CAR T-cell therapy for gliomas

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Purpose of review
To review the landscape of chimeric antigen receptor T-cell (CAR T) therapy for gliomas as seen in recently published trials and discuss on-going challenges with new cancer immunotherapy treatments.

Recent findings
Given how CAR T therapy has revolutionized the treatment of several hematologic malignancies, there has been increasing interest in using immunotherapy, and particularly CAR T therapy for gliomas. Within the past decade, several first in human trials have published early patient experiences showing treatment is generally well tolerated but with limited efficacy, which may be improving with newer evolutions in CAR T design to overcome known resistance mechanisms in glioma treatment.

Summary
CAR T therapy is a promising avenue of treatment for high-grade gliomas, which have a universally poor prognosis as well as limited therapeutics. There are a growing number of CAR T clinical trials for CNS tumors and thus, an understanding of their treatment strategies, toxicity management, and overcoming resistance mechanisms will be important for both clinical practice and to identify areas for future research.

Keywords
chimeric antigen receptor T-cell therapy, gliomas, immunotherapy

INTRODUCTION

Chimeric antigen receptor T-cell (CAR T) therapy has revolutionized the treatment of hematologic malignancies in the past decade. The first CAR T cells were developed more than 30 years ago [1,2], but early human trials were unsuccessful [3,4] possibly because of poor CAR T persistence. However, with the development of second-generation CARs with a CD28 or 4-1BB costimulatory domain, there has been improved persistence and efficacy of CAR T therapy leading to Food and Drug Administration (FDA) approval of six CAR T products for hematologic malignancies targeting CD19 and B-cell maturation antigen (BCMA) [5].

Despite the success of CAR T therapy in hematologic malignancies, there have been a number of challenges in the treatment of solid tumors such as tumor antigen heterogeneity, T-cell trafficking and persistence, and the immunosuppressive microenvironment [6,7]. For central nervous system (CNS) tumors, the immunologically distinct environment of the CNS and its complex interaction with the immune system present additional distinct immunotherapeutic challenges [8]. With an increasing understanding of immunotherapy and the unique immune environment of the brain, advancing glioma treatment via CAR T therapy is being explored in both preclinical studies as well as early phase clinical trials.

CLINICAL TRIALS OF CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY FOR GLIOMAS

CAR T therapy is being investigated for high-grade gliomas (HGGs) in both adult patients with glioblastoma (GBM) as well as pediatric patients with diffuse midline gliomas (DMGs), medulloblastoma, and ependymoma. A key consideration for CAR T therapy in gliomas is the choice of antigen target, which would ideally demonstrate high expression on the tumor and low or absent expression on normal tissue to decrease off-tumor, on-target toxicities [9]. A number of tumor antigen targets are

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KEY POINTS

- Phase I clinical trials of CAR T therapy for glioma targeting IL13Rα2, EGFRvIII, HER-2, B7H3, and GD2 have demonstrated safety and in some instances promising antitumor activity.
- Through these and other preclinical and clinical work, the optimal CAR T construct, dosing, route of administration, and preconditioning regimen are actively being determined.
- Treatment-related toxicities are generally mild, and are managed with supportive care, anakinra and corticosteroids.
- Resistance mechanisms need to be more fully understood and addressed in order to deliver lasting effective CAR T therapies for gliomas.

being tested with published clinical trials exploring a variety of these strategies.

IL13Rα2 chimeric antigen receptor T cells

IL13Rα2 is highly expressed in WHO grade 4 gliomas (approximately 58% in GBM) [10] as well as brainstem gliomas with low expression in normal brain tissue [11], and preclinical models demonstrated robust antitumor activity *in vitro* and via intracranial administration in orthotopic immunodeficient mice [12]. As a result, IL-13 receptor α2 (IL13Rα2) was targeted in one of the earliest published studies for CAR T therapy for malignant gliomas in 2015 by Brown *et al.* [13].

The initial report described three patients with recurrent HGGs (rHGGs), who were treated with first-generation IL13Rα2 CAR T cells with weekly intratumoral infusions on an intrapatient dose escalation schedule [13]. The therapy was well tolerated with no DLTs. One patient developed grade 3 headache and 1 patient had transient grade 3 shuffling gait and tongue deviation that resolved with dexamethasone. Median survival was 11 months after relapse and recurrent tumor tissue showed decreased IL13Rα2 expression, suggesting CAR T-specific antigen loss as potential resistance mechanisms. Brown *et al.* [14], also reported in 2016 on a patient with recurrent GBM treated with second-generation IL13Rα2 CAR T, who had a complete response (CR) with intraventricular infusions that was durable for 7.5 months. In 2022, Brown *et al.* [15], investigated an allogenic first-generation, steroid-resistant IL13Rα2 CAR T, where six patients with rHGGs on daily dexamethasone (4–12 mg) were treated with intratumoral infusions of IL13Rα2 CAR T cells and IL2 (aldesleukin). The therapy was well tolerated with no DLTs, but there was no clear objective clinical response or survival benefit with a median survival of 2.9 months after treatment initiation, and one patient surviving 11.3 months.

In 2024, Brown *et al.* reported on 65 patients with rHGGs treated with IL13Rα2 CAR T cells [16▪▪]. Patients were predominantly not only adults with IDH-wildtype GBM (72%), but also included H3K27M-altered DMGs (two patients), IDH-mutant grade 4 astrocytoma (six patients), grade 4 diffuse astrocytoma (one patient) and grade 3 glioma (seven patients). There were five treatment arms, with arm 1 receiving intratumoral infusion after biopsy, arm 2 receiving intratumoral infusions after maximal resection, arm 3 receiving intracerebroventricular (ICV) infusions, arm 4 and arm 5 receiving combination intratumoral and ICV infusions. Arm 5 patients received CAR T cells on a new manufacturing platform using CD62L+ enriched naive, stem cell memory, and central memory T cells (Tn/mem).

Patients received weekly infusions for 3 weeks with additional optional infusions on a dose-escalation regimen from 2 to 200×10^6 CAR + T cells. There were no DLTs, but two patients experienced transient grade 4 cerebral edema after cycle 1 that was managed with dexamethasone as well as one grade 3 ataxia and grade 3 encephalopathy. Half of the patients achieved stable disease (SD) (22% for ≥90 days). Of three patients with IDH-mutant gliomas, two patients had a partial response (PR) and one patient had a CR. Median overall survival (OS) was 8 months (7.7 months in recurrent GBM). Arm 5 had higher OS at 10.2 months as well as increased quality of life. The majority of patients had detectable CAR T cells in the CSF for at least one infusion with arm 5 demonstrating highest peripheral detection of CAR T cells. While a recommended phase 2 dose (RP2D) is still being refined, the investigators have found the intratumoral + ICV delivery route in arm 5 the most promising and are planning dual delivery for future trials with IL13Rα2 CAR T cells. Several clinical trials with IL13Rα2 CAR T cells are in process for leptomeningeal disease, medulloblastoma, ependymoma (NCT04661384), in combination with ipilimumab/nivolumab (NCT04003649) and in pediatric tumors (NCT04510051).

Epidermal growth factor receptor variant III chimeric antigen receptor T cells

Epidermal growth factor receptor variant III (EGFRvIII) is a common mutant of EGFR that is highly expressed (30–50%) in gliomas including GBM with low expression in normal brain tissue [17–19]. Preclinical studies with using a second-generation CAR T in an orthotopic murine model of GBM showed

~80% reduction of tumor volume [18]. As a result, a phase I clinical trial was conducted in 2017 by O'Rouke *et al.* [20]. Ten adult patients with recurrent GBM were screened for presence of EGFRvIII and treated with one dose of intravenous CAR T therapy. Three patients did not undergo surgery, three patients underwent 'late' surgery because of concern for recurrence and four patients underwent 'early' surgery for symptomatic progression. There were no DLTs on the trial, but one patient had grade 3 seizures treated with siltuximab, corticosteroids, and antiepileptics, one patient had grade 4 cerebral edema treated with high-dose corticosteroids and ultimately found to have progressive disease, and one patient had postoperative intratumoral hemorrhage that improved with observation. OS was ~8 months and one patient survived 36 months after recurrence and demonstrated persistent EGFR CAR T cells in peripheral circulation at 29 months of follow-up [21]. EGFR expression declined in 5/7 patients with tissue sampling, which suggested an on-target effect of CAR T. However, there was also upregulation of immunosuppressive molecules such as IDO1, FoxP3, IL-10, PD-L1, and/or TGFβ, which may have contributed to treatment resistance.

Goff *et al.* [22] also used targeted EGFRvIII with a third-generation CAR T in 18 patients with recurrent GBM. Patients underwent lymphodepleting chemotherapy followed by intravenous infusion of CAR T along with interleukin-2 (IL-2) administration 24 h after cell infusion. A DLT occurred at the highest dose of 6×10^6 with hypoxia leading to intubation and patient death with significant pulmonary edema found postmortem. Ten patients developed grade 2 neurologic symptoms and/or seizures managed with corticosteroids and/or antiepileptics. There were no objective responses on MRI, and median OS was 6.9 months with median PFS of 1.3 months.

Enhancing Epidermal growth factor receptor variant III-based chimeric antigen receptor T-cell therapy

Given the increased immunosuppressive microenvironment found after CAR T treatment in the 2017 O'Rouke trial with EGFRvIII [20], a phase I trial was conducted with EGFRvIII in combination with pembrolizumab [23]. Seven patients received intravenous CAR T cells with pembrolizumab for three cycles followed by a fourth cycle of pembrolizumab only. There were no DLTs, but there was one instance of high-grade cerebral edema and encephalopathy and one patient developed severe immune-related adverse event (irAE) likely related to pembrolizumab with acute liver and kidney injury that was managed on intravenous methylprednisolone. However, there was no clear clinical benefit with median OS of 11.8 months and median PFS of 5.2 months. Additionally, peripheral CAR T engraftment was lower than the initial O'Rouke trial in 2017.

In order to target both antigen heterogeneity and overcome the immunosuppressive tumor microenvironment in GBMs, Bagley *et al.* [24] used a bivalent CAR T, targeting EGFRvIII and IL13Rα2 [24]. The study reported on the initial six adult patients, with recurrent GBM, treated with a single dose of ICV infusion at two dose levels as part of a 3 + 3 dose-escalation design. All patients had early and moderate–severe neurotoxicity that was a combination of immune effector cell-associated neurotoxicity syndrome (ICANS) and tumor inflammation-associated neurotoxicity syndrome (TIANS), which were managed with dexamethasone and anakinra. One patient had a DLT at DL2, which manifested with grade 3 fatigue, muscle weakness, and anorexia that lasted for 8–14 days and required monitoring in the ICU. One patient at DL2 developed right-sided hemiplegia and worsening expressive aphasia and was treated with bevacizumab to facilitate rehabilitation and decrease dexamethasone requirement. At least 30% tumor shrinkage was seen in three of six patients and stable disease seen in three of four patients with at least 2 months follow-up, but none meet the criteria for objective response. Of the six treated patients, two also had transiently increased enhancing tumor, suggesting pseudo-progression.

Choi *et al.* [25▪] used another approach to target the challenges to antigen heterogeneity and immunosuppressive microenvironment with a second-generation EGFRvIII CAR T cell that also secretes a T-cell-engaging antibody molecule against wild-type EGFR (CARv3-TEAM-E). Preclinical models had shown CARv3-TEAM-E to decrease tumor burden in orthotopic murine models with heterogenous EGFR expression [26]. The phase I trial described the initial three patients, who received an ICV infusion (one patient had two infusions). There were no DLTs although one patient had grade 3 encephalopathy and one patient had grade 3 fatigue. One patient showed radiographic reduction on MRI day 1 after the infusion that was confirmed 2 weeks later, but had tumor progression on day 72 confirmed on biopsy. The patient later died 63 days after study discontinuation from bowel perforation while receiving bevacizumab and dexamethasone. One patient had an 18.5% tumor reduction on day 2 MRI, which continued to 60.7% reduction on day 69 that was durable at 150 days. Patient 3 had tumor regression on MRI on day 4, but recurrence occurred at 1 month.

Human epidermal growth factor receptor 2 chimeric antigen receptor T cells

Human epidermal growth factor receptor 2 (HER2) is another appealing target for immunotherapy given high expression on multiple CNS tumors including GBM [27] and medulloblastoma [28]. Ahmed *et al.* [29] treated 17 patients (10 adult patients, 7 pediatric patients) with recurrent GBM with intravenous HER2 CAR T in a phase I trial. Patients were planned for up to six doses depending on response with 6 of 17 patients receiving multiple doses. There were no DLTs, and of the 16 evaluable patients, the median OS was 11.1 months after CAR T infusion and with one PR lasting for more than 9 months, 7 with stable disease, and 8 with progressive disease (PD).

Vitanza *et al.* [30] also used a HER2 CAR T in a phase I trial for pediatric patients with CNS tumors. Three patients (one anaplastic astrocytoma, WHO grade III, two ependymoma, WHO grade III) were treated with either weekly ICV or intratumoral CAR T infusions on an intrapatient dose-escalation schedule up to 18 doses. There were no DLTs, but one had grade 2–3 headaches and grade 1–2 transient worsening of neurologic symptoms. Best responses were stable disease (one), and progressive disease in two patients.

GD2 chimeric antigen receptor T cells

GD2 is a disialoganglioside that is highly expressed not only in CNS tumors, particularly pediatric gliomas [31,32], but also in adult GBMs [33,34]. Preclinical models demonstrated robust killing of tumor cells with GD2 CAR T in both orthotopic murine models of pediatric DIPG [32] and increased survival [34] and tumor reduction [33] in murine models of GBM. For pediatric DIPG, a clinical trial by Majzner *et al.* [35] in 2022 reported on the first four pediatric patients with H3K27M-mutated DMGs treated with initial intravenous infusion followed by ICV infusions of GD2 CAR T cells. Patients experienced transiently increased neurologic deficits as well as tumor swelling and edema leading to increased intracranial pressure consistent with TIANS. Increased clinical symptoms also correlated with increased edema seen on MRI that resolved alongside clinical improvement. One patient was in rapid progression upon starting treatment and did not show a response, but the other three patients had both clinical and radiographic benefit following treatment. Clinical symptoms improved from pretreatment baseline at 1 month follow-up.

GD2 CAR T therapy has also been studied in a phase I trial for WHO grade 4 gliomas by Liu *et al.* [36] in 2023, which included GBMs. Eight patients (four adults, four pediatric patients) received lymphodepleting chemotherapy followed by either intravenous CAR T infusion or intravenous + intratumoral if patients received repeat surgery (three patients). There were no DLTs, but one patient had grade 2 seizures and grade 3 headache. The median OS was 10 months with four patients had a PR with one response lasting 24 months. One patient had SD, but died from hydrocephalus 4 months after infusion and three patients had progressive disease.

B7H3 chimeric antigen receptor T cells

B7H3 is a checkpoint molecule that is highly expressed on pediatric gliomas [31,37] as well as adult gliomas including GBM with no expression in normal brain tissue [38]. Preclinical studies showed efficacy in orthotopic murine mouse models for GBM [38] as well as pediatric glioma [31]. In clinical trials, Tang *et al.* [39] in 2021 treated one patient with recurrent GBM using a third-generation CAR T with weekly ICV infusions. The patient initially had reduction in tumor volume after the first infusion, which was sustained for 50 days prior to tumor recurrence and received an additional two cycles before discontinuing the study. The treatment was well tolerated other than grade 2 headaches.

Vitanza *et al.* [40] also use a B7H3 CAR T to target DIPG in a phase 1 trial. Three patients were treated with weekly ICV infusions. There were no DLTs on the trial, but patients had headaches, nausea/vomiting, and fever within 24 h after the infusion with one patient experiencing transient worsening of preexisting pontine-related symptoms that resolved with 48 h. Patients were treated with supportive therapy and did not require anakinra, bevacizumab, dexamethasone, or ICU monitoring. One patient had a 19.4% decrease in tumor size and improvement in their facial nerve palsy sustained for 12 months and was alive 16 months from enrollment. Two patients had progressive disease but remained alive 16 and 12 months from enrollment.

Other clinical trials

Numerous other tumor antigens are potential candidates for CAR T therapy. One target is Ephrin type A receptor 2 (EphA2), which is also highly expressed in GBM with low expression in normal brain tissue [41]. A phase I trial in 2021 treated three adult patients with recurrent GBM with EphA2 CAR T cells [42]. Patients received lymphodepleting chemotherapy followed by a single dose of intravenous CAR T-cell infusion. Two patients developed CRS (one with fever and hypotension, one with fever)

and two of three patients also developed pulmonary edema that resolved with dexamethasone. Two patients had progressive disease and one patient had SD (Table 1).

CHALLENGES IN CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY FOR GLIOMAS

Sustaining treatment response

Although these phase I clinical trials have demonstrated safety and feasibility of CAR T therapy, ultimately a sustained antitumor effect is critical for this therapy to benefit patients. Early imaging and clinical responses are encouraging, and the persistence of CAR T cells for weeks or months following therapy holds the potential to prevent tumor recurrence. Although studies to date have not been powered to assess survival outcomes, they do provide clues into the mechanisms of treatment failure. For example, loss of tumor antigen on posttreatment tumor specimens is one element of tumor heterogeneity that may indicate one potential escape mechanism. Insufficient CAR T expansion or CAR T exhaustion may contribute to limited efficacy as has been seen in hematologic malignancies. A tumor microenvironment that is hostile to CAR T activity, which could include microglial activation or immunosuppressive Treg activity may also impact CAR T activity. Optimizing CAR T design, including dosing, route of administration, and the structure of the antigen binding and costimulatory domains for gliomas may circumvent some of these barriers. Administration of lymphodepleting drugs also promotes a more favorable immune environment to promote CAR T function. Some trials have implemented administration of IL2 to promote T-cell activity following CAR T infusion.

Treatment-related toxicities

Given that patients with malignant gliomas almost universally have baseline neurologic deficits and structural brain injury before starting CAR T treatment, an understanding of the neurologic toxicities associated with therapy is especially important to delineate, but also complicated by the challenge of distinguishing neurotoxicity, pseudoprogression, and tumor progression. Cytokine release syndrome (CRS) and ICANS have been well described with CAR T therapy targeting hematologic malignancies [44]. CRS has been seen in glioma-directed CAR T therapy, but appears to be associated with intravenous administration such as seen in Goff *et al.* [22], initial intravenous infusion in Majzner *et al.* [35], and Lin *et al.* [42]. TIANS is a more recently identified neurologic toxicity described with ICV infusion of CAR T-cell therapy [45▪]. TIANS is characterized as having two types: type 1, which is a result of tumor inflammation and edema leading to increased intracranial pressure as well as concern for mechanical space constrains and herniation, and type 2, which is due to localized electrophysical neuronal network dysfunction, leading to worsening of preexisting neurologic symptoms [45▪].

Although most trials report neurologic toxicities posttreatment, distinguishing ICANS versus TIANS can be difficult. Bagley *et al.* [43▪], for example, report a combination of both ICANS and TIANS post treatment. ICANS almost universally follows CRS, is characterized predominantly by encephalopathy, and is associated with elevation of systemic inflammatory markers (erythrocyte sedimentation rate, C-reactive protein, ferritin). TIANS may also be associated with fever and headache, but most commonly presents clinically with regional cerebral dysfunction referable to the tumor location. The reason this may be difficult to distinguish is because patients may experience localized inflammation consistent with TIANS manifesting with more global symptoms, especially in the case of multifocal disease, language dysfunction, or extensive brainstem involvement that may lead to confusion or depressed level of consciousness, resembling ICANS clinically. However, given likely different underlying mechanisms of inflammation, the distinction will be important to understand, as each may respond to different treatment strategies.

Though controversial, the mainstay of treatment to mitigate the effects of ICANS and TIANS in most studies continues to be dexamethasone. In hematologic malignancies, the effect of dexamethasone on CAR T efficacy is still unclear. Although there are some studies that show no effect of steroids on treatment response [46], other studies indicate that higher cumulative doses may be associated with earlier disease progression and shorter survival [47]. In solid tumors where there is less overall experience, the effect of steroids is even more unclear. One preclinical study showed that in murine models, lower steroid doses may be better tolerated than high doses, which can potentially impact CAR T activity [48].

Additional therapies for CAR T-related neurotoxicities are also being employed such as anakinra, which is an interleukin1 receptor antagonist that has been used in refractory CRS and ICANS for CAR T therapy in hematologic malignancies [49]. It is also increasingly used in the management of CAR T for glioma such as in a continuous intravenous infusion [25▪,35,43▪]. Other therapies being explored to mitigate CAR T toxicities include dasatinib [50],

Table 1. Chimeric antigen receptor T-cell therapy clinical trials for gliomas

Clinical trial	CAR[a]	Tumor antigen	Tumor type	Patients	LD	CAR delivery	Neurotoxicity	Endpoints
Brown *et al.*, 2015 [13]	First gen	IL13Rα2	rHGG (1st recurrence)	3 adults	No	Weekly IT, Intrapatient dose escalation	Headache (grade 3), Neurologic symptoms with shuffling gait and tongue deviation (grade 3)	No DLTs, Mean survival of 11 months after relapse, 14 months in one patient, Decrease in IL13Rα2 expression after CAR T
Brown *et al.*, 2016 [14]	Second gen, 4-1BB	IL13Rα2	rGBM	1 adult (50M)	No	IT (5 infusions) ICV (10 infusions	Headache, fatigue, myalgia, olfactory aura (grades 1–2)	CR that persisted for 7.5 months
Ahmed *et al.*, 2017 [29]	Second gen, CD28	HER2	rGBM (multiple recurrence allowed)	17 pts (10, >18 years old, 7 <18 years old)	No	IV, up to 6 doses if response	Headache (grade 2), hydrocephalus (grade 3), hyponatremia (grade 3), cerebral edema (grade 4)	No DLTs, Median OS 11.1 months after CAR T infusion, Median time to progression 3.5 months, 1 pt with partial response >9 months, 7 pts stable disease for 8 weeks to 29 months, 8 progressive disease
O'Rourke *et al.*, 2017 [20]	Second gen, 4-1BB	EGFRvIII	rGBM (multiple recurrence allowed)	10 adults	No	IV, single dose	Seizure (grade 3), headache, weakness (grade 3), cerebral edema (grade 4) Hemorrhage in tumor bed	No DLTs, median OS ~8 months, Decrease in EGFRvIII expression in 5/7 patients, Detection of peripheral CAR T in all patients, Increase in immunosuppressive regulatory T cells in post-CAR T tissue
Goff *et al.*, 2019 [22]	Third gen, 4-1BB	EGFRvIII	rGBM	18 adults	Yes	IV, single dose + IL-2 administration	Neurotoxicity (grade 2) in 10 patients	DLT at 6 x10^{10} highest dose with severe hypoxia, intubation and death, Median OS 6.9 months, PFS 1.3 months, no clear ORR 1 pt alive at 59 months, 2 pts survived >1 year
Tang *et al.*, 2021 [39]	Third gen, 4-1BB and CD28	B7H3	rGBM	1 adult (56F)	No	Weekly ICV	Headache (grade 2)	No DLTs Initial reduction in tumor volume, then recurrence around cycle 6/7 with clinical progression stopping after cycle 7
Vitanza *et al.*, 2021 [30]	Second gen, 4-1BB	HER2	CNS tumor (1 anaplastic astrocytoma, 2 ependymoma)	3 pts (16,19, 26)	No	Weekly ICV (2 pts) or weekly IT (1), intra-patient dose escalation	Headache, worsening of baseline neurologic deficits, pain at metastatic sites of spinal disease	No DLTs, 2 pts with progressive disease, 1 with stable disease
Lin *et al.*, 2021 [42]	Second gen, 4-1BB	EphA2	rGBM	3 adults (45, 38, 30)	Yes	IV, single dose	Fever, CRS and pulmonary edema	1 pt with transient decrease in tumor size, 1 with SD (OS 164 days), 2 with PD (survival 181 days, 86 days)
Majzner *et al.*, 2022 [35]	Second gen 4-1BB	GD2	DIPG	4 patients (5–25)	Yes	IV (1 infusion), ICV (subsequent infusions)	Elevated intracranial pressure, hydrocephalus Headache, worsening of baseline neurologic deficits consistent with TIAN	Therapy was well tolerated with no on-target, off tumor toxicity, 3/4 patients had clinical and radiographic benefit, ICV infusion had less systemic toxicity compared with IV infusion

Table 1 (*Continued*)

Clinical trial	CAR[a]	Tumor antigen	Tumor type	Patients	LD	CAR delivery	Neurotoxicity	Endpoints
Brown *et al.*, 2022 [15]	Allogenic 1st gen, dexamethasone resistant	IL13Rα2	rHGG	6 adults	No	IT (4 doses over 2 weeks + IL-2)	Fatigue, headache and confusion were common 1 pt had grade 3 weakness and a stroke distant from infusion 2 weeks after final infusion	No DLTs, Median survival of 2.9 months from CAR T, Longest survival of 11.3 months from CAR T, Mean OS from diagnosis was 29 months (8.6–82 months) No clear objective clinical responses or survival benefit
Vitanza *et al.*, 2023 [40]	Second gen, 4-1BB	B7H3	DIPG	3 pts (18, 22, 10)	No	Weekly ICV, up to 18 infusions	Headache, nausea/vomiting, and fever within 24 h of infusion, Worsening of baseline neurologic deficits including gait disturbance and dysphagia	No DLTs, 1 pt (IDH-mut, anaplastic astrocytoma) had 19.4% decrease in tumor size in 1 dimension and improvement in facial nerve palsy, and was alive for 16 months from enrollment, 20 months from diagnosis, 1 patient was alive 17 months from enrollment, 36 months from diagnosis, 1 pt was alive 12 months from enrollment and 26 months from diagnosis
Liu *et al.*, 2023 [36]	Fourth gen, 4-1BB	GD2	WHO IV glioma	4 adults (29–63, 4 pediatric (3–6)	Yes	IV + IT if surgery (3/8 pts), IV if no surgery	Seizure (grade 2), headache (grade 3)	Median OS 10 months from CAR T, 4 pts had decreased tumor size after infusion, 1 pt had PR for 24 months 1 pt had SD but died of hydrocephalus 4 months later, 3 pts had PD, but survived >6 months, 1 pt had re-resection 6 weeks after infusion with decrease GD2 expression
Bagley *et al.*, 2024 [23]	Second gen, 4-1BB	EGFRvIII	New diagnosis GBM	7 adults	No	IV + pembro for 3 cycles, then 1 cycle of pembro alone	Cerebral edema (grade 3–4), encephalopathy (grade 3–4), left sided weakness (grade 3–4), seizure (grade 3–4), 1 patient with liver and kidney toxicity consistent with irAE	No DLTs, median PFS 5.2 mons, median OS 11.8 months, reduction in EGFRvIII in 6/7 patients post-CAR but no clear clinical efficacy, CAR T detected peripherally, but no clear CAR expansion
Brown *et al.*, 2024 [16▪▪]	Second gen, 4-1BB	IL13Rα2	rHGG (75% were second recurrence or later)	64 adults, 1 patient <18 years old	No	Arm 1: IT after biopsy Arm 2: IT after maximal resection Arm 3: ICV Arm 4: IT + ICV Arm 5: IT + ICV (new manufacturing, Tn/mem)	Ataxia (grade 3), encephalopathy (grade 3) Transient cerebral edema (grade 4) after cycle 1 in 2 pts	No DLTs, SD in 50% (29/58) pts (13 pts >90 days) Median OS 8 months (7.7 months for rGBM), arm 5 median OS 10.2 months, 2 pts with PR, 1 pt with CR (IDH-mut, 2/3 grade III glioma), Quality of life questionnaires showed increase for arm 5 compared with 1–4 but also arm 5 also had longer OS
Choi *et al.*, 2024 [25▪]	CARv3-TEAM-E, 4-1BB	EGFRvIII	rGBM	3 adults	No	ICV (2 infusions in 1 patient)	Encephalopathy (grade 3), transient, asymptomatic pulmonary nodules and ground glass opacities	No DLTs, 1 pt with decrease on MRI 1 day after infusion that persisted for 2 weeks, 1 pt with 60.7% decrease in tumor size for 150 days, 1 pt with reduction on day 5, but disease recurred in 1 month

Table 1 (*Continued*)

Clinical trial	CAR[a]	Tumor antigen	Tumor type	Patients	LD	CAR delivery	Neurotoxicity	Endpoints
Bagley *et al.*, 2024 [43▪▪]	Bivalent, 4-1BB	EGFRvIII + IL13Rα2	rGBM	6 adults	No	ICV, single dose	All pts had ICANS/ TIAN Transient worsening of neurologic symptoms	DLT in 1 pt at DL2 (anorexia, fatigue, muscle weakness), 3/6 patients at 30% shrinkage (but does not fit criteria for ORR), 3/4 patients had SD who had at least 2-month follow-up

CR, complete response; DLT, dose-limiting toxicity; EGFRvIII, epidermal growth factor receptor variant III; EphA2, ephrin type A receptor 2; Gen, generation; HER2, human epidermal growth factor receptor 2; ICV, intracerebroventricular infusion; irAE, immune-related adverse event; IT, intratumoral; IV, intravenous; LD, lymphodepleting chemotherapy; OS, overall survival; PD, progressive disease; pembro, pembrolizumab; PFS, progression-free survival; PR, partial response; pt, patient; rGBM, recurrent glioblastoma; rHGG, recurrent high-grade glioma; SD, stable disease.
[a]CAR generation and co-stimulatory molecule is included.

which may cross the blood–brain barrier [51], and potentially reversibly inhibit CAR T proliferation and toxicity. With increasing interest in CAR T therapy for gliomas, management strategies will need to be refined, and additional treatments identified with special considerations for the immunologically unique environment of the CNS.

CAR T-cell delivery

Other considerations with CAR T therapy for solid tumors, and especially glioma-directed CAR T therapy are the addition of lymphodepleting chemotherapy as well as route of delivery, both of which are actively being investigated. There is considerable variation among published clinical trials thus far. In hematologic malignancies, lymphodepletion is established for all FDA-approved commercial products and is an important factor for durable responses with improved CAR T persistence and expansion [52]. However, the utility of lymphodepleting chemotherapy for CAR T in gliomas is unclear, especially given the distinct immune environment of the CNS. Some studies have shown that lymphopenia following treatment with chemotherapy and radiation may benefit antitumor responses [53]. In addition, given increasing appreciation of the crosstalk between the CNS and immune system, the role of lymphodepleting chemotherapy with immunotherapy will need to be explored.

The route of delivery in CAR T therapy is another ongoing consideration. Although CAR T therapy has been given intravenously for hematologic malignancies, preclinical murine models have suggested that direct intracranial delivery is more efficient with improved trafficking to the tumor site [48]. The same study also suggested that ICV delivery may help with tumor control in more distant tumor sites. In recently published CNS CAR T trials, route of delivery has been moving towards intracranial delivery with a mix of intratumoral and ICV delivery. In particular, Brown *et al.* [16▪▪] favored an intratumoral + ICV approach stating that intratumoral provided improved control of large intraparenchymal tumors whereas ICV helped with small multifocal subpial tumors. This observation requires more prospective validation to demonstrate an impact on clinical outcomes. With more patient experience and maturation of trial data, the optimal route of CAR T delivery for CNS tumors will hopefully be elucidated, and may vary based on tumor type and location.

CONCLUSION

Given promising safety data in phase I clinical trials and some evidence of antitumor activity based on

imaging and clinical responses, there is an increasing interest in immunotherapy for solid tumors. However, there will be challenges for solid tumors and especially gliomas given the distinct immune environment. However, there are numerous trials planned for CNS tumors targeting a wide variety of antigens. Although there is initial safety data, resistance to treatment is also emerging and thus new methods are being investigated to overcome resistance mechanisms such as antigen heterogeneity, immunosuppressive microenvironment, CAR T trafficking, and exhaustion [54]. In addition, constructs such multivalent CAR T cells are being explored [55] and tested in clinical trials targeting B7-H3, EGFR806, HER2, and IL13-zetakine (NCT05768880). Other strategies include CAR-NK cells, which may be more amenable to allogeneic use [56,57]. With increased understanding of which resistance mechanisms may be impeding treatment efficacy, a variety of strategies can be employed to enhance the efficacy and durability of immunotherapy for glioma treatment.

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There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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