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Chimeric antigen receptor (CAR) T-cell therapy for glioblastoma (GBM): current clinical insights, challenges, and future directions

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ABSTRACT

Glioblastoma (GBM) remains the most lethal primary brain cancer with a median survival of under 2 years despite current best treatment practices. Early immunotherapies, including checkpoint blockade and vaccines, showed safety and immunogenicity but no survival benefit. Chimeric antigen receptor (CAR) T treatments in GBM trials have yielded feasibility and antitumor signals but still lack long-term control. This review synthesizes recent clinical and mechanistic data to establish priorities for clinical trial design, patient selection, and treatment development aimed at achieving durable responses in GBM.

Recent trials highlight two consistent observations regarding the delivery of CAR T treatment. First, that CAR T cells can be effectively delivered peripherally rather than requiring direct intracranial administration. And second, multi-antigen, regionally delivered products can induce measurable intracranial responses. These findings indicate that access across the blood-brain barrier is feasible, but persistent function is limited by tumor antigen heterogeneity and an immunosuppressive, myeloid-dominated microenvironment that accelerates T-cell exhaustion.

Emerging development strategies reflect these constraints. Broader antigen recognition is being pursued through bivalent and engager-secreting constructs. Locoregional delivery through cerebrospinal fluid spaces enables repeated exposure at multifocal sites. Resistance modules targeting TGF-β (Transforming Growth Factor-beta) signaling and myeloid suppression are being investigated to prolong persistence. Cerebrospinal fluid pharmacodynamic monitoring, such as measuring cytokines, chemokines, and CAR cell kinetics, may support adaptive dosing and minimize corticosteroid use. Patient selection criteria increasingly favor individuals with confirmed target expression, sufficient intratumoral T-cell infiltration, and minimal steroid exposure.

Advances in manufacturing, including point-of-care platforms, allogeneic products, and in vivo CAR engineering, aim to shorten production timelines and improve access. Collectively, regional delivery, multi-antigen recognition, and microenvironment resistance constitute the current framework for translating CAR T therapy in GBM from transient responses toward sustained benefit.

INTRODUCTION

Glioblastoma (GBM) remains the most lethal primary brain cancer, with a median survival of under 2 years despite maximal resection and chemoradiation.[1] Experiments and trials with immunotherapy approaches in GBM, including immune checkpoint inhibitors and vaccine strategies, have demonstrated safety and immunogenicity but have failed to show survival benefit.[2 3] Chimeric antigen receptor (CAR) T cells offer targeted cytotoxicity by engineering autologous T cells to recognize GBM antigens, a strategy that achieves up to 90% remission in CD19-positive hematologic cancers.[4] Early CAR T GBM trials show feasibility, safety, and measurable antitumor activity, but also have not significantly improved survival, underscoring the rationale to further develop and refine CAR T therapy for this disease.[5 6]

Over the last 2 years, the conceptual framework of CAR T-cell therapy for GBM has advanced rapidly with reproducible intracranial signals in adult GBM, innovations in antigen targeting, delivery route, and tumor microenvironment (TME) modulation (figure 1). Recent trials have established two key principles: (1) CAR T cells can be effectively delivered peripherally rather than requiring direct intracranial administration, and (2) multi-antigen, regionally delivered products can induce measurable intracranial responses. However, these innovations have yet to comprehensively overcome GBM's intratumoral heterogeneity and immune-exclusionary microenvironment. This review synthesizes recent clinical and mechanistic data to identify design recommendations, patient selection strategies,

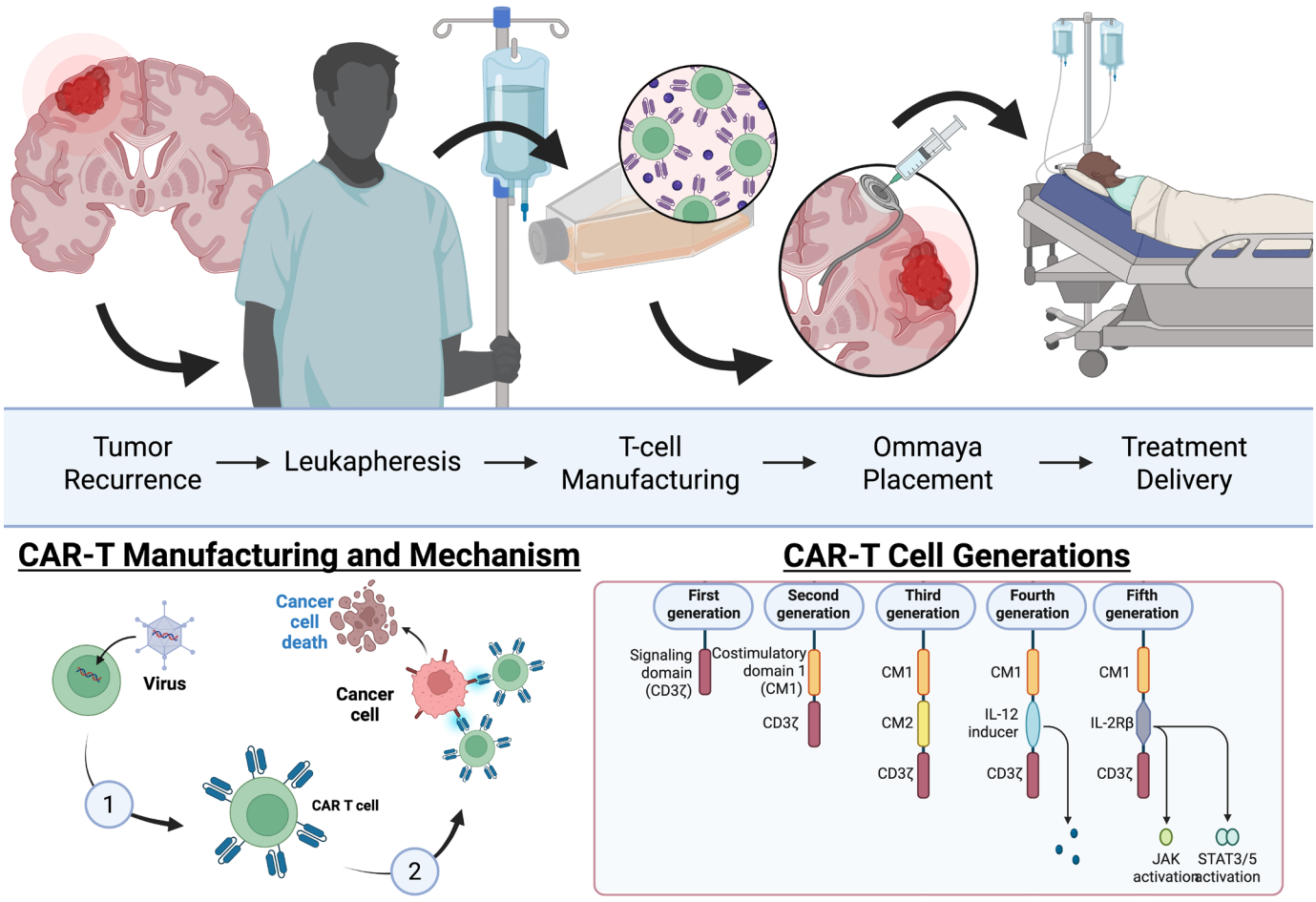

Figure 1 Overview of CAR T therapy for glioblastoma, from tumor diagnosis through leukapheresis, ex vivo CAR T manufacturing, Ommaya reservoir placement, and intrathecal delivery. Below, CAR design and mechanism—viral transduction endows T cells with tumor antigen recognition, while successive generations add co-stimulatory domains, cytokine modules, safety switches, and cytokine-receptor modules that engage JAK-STAT pathways. CAR, chimeric antigen receptor; IL, interleukin; JAK-STAT, Janus kinases, Signal Transducer and Activator of Transcription.

and trial design priorities for moving CAR T therapy from transient responses toward sustained benefit in GBM.[4 7–9]

ANTIGEN-SPECIFIC CAR T APPROACHES IN GBM

EGFRvIII and EGFR: monovalent versus engager or dual-target designs

Evidence summary

The therapeutic development of CAR T-cell therapy for GBM presents a dichotomy between delivery modalities and clinical efficacy. Systemic administration of Epidermal Growth Factor Receptor variant III (EGFRvIII) CAR T cells via intravenous injection demonstrates blood-brain barrier (BBB) penetration and tumor engagement. But clinical benefit remains negligible, a failure attributable to rapid antigen escape mechanisms and adaptive resistance pathways. The addition of anti-programmed cell death protein-1 (PD-1) checkpoint blockade fails to overcome these limitations in newly diagnosed EGFRvIII-positive GBM (NCT03726515),[10] suggesting that peripheral immunomodulation cannot address the core challenge of intracranial antigen heterogeneity. Regional delivery strategies induce a change in bioactivity profiles. Intraventricular administration of CARv3-TEAM-E (T cell Engager Antibody Molecule-EGFR), which broadens target recognition through secretion of an EGFR-binding engager, produces rapid radiographic regressions without dose-limiting toxicity, though responses remain transient (NCT05660369).[11] Key evidence emerges from bivalent intracerebroventricular CAR therapy targeting both EGFR epitope 806 and IL13Rα2, which achieves radiographic regression in 62% of patients with measurable disease, including one confirmed partial response and one patient with stable disease extending beyond 16 months (NCT05168423).[3] The progression-free survival was 1.9 months, and toxicity profiles revealed grade 3 neurologic events in 56% of patients, with the absence of grade 4 or 5 events suggesting a manageable safety threshold.[3]

As such, single-antigen systemic therapy proves insufficient. At the same time, multi-antigen recognition coupled with cerebrospinal fluid-space delivery

generates reproducible intracranial bioactivity, although with transient durability that underscores the need for further mechanistic refinement.[3 10 11]

Mechanistic interpretation

Failure after intravenous EGFRvIII CAR reflects two forces. First, spatial heterogeneity and rapid downregulation of EGFRvIII under immune pressure drive antigen escape. Second, a myeloid-dominant, TGF-β (Transforming Growth Factor-beta)–rich niche that accelerates exhaustion and limits persistence. TEAM-E partially offsets heterogeneity by recruiting bystander T cells to wild-type EGFR. The bivalent 806 epitope extends EGFR coverage while sparing normal ligand-bound conformations. Neither approach alone solves persistence. The limiting step is sustained function within the TME, not access or initial cytolysis.[3 6 8 9 12–14]

Safety profile

Neuroinflammatory toxicities predominate negative outcomes, reflecting intracranial cytokine activity. Regional EGFR axis products show frequent grade 1–3 neurologic events that are manageable with standardized support. TEAM-E shows no dose-limiting toxicity in first-in-human use. The bivalent intracerebroventricular (ICV) program defines a window with common grade 3 events and no grade 4–5 events. These boundaries should guide dose, frequency, and escalation rules.[8 9 12]

Implications for product design

Three axes of development stand out from EGFRvIII trials: (1) broaden antigen recognition beyond EGFRvIII using engager secretion or dual targeting to prevent vIII negative clone outgrowth; (2) use cerebrospinal fluid (CSF)-space delivery on repeatable schedules to maximize multifocal exposure; (3) incorporate resistance modules against myeloid suppression and TGF-β to extend persistence.[3 8 9 12 15 16]

Evidence gaps and next tests

Key gaps include optimal cadence for ICV redosing, thresholds for switching or layering antigens when EGFRvIII signal wanes, and validated persistence biomarkers that predict progression-free survival. Next trials should randomize route or valency when feasible, incorporate paired CSF and tumor sampling, and prespecify steroidsparing and edema protocols to protect efficacy signals.[8–10 12 14 17 18]

IL13Rα2: maturing locoregional evidence

A phase 1 investigation of IL13Rα2-targeted CAR T therapy establishes the foundational parameters for locoregional immunotherapy in GBM. The 65-patient single-center trial (NCT02208362) employed memory-enriched CAR T cells administered through three distinct anatomical routes: intratumoral, intraventricular, or dual-route delivery, using implanted reservoirs and ventricular catheters for iterative dosing cycles without mandatory lymphodepletion.[7]

The safety profile demonstrates exceptional tolerability: zero dose-limiting toxicities emerged across all cohorts, with only two grade 3 neurologic events deemed treatment-related. The benign toxicity spectrum enabled sustained multicycle administration, unlike systemic CAR T therapy, where severe cytokine release syndrome often limits therapeutic intensity.

Clinical efficacy metrics emphasize biological activity, as~50% of patients achieved disease control or superior outcomes by RANO-HGG criteria, including two partial responses and two complete responses, exceptional results for recurrent GBM immunotherapy. The optimized dual-route delivery arm achieved a median overall survival (OS) of 10.2 months versus 7.7 months for the aggregate cohort, suggesting that simultaneous intratumoral and intraventricular administration creates synergistic therapeutic pressure.[7]

Pharmacodynamic surveillance uncovered critical mechanistic insights through cerebrospinal fluid biomarker kinetics. Quantifiable CAR T-cell persistence coincided with dynamic cytokine signatures. Interferon (IFN)-γ exhibited pulsatile elevation synchronized with dosing intervals before inter-cycle attenuation, while CXCL (C-X-C Motif Chemokine Ligand) 9/10 chemokine levels demonstrated direct correlation with radiographic response trajectories. This temporal coupling between immunologic activation and anatomical regression provides molecular validation of the therapeutic mechanism.

These data suggest IL13Rα2 as the most clinically mature target for locoregional CAR T therapy in GBM. The program supports the clinical validity of locoregional cellular immunotherapy, achieving~50% disease control with negligible dose-limiting toxicity through iterative intratumoral/intraventricular delivery. This establishes the molecular framework for next-generation multivalent targeting strategies to overcome the transient response kinetics that remain the field's rate-limiting constraint.

Translational correlates

Pretreatment intratumoral CD3 density correlated with OS and was prespecified as a biomarker, supporting immune-contexture-based selection. Baseline steroid exposure was minimized per-protocol to preserve T-cell fitness. Together, these findings provide actionable criteria for patient enrichment. Adaptive schedules could be guided by pharmacodynamics: intensify dosing when CSF cytokine induction is absent and de-escalate when inflammatory surges occur without radiographic improvement.[7]

Safety profile

Locoregional IL13Rα2 CAR T dosing repeatedly triggered cerebrospinal fluid (CNS) cytokine surges and neuroinflammation. Most events were manageable, but became clinically significant near eloquent cortex. Across trials, steroid-sparing supportive care and aggressive intracranial pressure control were required. However,

repeated intratumoral and ventricular dosing schedules were completed without prohibitive toxicity.[9 12]

Delivery

IL13Rα2 appears most effective when paired with other antigens and supported by armoring. Bicistronic EGFR/IL13Rα2 CARs co-expressing dominant-negative TGF-βRII improve proliferation and in vivo control in xenografts.[15] Bispecific IL13Rα2/TGF-β converters further enhance infiltration and resist suppression.[19] These designs target the observed persistence bottleneck. Trial schemas should prespecify dual intratumoral plus intraventricular access when anatomy allows, include CSF biomarker panels (IFN-γ, CXCL9/10, CAR-quantitative PCR), and define early switch criteria for rapid regrowth or inadequate cytokine induction.

Evidence gaps and next tests

Current studies are single-center, non-randomized, and heavily pretreated populations, which limits generalizability. Next steps include validating pretreatment CD3 thresholds for selection, defining minimal effective CSF exposure per cycle, and testing whether TGF-β–resistant IL13Rα2 products extend persistence in humans. Combinatorial strategies with radiation or myeloid-modulating agents should be evaluated. Future trial designs should incorporate crossover to alternate antigens when IL13Rα2 expression wanes, guided by repeat biopsy or liquid biopsy.[9 11 12 15 19]

HER2

There have been no new peer-reviewed adult GBM CAR T clinical publications focused on HER2 (Human Epidermal Growth Factor Receptor 2) in the past 2 years; pediatric HER2 CAR T experience predates this window and is not summarized here. The gap reflects a lack of recent evidence, not resolved futility. Historical on-target off-tumor risk remains a design concern. Although best known in breast and gastric cancers, immunohistochemistry and gene-expression profiling show that up to~80% of GBM overexpress HER2, a major EGFR dimerization partner; systematic screening linked HER2 levels to outcomes, implicating it in proliferation,[20] and its minimal expression in normal brain parenchyma suggested a therapeutic window. Early trials using virus-specific HER2 CAR T cells were generally tolerable. They showed sustained T-cell persistence with stable disease in~50% of patients. Still, low-level HER2 in organs such as the heart and lungs caused on-target/off-tumor toxicity,[14 20 21] including a fatal high-dose event that highlighted the risk of targeting a receptor overexpressed in GBM yet not entirely tumor specific.[22] HER2 remains a biologically relevant but high-risk GBM target, and future CAR T development will require safety switches, logic-gated or multi-antigen constructs to mitigate off-tumor toxicity while leveraging its high prevalence and role in EGFR signaling.

B7H3: pediatric brainstem disease informs feasibility

In the completed BrainChild03 Arm C phase 1 trial, 21 children and young adults with diffuse intrinsic pontine glioma received repeated ICV B7H3 CAR T cells without lymphodepletion (NCT04185038).[23] Feasibility and tolerability endpoints were met across 253 doses. The maximally tolerated dose regimen escalated to 1×10^8 cells per infusion. Median survival from first CAR T infusion was 10.7 months, and from diagnosis 19.8 months; one partial response was documented, and multiyear dosing was feasible in select patients. However, doselimiting hemorrhage did occur once. Although pediatric and not GBM, these data establish the real-world practicality of repetitive CSF delivery against a GBM relevant antigen highly expressed in adult gliomas.

GD2: sustained regressions in diffuse midline glioma with sequential IV then ICV dosing

A phase 1 study reported outcomes for children and young adults with H3K27M+diffuse midline gliomas using sequential intravenous followed by ICV GD2 (Disialoganglioside 2) CAR T cells (NCT04196413).[24] The trial documented radiographic regressions and neurological improvements in several participants, with clinical activity observed after both intravenous and ICV dosing; the publication emphasizes feasibility of repeated ICV dosing after initial intravenous exposure. The work is not adult GBM, but it demonstrates that repeated CSF space dosing of CAR T cells against a glycolipid antigen can be safe and clinically meaningful in primary CNS malignancies.[21] GD2 is present in subsets of high-grade gliomas; the diffuse midline glioma experience supports that the CNS route generalizes to glycolipid targets in high-grade glioma subsets.

EphA2: on-target toxicity limits clinical development

EphA2 (Ephrin Type-A Receptor 2), a receptor tyrosine kinase implicated in cell proliferation and invasion, is overexpressed in a majority of GBM specimens.[25 26] There are no new peer-reviewed GBM clinical publications for EphA2-directed CAR T cells in the last 2 years. Preclinically, 2024 work with EphA3 targeted CAR T cells in resected human GBM tissue and in vivo models showed specific recognition and coverage of neurotropic tumor zones, suggesting the EphA family remains a viable avenue, but clinical translation for EphA2 in GBM has not been newly reported in this period.[8 27 28] Previous trials of intratumoral EphA2 CAR T cells have shown transient periods of stable disease in patients with recurrent GBM. However, occasional on-target/off-tumor toxicities underscore the challenges of targeting receptors with low-level expression in healthy tissues.[5 11 12 29]

Preclinical targets

CAR T cells directed against NKG2D (Natural Killer Group 2D) ligands are under preclinical evaluation; these ligands are upregulated in response to cellular stress and are commonly expressed on tumor cells.[30] This strategy

leverages the widespread stress-related expression of NKG2D ligands to provide a more comprehensive attack against heterogeneous tumor populations. The integration of CAR T cells with other treatment modalities is an emerging strategy to achieve synergistic antitumor effects. Oncolytic viruses can lyse tumor cells and release neoantigens, thereby enhancing local immune responses that potentiate CAR T-cell activity.[31 32] Similarly, radiotherapy not only upregulates tumor antigen presentation but also transiently disrupts immunosuppressive barriers, offering a window in which CAR T cells can more effectively infiltrate and eradicate tumor cells.[32 33] Metabolic modulators have also shown promise; by inhibiting pathways that impair T-cell function (such as arginase or indoleamine 2,3-dioxygenase), these agents can relieve immunosuppression and enhance CAR T-cell efficacy.[34] Moreover, innovative delivery platforms, such as biodegradable hydrogels implanted into the tumor resection cavity and the use of focused ultrasound to transiently open the BBB, further augment local CAR T-cell concentrations and improve tumor accessibility.[35]

The temporal evolution of CAR T-cell therapy for GBM reveals a recent developmental shift. The pre-2023 foundational period established critical proof-of-concept parameters: demonstrable BBB penetration, preliminary safety profiles across multiple antigenic targets, and initial cytotoxic validation. However, treatments remained constrained by two systematic limitations, exclusive reliance on monotherapy constructs and predominant utilization of systemic delivery paradigms, resulting in uniformly transient responses and negligible survival extension. Recent efforts represent a research shift characterized by four convergent innovations that have redefined therapeutic architecture. First, regional delivery modalities via ICV and intratumoral routes have emerged as the dominant administration strategy, generating reproducible signals with superior pharmacokinetic profiles compared with peripheral infusion. Second, multi-antigen targeting through bivalent CAR constructs and T-cell engager secretion mechanisms directly addresses the intratumoral heterogeneity that previously enabled rapid clonal escape. Third, implementation of pharmacodynamic monitoring protocols, particularly cerebrospinal fluid cytokine kinetics and quantitative CAR T-cell persistence metrics, enables adaptive dosing algorithms that optimize therapeutic pressure while managing neurotoxicity. Fourth, these integrated approaches have yielded median survival metrics that extend beyond historical benchmarks, with optimized dual-route delivery arms achieving higher OS in aggregate cohorts. This combined approach marks a definitive conceptual transition: the field has progressed from validating whether CAR T cells can access intracranial targets to elucidating how to sustain their functional persistence within the immunosuppressive TME (table 1). Current evidence establishes cerebrospinal fluid-space delivery coupled with multi-target antigen coverage as the minimal viable framework on which next-generation optimization must build.

NEXT TRIAL DESIGNS AND ENDPOINTS

Resistance mechanisms are dominated by spatial and temporal antigen heterogeneity, clonal downregulation under immune pressure, and a suppressive myeloid network that drives terminal T-cell exhaustion (figure 2). Human datasets place TGF-β signaling and myeloid antigen presentation at the center of functional attrition. This explains fast on-target cytoreduction with TEAM or bivalent constructs but short progression-free intervals, and why dose escalation alone has not solved durability.[6 13] Across IL13Rα2, dual EGFR/IL13Rα2, B7-H3, and GD2 CAR programs, ICV or combined intratumoral plus intraventricular delivery consistently demonstrates superior bioactivity signals and operational feasibility compared with peripheral infusions, with acceptable neurotoxicity when dose and schedule are optimized.[7 11 12 21 23] Antigen escape and microenvironmental immunosuppression remain dominant resistance mechanisms. While TEAM-secreting and dual-target CARs can achieve rapid tumor debulking, durability is often poor, likely due to adaptive antigen modulation and myeloid-driven T-cell exhaustion programs now characterized in human GBM.[6 11–13]

Delivery methods

CAR T cells have been administered either systemically (intravenous) or via locoregional routes (direct intracranial infusions) in GBM, and each delivery strategy yields distinct outcomes. Early trials of intravenous delivery confirmed that circulating T cells can traffic across the BBB and infiltrate tumors, but they showed minimal clinical efficacy. Tumor antigen loss was observed without sustained responses, and median survival remained on the order of 6–8 months, no better than historical controls.[10] In contrast, recent locoregional approaches, such as delivering CAR T cells into the resection cavity or ventricular system, have produced stronger initial antitumor effects. The regional delivery trials demonstrate that direct CNS administration can achieve rapid tumor debulking that intravenous infusion alone has not, but the remissions have been fleeting in most cases, underscoring that no route has yet overcome GBM's recurrence.

These findings suggest that combining systemic and regional delivery may maximize tumor exposure, but such approaches are still experimental. Ongoing investigations are exploring novel delivery modalities. For example, implanting CAR T cells within bioengineered hydrogels at the resection cavity to prolong their local persistence, or using focused ultrasound to transiently open the BBB and enhance CAR T trafficking into the brain.[36 37] However, it remains undetermined which route or combination will ultimately prove most effective for sustained GBM remission.

Manufacturing and timing

Autologous CAR T-cell therapy for GBM involves a complex "vein-to-vein" manufacturing cycle. T cells must be harvested via leukapheresis, genetically engineered with a CAR construct, expanded to sufficient numbers,

Table 1 This table summarizes the therapeutic landscape of CAR T-cell targets for GBM multiforme and related brain tumors, presenting the biological rationale, historical clinical outcomes (pre-2023), recent clinical advances (2023–2025), and current strategic positioning for each target antigen

Target	Biological rationale	Historical clinical experience (pre-2023)	Recent advances (2023–2025)	Current strategic position
EGFR/ EGFRvIII	► Tumor-specific vIII mutant (~25–30% GBMs) ► Wild-type EGFR amplified in~60% ► Minimal normal brain expression	**IV monotherapy (NCT01454596):** BBB penetration confirmed but rapid antigen escape, limited persistence **Delivery:** primarily intravenous	**CARv3-TEAM-E (NCT05660369):** intraventricular delivery with EGFR engager secretion yields rapid regressions without DLT **Bivalent EGFR-806/IL13Rα2 (NCT05168423):** 62% radiographic regression rate via ICV route; median OS unreached at 8.1 months	Multi-target engagement via engager secretion or bivalent constructs now standard; CSF delivery proven superior to systemic
IL13Rα2	► Overexpressed in >75% GBMs ► Absent in normal brain parenchyma ► High target specificity	**Early experience:** single-patient complete response (NEJM 2016); established locoregional feasibility	**65-patient phase 1 (NCT02208362, 2024):** 50% disease control rate; dual intratumoral/ICV route achieves 10.2 vs 7.7 months OS; CSF cytokine kinetics validated **Combined with EGFR-806:** see bivalent data above	Most clinically mature locoregional target; iterative dosing protocols established; pharmacodynamic biomarkers validated
HER2	► Expressed in~80% GBMs ► EGFR dimerization partner ► Cross-cancer validation	**VST-based trials (NCT01109095):** ~50% stable disease; persistence up to 12 months **Safety concerns:** fatal high-dose event; cardiac/ pulmonary risk	**No new adult GBM publications 2023-2025** Development stalled due to off-tumor toxicity	Requires safety switches or logic-gating; historical risk profile limits monotherapy development
B7-H3	► Overexpressed in GBM ► Minimal normal CNS expression ► Poor prognosis marker	**Limited adult data** Preclinical validation only	**BrainChild-03 Arm C (NCT04185038, 2023):** 21 pediatric patients with DIPG; 253 ICV doses tolerated; median OS 10.7 months from first dose **Preclinical (2024):** nanobody CARs show on-target/off-tumor toxicity in mice	Pediatric feasibility established for repetitive ICV dosing; adult translation pending; sensitivity optimization creates toxicity risk
GD2	► Glycolipid antigen ► H3K27M-mutant DMG expression ► Limited normal tissue	**Pediatric focus** No significant adult GBM data	**DMG trial (NCT04196413, 2025):** sequential IV→ICV dosing produces radiographic regressions and neurological improvement	Demonstrates glycolipid targeting feasibility in CNS; sequential systemic-to-regional delivery paradigm validated
EphA2	► RTK overexpressed in majority GBMs ► Invasion/proliferation driver	**Phase 1 (NCT03423992):** transient stable disease; sporadic toxicities	**No new clinical publications 2023-2025** **Preclinical (2024):** EphA3 CARs show neurotropic zone coverage	Clinical development lagging; EphA family remains viable but untested at scale
Emerging targets	**NKG2D ligands:** stress-induced expression **TAMs:** microenvironment constant **Oncolytic viruses** **Radiotherapy:** improved permeability	**Preclinical validation only**	**Active preclinical development** Focus on circumventing heterogeneity	Represent next-generation strategies targeting non-tumor compartments

BBB, blood-brain barrier; CAR, chimeric antigen receptor; CNS, Central Nervous System; CSF, Cerebrospinal Fluid; DIPG, diffuse intrinsic pontine glioma; DLT, dose-limiting toxicity; DMG, diffuse midline glioma; EGFR, Epidermal Growth Factor Receptor; GBM, glioblastoma; GVHD, Graft-versus-Host Disease; HLA, Human Leukocyte Antigen; ICV, intracerebroventricular; IDH, Isocitrate Dehydrogenase; IL, interleukin; IV, intravenous; MGMT, O6-Methylguanine-DNA Methyltransferase; OS, overall survival; PTEN, Phosphatase and Tensin Homolog; RTK, receptor tyrosine kinase; TAMs, tumor-associated macrophages; TCR, T Cell Receptor; TEAM-E, Tumor Environment and Metastasis - EGFR; VST, virus-specific T cells.

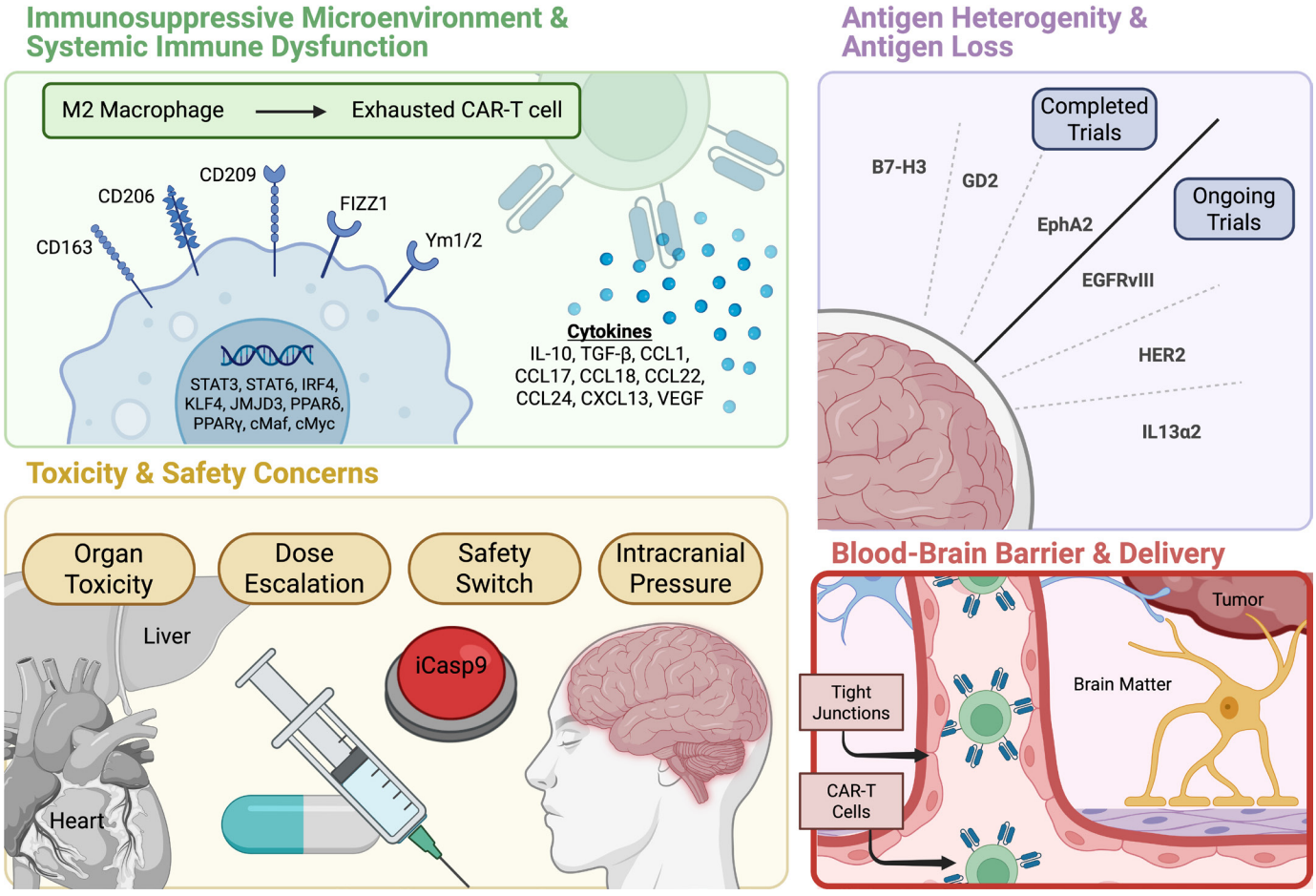

Figure 2 Major challenges to CAR T therapy in glioblastoma: (1) an immunosuppressive microenvironment and systemic immune dysfunction driving T-cell exhaustion; (2) antigen heterogeneity and loss limiting sustained tumor targeting; (3) toxicity and safety concerns (organ damage, dose escalation, safety switches, intracranial pressure); and (4) the blood-brain barrier impeding efficient cell delivery. CAR, Chimeric Antigen Receptor; CCL, C-C Motif Chemokine Ligand; CXCL, C-X-C Motif Chemokine Ligand; EGFRvIII, Epidermal Growth Factor Receptor variant III; EphA2, Ephrin type-A receptor 2; GD2, Disialoganglioside; HER2, Human Epidermal Growth Factor Receptor 2; IL, Interleukin; TGF, Transforming Growth Factor; VEGF, Vascular Endothelial Growth Factor.

and then shipped back for infusion. This process typically spans several weeks, which poses a major challenge in GBM. Patients with recurrent GBM often deteriorate quickly, and there is only a narrow window when their performance status is adequate for experimental therapy. In the EGFRvIII CAR T trial at Penn, 17 patients consented to leukapheresis, but several experienced clinical decline and could not proceed to infusion.[17 38] Therefore, if manufacturing takes too long, patients risk progressing or becoming too ill to be treated by the time the CAR T product is ready.

Recent advances are exploring ways to shorten this timeline. Commercial CAR T products for hematologic cancers have achieved manufacturing in~2–3 weeks, and similar or even faster turnarounds are being pursued in GBM. For example, one GBM CAR T trial tested a fixed 12-day production process and even included a study arm with mandated cell cryopreservation prior to infusion to assess logistical feasibility. Decentralized, point-of-care manufacturing at the treating hospital is another strategy under development to reduce vein-to-vein time.[35] An alternative approach is to use healthy donor-derived "off-the-shelf" CAR T cells that are gene-edited to prevent graft-versus-host disease. Such allogeneic CAR T cells could be banked and immediately deployed on need, bypassing the lengthy autologous production. Allogeneic CAR T cells engineered to minimize GVHD (Graft-versus-Host Disease) and rejection are being investigated as a readily available therapy for GBM. For instance, an IL13Rα2-specific allogeneic CAR T product (GRm13Z40-2) was developed with the TCR (T Cell Receptor) and glucocorticoid receptor knocked out to enhance safety and steroid resistance. In a first-in-human trial, this off-the-shelf CAR T was administered intracranially (with adjunct interleukin-2 and dexamethasone) to six patients with GBM, and two-thirds of them showed transient tumor regressions or necrosis at the injection sites without any graft-versus-host disease. Such results demonstrate the feasibility of allogeneic CAR T therapy in GBM, offering the promise of rapid deployment without the delays of manufacturing.[39]

In addition, in vivo CAR T-cell engineering is being explored to eliminate the ex vivo manufacturing phase

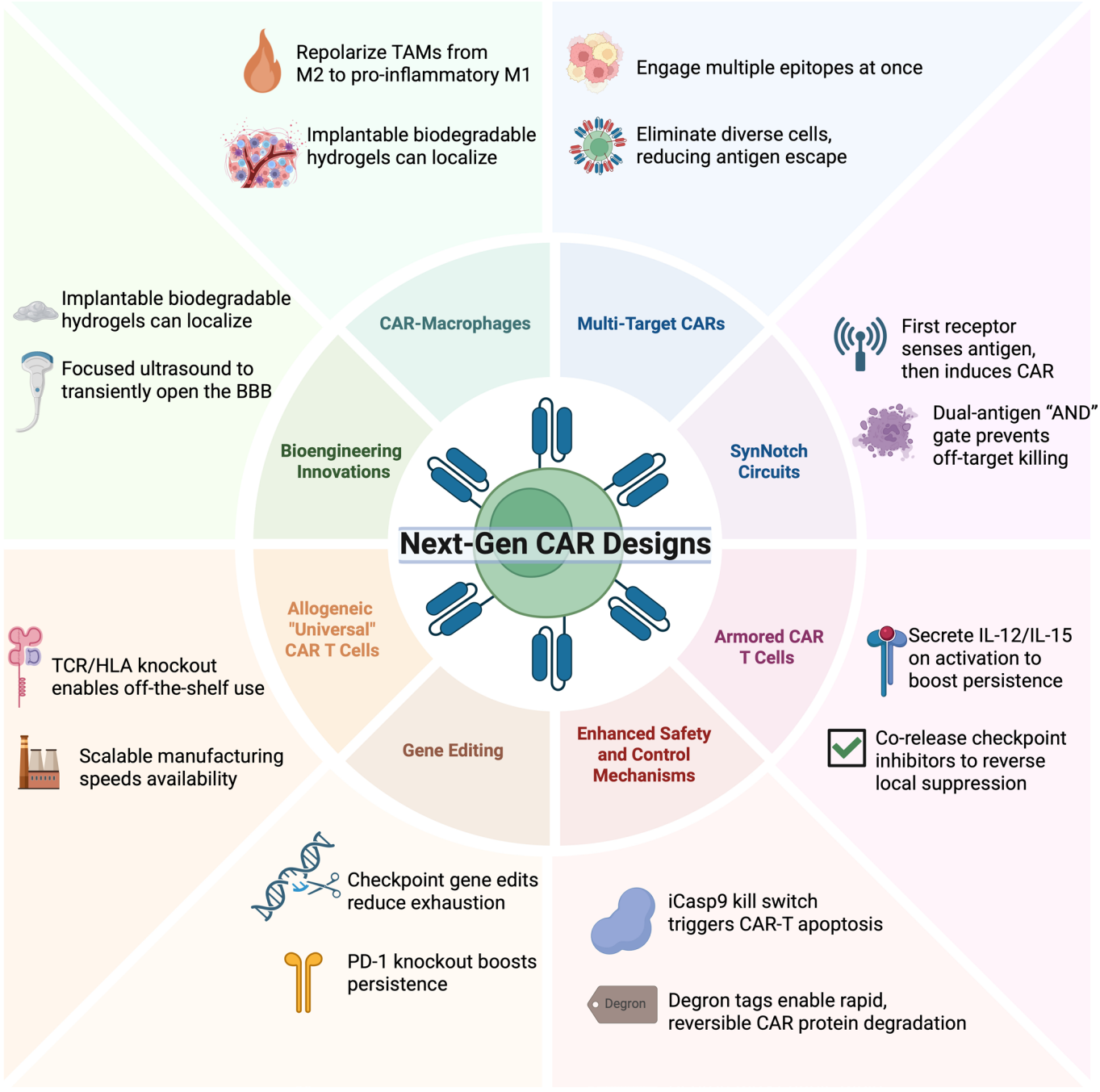

Figure 3 Next-generation CAR strategies integrate cellular, genetic and engineering advances to tackle GBM: CAR macrophages reprogram TAMs, while multitarget CARs and SynNotch "AND" circuits boost antigen coverage and specificity. Armored CARs secrete IL-12/IL-15 and checkpoint inhibitors, paired with safety switches (iCasp9, degron tags) for controllable activity. Gene-edited universal CARs enable off-the-shelf use, and bioengineering tools (biodegradable hydrogels, focused ultrasound) enhance localized delivery and BBB penetration. BBB, blood-brain barrier; CAR, chimeric antigen receptor; GBM, glioblastoma; HLA, Human Leukocyte Antigen; IL, interleukin; PD-1, programmed cell death protein-1; TAM, tumor-associated macrophage; TCR, T Cell Receptor.

altogether. Instead of fabricating CAR T cells in the lab, these approaches would generate CAR T cells directly inside the patient's body using gene delivery vectors or nanoparticles. Recent preclinical studies have shown the potential of this strategy: for example, a targeted lipid nanoparticle system carrying CAR DNA and a transposase successfully produced CAR-modified T cells in vivo after a single infusion, resulting in robust tumor control and improved survival in mouse models. Other in vivo CAR platforms using messenger RNA (mRNA) have likewise demonstrated efficient CAR T-cell formation and anti-tumor activity in mice without the need for any lympho-depleting preconditioning. If translated to humans, in vivo CAR T generation could drastically compress the vein-to-vein timeline. As a result, patients could receive CAR therapy immediately via an injection, rather than

waiting weeks, and thus treat patients before their disease progresses beyond eligibility. This nascent strategy may broaden access and accelerate the delivery of therapy for patients with GBM who cannot afford delays.

Beyond manufacturing speed, the timing and preparation of the patient are being refined. Traditionally, lymphodepleting chemotherapy is administered before CAR T-cell infusion to enhance T-cell engraftment; however, this approach also introduces additional toxicity and delays. Tennant *et al*[40] demonstrated in mouse models that transiently activating the STAT5 pathway in CAR T cells via mRNA electroporation can enable robust, cell-intrinsic engraftment in fully lymphoreplete hosts.[40] In other words, by briefly expressing a constitutively active STAT5b in the T cells, the need for patient preconditioning was abrogated entirely. This approach could spare patients with GBM from lymphodepletion and expedite therapy delivery, an important advantage given they cannot afford treatment delays. Ongoing translational research is evaluating how such strategies might be applied in clinical GBM trials, potentially allowing CAR T infusion on rapid timeframes without intensive preconditioning.

PATIENT SELECTION: POST-PRIMARY RESECTION AND EARLY RECURRENCE

Prime candidates are newly diagnosed or immediate post-resection patients before recurrence. This window preserves performance status and T-cell fitness, reduces treatment-induced lymphopenia, and enables intraoperative placement of intratumoral or intraventricular access for regional dosing. To date, most trials have treated patients with recurrent GBM. Recent trials have found great success when exploring CAR T therapy in the upfront or adjuvant setting for high-risk cases. In these studies, patients undergo standard surgery to debulk the tumor and then receive CAR T-cell infusion either postoperatively or after initial chemoradiation, rather than waiting for overt recurrence. Several trials have evaluated therapy as an adjuvant following resection or even upfront in high-risk cases, contingent on confirmed target antigen expression determined by pathology[17 41] (figure 3).

Beyond target expression, classic molecular markers in GBM might further refine which patients are most suitable for CAR T therapy. One factor is MGMT (O6-Methylguanine-DNA Methyltransferase) promoter methylation, an epigenetic status of a DNA repair gene that predicts responsiveness to temozolomide chemotherapy. Patients with unmethylated MGMT have tumors that are resistant to standard chemo and then face a worse prognosis.[33] Arguably, these patients could be prioritized for innovative treatments like CAR T cells earlier in their disease course, since conventional therapy offers them limited benefit. Another factor is IDH (Isocitrate Dehydrogenase) mutation status. IDH-mutant gliomas (which are typically lower-grade or secondary GBMs) have a very different tumor biology and immune environment than IDH-wild-type GBMs. Initial observations had suggested that IDH-mutant gliomas might be somewhat less immunosuppressive. For instance, one early study noted lower levels of certain immunosuppressive cytokines and higher lymphocyte infiltration in IDH-mutant tumors compared with IDH-wild type.[42] This raised the hypothesis that IDH-mutant gliomas could potentially respond better to immunotherapies like CAR T cells. However, more recent research has revealed a more complex picture. The oncometabolite produced by mutant IDH (D-2-hydroxyglutarate) can impede antitumor immunity by suppressing T-cell-attracting chemokines. IDH-mutant tumors tend to be "immune-cold", showing significantly reduced $CD8^+$ T-cell infiltration relative to IDH-wild counterparts.[43] Future trials could include stratification by biomarkers such as IDH, and perhaps other markers like EGFR amplification or PTEN (Phosphatase and Tensin Homolog) loss, to allow subgroup analyses.

CONCLUSION

CAR T-cell therapy for GBM shows promise despite significant challenges. Early clinical experiences validate the safety and potential of these engineered cells to infiltrate the BBB and elicit measurable antitumor activity. However, treatments have yet to overcome tumor heterogeneity, the immunosuppressive microenvironment, and technical complexities in cell manufacturing. Emerging strategies, such as multi-target CAR constructs, AND-gate circuitry, armored CAR T cells, gene-edited universal products, combination therapies, and the novel application of CAR-macrophages, offer exciting avenues to overcome these obstacles. Ultimately, sustained interdisciplinary research and rigorous clinical translation will be fundamental to refining these approaches and improving outcomes for patients with this devastating disease.

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