusions in trials targeting recurrent high-grade gliomas.

Reservoir placement is a well-established neurosurgical procedure, with the ventricular catheter usually inserted into

the frontal horn of the lateral ventricle and secured to the skull. For intratumoral or ICT delivery, catheters can be placed during a tumor biopsy or resection. In the trial by Vitanza et al. [6], catheters were left in the resection cavity for monthly CAR T-cell injections. Brown et al. [8] demonstrated that stereotactic catheter placement is feasible only when a tumor biopsy is performed.

8.2 Dual-Access Setups

Concurrent use of intratumoral and ICV catheters requires careful surgical planning. Typically, the intratumoral catheter is placed during tumor resection, while the ventricular catheter may be implanted during the same operation or as a staged procedure. In the trial by Li et al. [7], both reservoirs were placed in a single surgery with doses split between routes, whereas Brown et al. [8] used ventricular access upfront and added intratumoral catheters selectively. Dual access provides flexibility, enabling cavitary delivery for localized disease and ventricular administration for diffuse involvement. Differences in pressure dynamics between closed cavities and the ventricular system necessitate slow low-volume infusions, which have been well tolerated in clinical trials. [4, 8, 27] Ongoing catheter patency and positioning must be carefully monitored to ensure safe and reliable CAR T-cell delivery.

9 Safety, Tolerability, and Toxicities

9.1 Cytokine Release Syndrome (CRS)

Cytokine release syndrome is a systemic inflammatory response caused by rapid immune effector cell activation and excessive cytokine release. It is the most common systemic toxicity of CAR T-cell therapy. Key mediators include IL-6, IFNγ, tumor necrosis factor, IL-2, IL-2-receptor-α, IL-8, and IL-10. Clinically, CRS presents with fever, hypotension, tachycardia, and hypoxia, and can be fatal in severe cases. Laboratory findings often include elevated C-reactive protein and ferritin. Severity is graded according to the ASTCT 2019 consensus criteria [35], in which Grade 1 is defined by fever only (body temperature 38 °C or higher), and Grade 4 by life-threatening hypotension requiring multiple vasopressors and/or hypoxia requiring mechanical ventilation [36].

Management follows established protocols using supportive care, tocilizumab, and corticosteroids. In contrast to systemic CAR T-cell therapy, locoregional CAR T-cell delivery for rGBM rarely causes clinically significant CRS despite robust local cytokine responses. Large trials reported CSF cytokine peaks without dose-limiting systemic CRS, with most adverse events being low grade and manageable [4, 8, 9]. Some studies observed prominent local CAR T-cell expansion and CSF cytokine release, manifesting primarily as neurotoxicity rather than classic systemic CRS [4, 8, 9].

9.2 Tumor Inflammation-Associated Neurotoxicity (TIAN)

Tumor inflammation-associated neurotoxicity is a unique localized inflammatory response occurring near the tumor site after locoregional CAR T-cell infusion. It results from localized immune activation, vascular permeability changes through endothelial activation, and resultant edema in and around the tumor site due to BBB disruption [34]. Tumor inflammation-associated neurotoxicity symptoms may reflect neuronal dysfunction due to local inflammatory effects, and/or effects of transient local inflammation-induced brain edema (in contrast to generalized and diffuse cerebral edema as observed in severe ICANS) [34]. Tumor inflammation-associated neurotoxicity-related edema can cause tissue shifts resulting in CSF flow obstruction, increased intracranial pressure, and may even cause a herniation syndrome. Patients may consequently develop headache, focal neurological deficits, or decreased consciousness within days of treatment, often mimicking tumor progression on imaging [34]. Magnetic resonance imaging typically reveals peritumoral enhancement or edema without clear systemic cytokine elevation [37, 38]. However, cerebral edema is not necessarily present during TIAN, as primary local neural dysfunction can occur owing to the powerful direct effects of neural-immune interactions and inflammatory signaling molecules on neural cell function [39]. Tumor inflammation-associated neurotoxicity is therefore considered secondary to tumor inflammation and on-target effects within the CNS and should be distinguished from other immunotherapy-associated neurotoxicities [34].

Management consists of corticosteroids, osmotic therapy if raised intracranial pressure is suspected, and temporary interruption of CAR T-cell infusion. In severe cases, CSF drainage through the implanted reservoir may be necessary [34].

9.3 Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)

Immune effector cell-associated neurotoxicity syndrome is a more generalized neurotoxicity resulting from systemic cytokine exposure-end consequent endothelial activation, leading to disruption of the BBB [18, 40]. While more common in CAR T-cell therapy for hematologic malignancies, ICANS can occur in patients with GBN,

particularly when cytokine levels rise after repeated infusions or in cases of BBB breakdown [18, 40]. Immune effector cell-associated neurotoxicity syndrome typically manifests as a toxic encephalopathy, starting with mild symptoms such as word-finding difficulty or confusion [18, 40]. In more severe cases, it can progress to aphasia, impaired fine motor skills, somnolence, seizures, and even coma [18, 40]. Notably, most patients who develop ICANS have preceding CRS, which is often considered an "initiating event" or cofactor [40]. The severity of ICANS is graded using the Immune Effector Cell Encephalopathy score [35]. Distinguishing ICANS from TIAN is clinically important: ICANS presents with diffuse encephalopathy and systemic inflammatory markers, whereas TIAN manifests as focal symptoms localized to the infusion/tumor site [34]. Treatment of ICANS includes corticosteroids, supportive care, and seizure prophylaxis, while tocilizumab is typically ineffective as IL-6 blockade does not fully address neuroinflammation [4, 18, 41].

10 Future Directions for Locoregional CAR T-Cell Therapy in rGBM

Early locoregional CAR T-cell trials in rGBM have established the feasibility of repeated intracranial administration and provided evidence of local immunologic bioactivity and occasional radiographic responses [4, 6–9, 27]. However, durable clinical benefit remains limited by antigen heterogeneity and escape, insufficient CAR T-cell persistence, and the profoundly immunosuppressive TME [4, 6–9, 24, 27, 42]. Future development should therefore focus on improving target coverage, enhancing functional persistence, optimizing delivery and dosing strategies, and integrating biologically informed combinations strategies.

10.1 Overcoming Antigen Heterogeneity and Improving CAR T-Cell Fitness

Given the spatial and temporal heterogeneity of antigen expression and the risk of antigen loss at relapse, single-antigen targeting is unlikely to be sufficient in most patients with rGBM [43, 44]. Multi-antigen approaches, including bispecific, tandem, or logic-gated CAR constructs, may broaden tumor coverage while reducing selective pressure on a single target [4, 9, 45, 46]. In parallel, armored CAR T cells designed to counteract local immunosuppression, for example through cytokine secretion, resistance to inhibitory signaling, or modulation of myeloid-mediated immunosuppression, represent a rational strategy to improve activity within the GBM TME [47]. Further optimization of CAR architecture, co-stimulatory domains, and T-cell differentiation state may also improve persistence and reduce functional exhaustion [17].

10.2 Optimizing CAR Design and Targeting, Manufacturing, Logistics, and Regulatory Processes

Clinical experience confirms that targeting a single antigen often yields transient tumor regression followed by relapse due to antigen-negative tumor variants. Future CARs should therefore address intratumoral heterogeneity through multi-specific or bispecific constructs [8, 9]. Early efforts are underway, demonstrating feasibility of multi-targeting CAR T-cells [8, 9]. Preclinical work also suggests benefits of trivalent or sequential CAR strategies to minimize escape [48]. In addition, armored CARs are engineered to resist immunosuppression or to express co-stimulatory cytokines and ligands, which may enhance potency [49–51]. Co-stimulatory domains (CD28, 4-1BB) and transmembrane regions can affect CAR T-cell persistence and exhaustion [50, 51]. Next-generation constructs incorporating 4-1BB or ICOS co-stimulation, or optimized single-chain IL-13 ligands for IL-13Rα2 should be further explored [49–51].

Clinical experience with allogeneic universal CAR T-cell products remains very limited in rGBM [52]. Autologous CAR T-cell therapy is constrained by individualized manufacturing requirements and potentially impaired T-cell fitness in heavily pretreated patients, providing a rationale for optimized manufacturing and allogeneic off-the-shelf platforms [4, 6–9, 27]. Consequently, developing universal CAR T-cell banks with HLA-knockout and immune-evasive features (HLA-E, CD47) represents a priority [53]. In a first-in-human phase I study of intrathecally administered, CRISPR-edited IL-13Rα2-targeted universal CAR T cells in recurrent high-grade glioma, disruption of the endogenous T-cell receptor was intended to reduce the risk of graft-versus-host disease, whereas HLA class I disrupt was designed to mitigate host-versus-graft rejection [52]. No graft-versus-host disease was observed in the five treated patients, and the off-the-shelf product permitted repeated intrathecal administration [52]. However, natural killer cell-mediated rejection remained a potential limitation. These preliminary findings support further development of universal CAR T-cell platforms as a strategy to shorten manufacturing time, overcome limitations of patient-derived T-cell products, and facilitate repeat dosing, but their clinical benefit and durability in rGBM require more information. [52] Given the complexity, these trials will be limited to specialized centers only. Early-phase studies should partner with neuro-oncology consortia to share protocols and harmonize data. Moreover, regulatory agencies should consider accelerated pathways for promising multi-center trials.

10.3 Delivery Route and Dosing Strategies

Locoregional delivery is likely to remain central to the further development of CAR T-cell trials for rGBM, as it bypasses the BBB and enables repeated administration directly into CNS compartments [4, 6–9, 27]. Intracavitary delivery may be most appropriate for dominant local tumor recurrences, whereas ICV administration may improve access to multifocal, distant, or CSF-disseminated disease [4, 6–9, 27]. The latter concept is supported by early clinical observations, including rapid radiographic responses after ICV infusions and regression of multifocal intracranial disease following repeated ICV treatment [4, 8]. Notably, comparative evidence is missing and therefore proven superiority of one route is still lacking.

Repeated dosing through implanted reservoirs is particularly attractive, as transient responses and limited CAR T-cell persistence remain major challenges. In the phase I study by Brown et al. [8], repeated locoregional administration (ICT, ICV, dual route) was feasible, and the dual-route cohort showed evidence of CNS immune activation, supporting further investigation of combined local and CSF-directed treatment. However, route-specific benefits cannot be separated from differences in CAR T-cell manufacturing and treatment cohorts. Moreover, dose escalation appears product dependent. While no DLT was reported in the trial of Choi et al. [4] (10×10^6 cells), clinically relevant toxicities (one DLT, neurotoxicity in all patients) occurred in the trial of Bagley et al. [9] using subsequent ICV administration (2×10^7 cells), emphasizing the need for careful neurological monitoring and product-specific escalation strategies.

Although a systematic review reported a lower rate of grade ≥3 adverse events (over 60% reduction) with locoregional CAR T-cell delivery compared with peripheral infusion, this finding is based on indirect comparisons across heterogeneous early-phase trials and should not be interpreted as definitive evidence of reduced neurotoxicity [54]. Future studies should therefore prioritize repeated and potentially dual-route administration, integrate serial CSF pharmacokinetic and immune-biomarker analyses, and prospectively evaluate the optimal balance between local tumor exposure, broad CNS coverage, and treatment-related neurotoxicity.

10.4 Patient Selection and Biomarkers

Correlative biomarkers studies may be essential for the identification of patients who most likely benefit from CAR T-cell therapy. Brown et al. [8] found higher intratumoral CD3+ T-cell density correlated with better survival after CAR T-cell therapy, suggesting that CAR T cells may work best in an already inflamed milieu. In recent locoregional CAR T-cell trials, treatment was associated with increased CNS inflammatory cytokines and chemokines, which were therefore associated with bioactivity [4, 6–9, 28]. Consequently, real-time monitoring using CSF analysis for CAR T-cell persistence and cytokine/chemokine profiling is invaluable. Future studies should include correlative endpoints to link immunologic changes with clinical and radiological outcomes. At present, IDH status, MGMT promoter methylation, and tumor mutational burden should be described as candidate stratification biomarkers rather than established predictors of CAR T-cell response because validated clinical evidence remains limited.

10.5 Combinatorial Strategies to Overcome the Immunosuppressive Tumor Microenvironment (TME)

Although locoregional delivery improves access of CAR T-cells to the CNS, it does not by itself overcome the immunosuppressive GBM TME [4, 6–9, 27]. Future strategies should therefore combine locoregionally delivered CAR T-cells with additional interventions that preserve T-cell function, increase tumor immunogenicity, or reprogram suppressive immune cells in the TME [49]. Notably, evidence for these combinations remain predominantly preclinical, and additive clinical benefit has not yet been demonstrated in patients with rGBM [10, 55, 56]. Immune checkpoint inhibition represents one rational combination strategy. Following peripheral EGFRvIII-directed CAR T-cell therapy in rGBM, post-treatment tumor specimens demonstrated antigen loss and adaptive immunosuppressive changes, supporting the hypothesis that checkpoint pathways may limit CAR T-cell activity [2]. In preclinical glioma models, checkpoint blockade improved the antitumor activity of IL-13Rα2-targeted CAR T-cells [55]. However, translation into clinical benefit has remained challenging. A phase I study of repeated peripheral EGFRvIII CAR T-cell infusions combined with pembrolizumab showed that treatment was feasible but did not demonstrate an efficacy signal [10]. Thus, checkpoint inhibitor combinations should currently be viewed as biologically plausible but clinically unproven.

Radiotherapy may provide a complementary immune-conditioning strategy for CAR T-cell therapy. Beyond cytoreduction, radiation can promote inflammatory signaling, antigen release, and immune cell recruitment within the TME [57]. In preclinical GBM models, radiotherapy enhanced the activity of NKG2D-based CAR T cells [58]. More recently, in an immunocompetent orthotopic glioma model, stereotactic radiation followed by intratumoral IL-13Rα2-targeted CAR T-cell administration increased antitumor activity and generated improved memory responses upon rechallenge [59]. These findings suggest that carefully timed focal radiation may enhance

both local CAR T-cell activity and endogenous immune responses against antigen-negative tumor compartments. Nevertheless, the optimal radiation dose, sequencing, and potential risks of treatment-related cerebral inflammation remain to be defined in a clinical context.

Moreover, myeloid targeting approaches are particularly relevant in GBM, where glioma-associated microglia and macrophages constitute a major component of the TME [49]. A recent preclinical study engineered EGFRvIII-directed CAR T-cells to secrete a SIRPγ-derived CD47 blocker, thereby combining tumor specific T-cell cytotoxicity with local disruption of the CD47-SIRPα axis, which represents a myeloid immune checkpoint [47]. Following locoregional injection, these armored CARs eradicated orthotopic EGFRvIII-mosaic GBM xenografts, enhanced glioma-associated myeloid cell-mediated phagocytosis, and promoted elimination of bystander tumor cells [47]. However, potential neuroinflammation and hematological toxicities of local CD47-axis modulation require careful evaluation in early-phase clinical trials [47].

11 Conclusions

Locoregional CAR T-cell therapy represents a paradigm shift in the treatment of rGBM, directly integrating neuro-oncology expertise into precision immune oncology. Early-phase clinical trials demonstrate that direct intracranial or CSF-based CAR T-cell delivery is feasible and generally safe, with evidence of biological activity and occasional radiographic responses while minimizing systemic CRS. Nevertheless, major challenges persist, including the immunosuppressive TME, antigen heterogeneity, and limited CAR T-cell persistence.

For neuro-oncology teams, successful implementation requires a new therapeutic framework that includes accurate catheter placement, stereotactic delivery, familiarity with intrathecal infusion strategies, and close interdisciplinary collaboration for toxicity management. Clinicians will play a key role in longitudinal patient care, including acquisition of biomaterials at re-resection to study mechanisms of response and resistance, and interpretation of evolving neuroimaging criteria specific to CAR T-cell treated patients. As ongoing trials refine delivery routes, multi-antigen targeting, and repeat dosing strategies, locoregional CAR T-cell therapy may become an integral component of multimodal GBM treatment, bridging neuro-oncologic care with cellular immunotherapy to improve patients' outcomes.

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