# Chimeric Antigen Receptor T-Cell Therapy: Updates in Glioblastoma Treatment

**Lisa Feldman, MD, PhD***
**Christine Brown, PhD‡§**
**Behnam Badie, MD***

*Division of Neurosurgery, City of Hope National Medical Center, Duarte, California; ‡Department of Cancer Immunotherapy & Tumor Immunology, City of Hope National Medical Center, Duarte, California; §Department of Hematology & Hematopoietic Cell Transplantation, City of Hope National Medical Center, Duarte, California

**Correspondence:**
Lisa Feldman, MD, PhD,
Division of Neurosurgery,
City of Hope National Medical Center,
MOB 2001,
1500 East Duarte Rd,
Duarte, CA 91010, USA.
Email: lfeldman@coh.org

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Glioblastoma multiforme (GBM) are the most common and among the deadliest brain tumors in adults. Current mainstay treatments are insufficient to treat this tumor, and therefore, more effective therapies are desperately needed. Immunotherapy, which takes advantage of the body's natural defense mechanism, is an exciting emerging field in neuro-oncology. Adoptive cell therapy with chimeric antigen receptor (CAR) T cells provides a treatment strategy based on using patients' own selected and genetically engineered cells that target tumor-associated antigens. These cells are harvested from patients, modified to target specific proteins expressed by the tumor, and re-introduced into the patient with the goal of destroying tumor cells. Here, we review the history of CAR T-cell therapy, and describe the characteristics of various generations of CAR T therapies, and the challenges inherent to treatment of GBM. Finally, we describe recent and current CAR T clinical trials designed to combat GBM.

**KEY WORDS:** Adoptive therapy, Chimeric antigen receptor (CAR) T cell, Clinical trials, Immunotherapy, Glioblastoma multiforme, Review

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High-grade gliomas, including glioblastoma multiforme (GBM), are the most common brain tumors in adults with an average incidence of 4.67 to 5.73 per 100 000 people.[1,2] These tumors pose a phenomenal challenge in neuro-oncology as they are extraordinarily difficult to treat and confer a grim 5-yr survival of approximately 5% of patients.[3] Immunotherapy, which takes advantage of the body's natural defense mechanism, is an exciting emerging field in neuro-oncology. In adoptive cell therapy, cells are harvested from patients, modified to target the tumor, and re-introduced into the patient with the goal of destroying tumor cells (Figure).

## HISTORY OF CAR T-CELL THERAPY

Chimeric antigen receptor (CAR) T cells were designed over 3 decades ago by genetically modifying T lymphocytes to recognize and eliminate cancer cells.[4] First-generation CARs consist of a targeting moiety (which most commonly involves a single-chain fragment variable [scFv] from a monoclonal antibody) connected to a spacer domain, a transmembrane region, and an intracellular CD3ζ chain (the signaling domain of a T-cell receptor [TCR]).[5,6] This construction not only allows recognition of a wide range of antigens, such as proteins and carbohydrates, but also works independent of major histocompatibility complex presentation, which often is downregulated by tumor cells.[7] Once the CAR construct binds its target antigen, T cells are activated leading to cytokine release, cytolytic degranulation, and proliferation.[8] Although first-generation CAR T cells were functional in preclinical in Vitro and animal studies, this treatment had limited effect in reducing tumor burden in human patients,[9,10] primarily because of poor persistence of T cells after administration.[11] Thus, second- and third-generation constructs were designed to include CD3ζ with 1 or 2 costimulatory domains (eg, CD28, OX40, and 41BB) to enhance their persistence and antitumor efficacy. Fourth-generation CARs include additional proteins such as cytokines,

**ABBREVIATIONS: BBB,** blood-brain barrier; **BiTE,** bispecific T-cell engager; **CAR,** chimeric antigen receptor; **CNS,** central nervous system; **CRS,** cytokine release syndrome; **GBM,** glioblastoma multiforme; **scFV,** single-chain fragment variable; **TCR,** T-cell receptor; **$T_{regs}$,** regulatory T cells

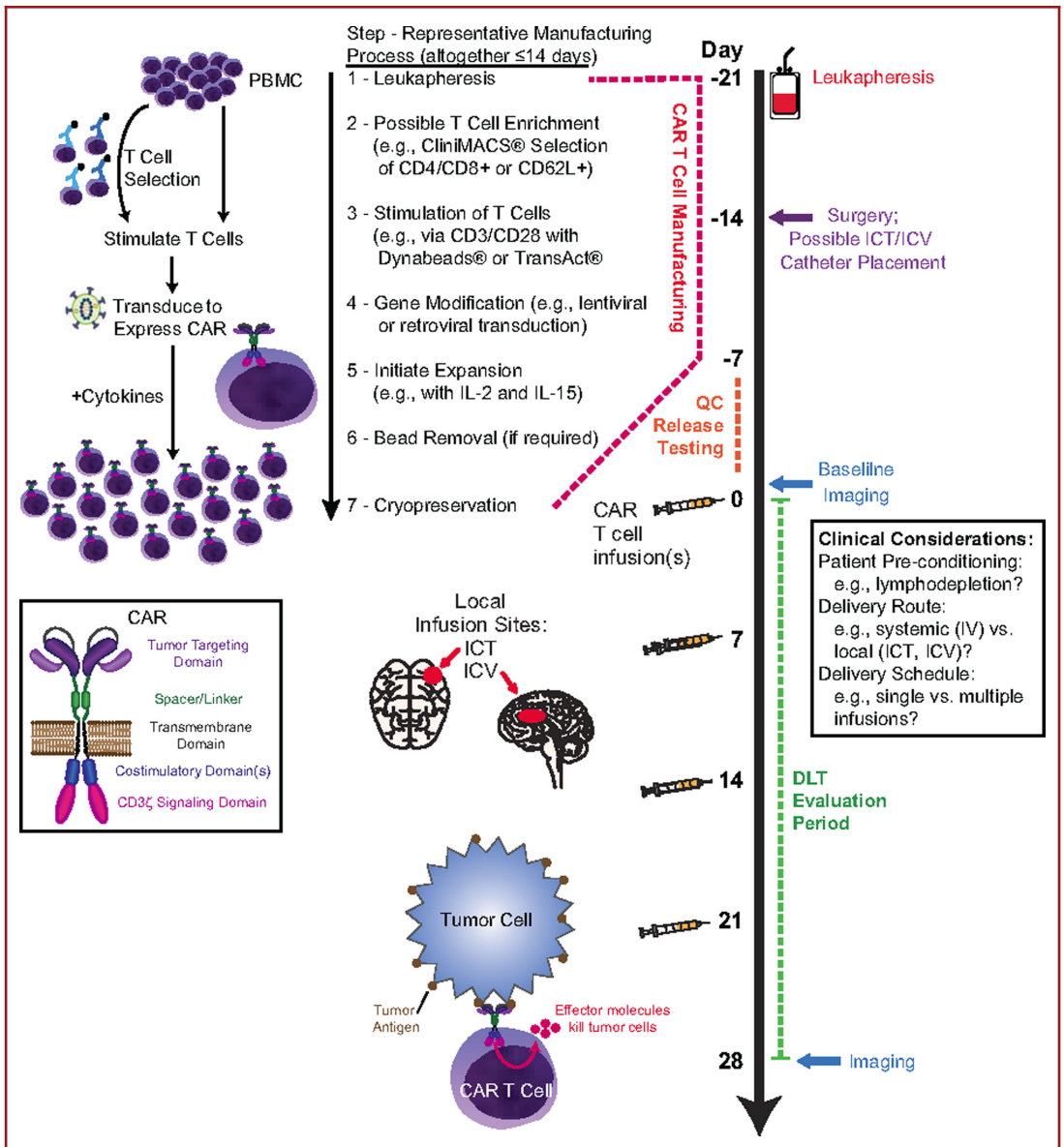


**FIGURE.** *Schematic of CAR T-cell generation and delivery to patient. Representative CAR T-cell manufacturing processes are depicted at the left, and a timeline of treatment during a clinical trial is depicted at the right. Insets include diagram of a CAR, depicting the tumor targeting, spacer, transmembrane, costimulatory, and signaling domains; schematic of local infusion sites, depicting where CAR T cells may be injected into the brain; schematic of a T-cell interacting with a tumor cell, where CAR recognition of tumor-associated antigen signals the T cells to release cytolytic effector molecules; and general considerations for the clinical application of CAR T-cell therapy. PBMC, peripheral blood mononuclear cell; QC, quality control; ICT, intracranial at the tumor site; ICV, intracranial into the ventricles; IV, intravascular; DLT, dose-limiting toxicity.*

homing receptors, or other biologics to enhance T-cell antitumor potency.[12] Recent clinical trials utilizing CAR T cells to target CD19 led to extraordinary remission in relapsed or refractory B-cell lymphomas,[13,14] including cases that involve extensive central nervous system (CNS) disease.[15] Indeed, the FDA has approved this treatment for pediatric[13] and refractory adult[16] acute lymphoblastic leukemia. Although CAR T is a validated treatment for hematological malignancies, the potential of this therapy has not yet been fully realized for the treatment of GBM.

## CHALLENGES IN IMPLEMENTING CAR THERAPY FOR GBM

Historically, the CNS was considered an immunologically privileged site with restricted access of immune cells to the brain and lack of resident dendritic cells,[17] suggesting that immunotherapy may be ineffective for brain tumors. More recent studies have revealed that activated T cells do cross the blood-brain barrier (BBB) and diffusely penetrate the brain parenchyma.[15,18,19] Once CAR T cells reach target tumor cells, however, the immunosuppressive tumor microenvironment may suppress their activity and proliferation by expressing inhibitory cell-surface molecules (ie, programmed death ligand 1 [PD-L1] and CD95)[20] or by releasing immunosuppressive tumor-derived soluble factors and cytokines (ie, prostaglandin E2, IL6, IL10, and TGF$\beta$).[21] The tumor microenvironment also preferentially promotes trafficking of suppressive cell populations, such as regulatory T cells ($T_{regs}$), tumor-associated macrophages, microglia, and myeloid-derived suppressor cells[7,22,23] and creates other physical and metabolic blockades.[24,25] Current standard GBM treatments with corticosteroids and chemotherapy further promote an immunological "cold" tumor microenvironment and lymphopenia.

CAR T cells are particularly successful at targeting and destroying B-cell malignancies because these T cells are engineered to bind to a single molecule that is uniformly expressed on the surface of all B-cell-derived tumors, CD19. GBM tumors, conversely, are notorious for having both intertumor and intratumor heterogeneity of cellular, genetic, and molecular signatures.[26-28] This tumor diversity makes their targeting with a single antigen more challenging. Nevertheless, early clinical trials using CARs for GBM directed to interleukin-13 receptor alpha 2 (IL-13R$\alpha$2),[29,30] EGFRvIII,[31] and human epidermal growth factor receptor 2 (HER2)[32] have reported promising results that support further development of this technology.

## RECENT CAR T CLINICAL TRIALS

### IL-13R$\alpha$2 CAR T Cells

IL-13R$\alpha$2 is a cancer-germline antigen expressed in the testes[7] as well as expressed in over 75% of GBMs,[33,34] making this an attractive target. IL-13R$\alpha$2 leads to activation of the phosphatidylinositol-3 kinase/AKT/mammalian target of rapamycin pathway,[35,36] resulting in increased tumor invasiveness and therefore worse prognosis.[37] In 2015, our group reported a first-in-human safety and feasibility trial using repeat doses of autologous $CD8^+$ T cells engineered to express a first-generation IL-13R$\alpha$2 CAR T. The IL-13 zetakine CAR T cells were injected directly into the tumor cavities of 3 postsurgical patients with recurrent GBM.[30] This study revealed that CAR T cells could be properly manufactured and administered directly into the tumor cavities of recurrent GBM patients through implanted reservoirs, with only mild side effects (headaches and neurological changes) that were managed with low-dose steroids. Moreover, evidence for CAR T-cell-mediated antitumor activity is supported by one patient showing a significant increase in necrotic tumor volume by imaging, and another patient showing reduction in IL-13R$\alpha$2 tumor cell expression.[30]

In 2016, our group published a remarkable case study on a patient with recurrent, multifocal GBM, who had failed standard treatment, including multiple surgical resections.[29] Targeted genomic analysis on both primary and recurrent tumor samples revealed similar genetic backgrounds and heterogeneous IL-13R$\alpha$2 expression. The patient underwent surgical resection of 3 of his intracranial tumors and subsequently received 6 weekly intracavitary infusions of second-generation CAR T cells (ie, containing a 4-1BB costimulatory domain) via an implanted reservoir/catheter device. Although the treated site remained tumor free, the nontreated lesions progressed, and new leptomeningeal tumors involving the spine were detected by imaging. The patient was then treated with 10 additional intraventricular infusions ($10 \times 10^6$ cells each) via a second reservoir/catheter placed in the lateral ventricle. Analysis of cerebrospinal fluid (CSF) revealed an influx in endogenous immune cells and an increase in 11 inflammatory cytokines by a factor of 10 or more, as compared to preinjection baseline levels including IFN$\gamma$, tumor necrosis factor $\alpha$, IL 2, 10, 5, 6, and 8, and a variety of additional chemokines. The patient tolerated both intracavitary and intraventricular infusions well, with mild side effects of headaches, generalized fatigue, and myalgia. Astonishingly, repeat imaging revealed all intracranial and spinal tumors completely regressed following treatment as assessed by radiographic imaging and quality-of-life measures. Interestingly, the patient's GBM tumor did not uniformly express the target antigen, suggesting that the treatment may have triggered immunity to other target antigens through epitope spreading.[7] Although the patient unfortunately subsequently developed new tumors, this study provides important data regarding safety of locoregional delivery of CAR T cells into the CSF and activation of host immune responses following locoregionally delivered CAR T-cell therapy.

### HER2 CAR T Cells

HER2 is a cell membrane receptor with tyrosine kinase activity and is critically important for cell proliferation, differentiation, motility, and adhesion.[38] Overexpression of this receptor in cancer is associated with a poor prognosis.[39] Because HER2 is expressed in up to 80% of GBM tumors,[40,41] including GBM cancer stem cells,[42] but it is only expressed at low levels by healthy CNS tissue,[43] this receptor is an attractive tumor-targeting antigen for CAR treatment.

In 2017, Ahmed et al[32] published a phase 1 trial using second-generation CAR T cells with an FRP5-based (anti-HER2) scFv and a CD28 costimulatory domain in 17 patients with HER2-expressing GBM. The polyclonal virus-specific HER2-CAR T cells were systemically administered to patients at every 6 to

12 wk. Other than manageable seizures and headaches, there were no cases of dose-limiting toxicity. Interestingly, blood quantitative polymerase chain reaction showed that HER2-CAR cells persisted more than 6 wk after the final infusion in 7 of 15 enrolled patients. Two patients had detectable HER2-CAR cells 12 mo following infusion, but none were detectable after 18 mo. Tumor response, assessed with magnetic resonance imaging (MRI) 6 wk following infusion, showed a partial response in 1 patient and stable disease in 7 patients while the median overall survival for the entire study was 11.1 mo, and the median progression-free survival was 3.5 mo for the group. In summary, this phase 1 study confirmed the safety and feasibility of peripherally infused, virus-based CAR T for GBM patients with encouraging antitumor efficacy.

### EGFRvIII CAR T Cells

EGFRvIII is a constitutively activated, mutated form of the wild-type EGFR receptor by deletion of exons 2 through 7, which results in the insertion of a glycine residue at the junction between normally disparate portions of the receptor.[31] This epitope serves as a strong tumor-restricted antigen, as it is expressed in 30%[44] to 40%[45,46] of human GBM tumors and is not expressed in healthy tissue.[7] O'Rourke et al[31] published a phase 1 study in 2017 involving 10 patients with EGFRvIII-positive GBM, 9 of which had multifocal disease, who each received a single dose of intravenously delivered second-generation CAR T cells with a humanized anti-EGFRVIII scFv and 4-1BB costimulatory domain. Of the 10 patients, 7 subsequently underwent surgery, which allowed for histopathological and molecular study of treated tumor tissue. No clear therapeutic response was seen on MRIs 4 wk following infusion, but 1 patient had stable disease for at least 18 mo postinfusion. This group demonstrated the safety of single-dose infusion of EGFRvIII CAR T cells, without any dose-limiting toxicity, no targeting of wild-type EGFR, or cytokine release syndrome (CRS). In Situ RNA hybridization assay confirmed the presence of CAR T cells in tumor from 4 of the 7 patients who underwent postinfusion surgery. Significant levels of non-CAR T cells also infiltrated the tissue, including unmodified T cells, and immune suppressive $T_{regs}$. Immunohistochemical staining revealed significant upregulation of a variety of immunosuppressive molecules such as indoleamine 2,3-dioxygenase (IDO) 1, PD-L1, transforming growth factor (TGF)-$\beta$, and IL-10. These results suggest that EGFRvIII-targeting CAR T cells trigger an immunosuppressive reaction in the tumor microenvironment. The 2 patients with the highest levels of both CAR T-cell and $CD8^+$ T-cell infiltration outlived the remaining patients. Except for one patient, most patients had reduced expression of EGFRvIII in tumor tissue following a single CAR T-cell infusion.

In another dose escalation trial, EGFRvIII CAR T cells were administered intravenously after lymphodepleting chemotherapy and were supported postinfusion with low-dose IL-2.[47] All patients experienced some expected transient leukopenia, thrombocytopenia, and anemia from chemotherapy; however, 2 patients developed severe hypoxia. The authors presume the respiratory symptoms developed because of congestion of pulmonary vasculature from activated T cells in a dose-responsive fashion. This treatment failed to induce objective tumor regression, nor did it delay progression or prolong survival in patients with recurrent GBM.[47] Clearly, the use of EGFRvIII-targeting CAR T cells to treat human GBM is still in its infancy, and methods to improve efficacy and safety are needed.

### Currently Active CAR T Clinical Trials

In addition to these published studies, there are currently at least 7 active clinical trials worldwide that are utilizing a variety of CAR constructs to treat GBM. Besides those currently enrolling in the USA as depicted in Table, Beijing Sanbo Brain Hospital is recruiting adult patients with recurrent glioblastoma to undergo lymphodepletion chemotherapy with fludarabine and cyclophosphamide, followed by intravenous administration of autologous anti-EGFRvIII CAR T cells.[48]

## FUTURE OF CAR T THERAPIES

As mentioned above, despite early promising results, several limitations have been identified that may hinder the efficacy of CAR T cells for GBM therapy. First, tumor heterogeneity and antigen escape are major contributors to failure of immunotherapy.[49,50] A creative strategy to combat this is to combine therapies to allow CAR T cells to simultaneously target multiple surface antigens.[51-53] For example, dual-targeting CAR T cells have been designed to co-target IL-13R$\alpha$2 and HER2 for GBM.[53] Indeed, a group in Boston genetically modified CAR T cells targeting EGFR to deliver bispecific antibodies (also known as bispecific T-cell engager [BiTE]) to tackle the heterogeneity in GBM tumors. EGFRvIII-targeting CAR T cells were unable to fully treat GBMs with heterogeneous EGFRvIII expression, resulting in expansion of EGFRvIII-negative, EGFR-positive GBM. This group has shown that EGFR-targeted BiTEs redirected CAR T cells, recruited bystander T cells to attack EGFR, and were successful in eliminating mouse models of GBM tumors.[54]

A variety of strategies are now also being exploited to overcome the immunosuppressive microenvironment of solid tumors including GBM. For example, most CARs have now been modified to include costimulatory signaling domains to increase T-cell survival (ie, the aforementioned second- and third-generation CARs). Other CAR T cells have been further modified to secrete stimulatory cytokines, such as IL-12,[55] or to constitutively express CD40[56] to support T-cell-mediated immune function. Additionally, a number of investigators are combining immunotherapeutic treatments to augment adoptive CAR T-cell therapy, such as co-delivering PD-1 checkpoint inhibitors with CAR therapy.[57,58]

Inflammation in response to CAR therapy poses another significant risk to patients. CRS, or the systemic elevation in several cytokines including IL-6 and IFN-$\gamma$, is a common

**TABLE.** Recent and Ongoing CAR T Clinical Trials for Glioblastoma in the United States

| CAR target | Freq in GBM | Homogeneity in GBM | Study | Institution | Phase | Identifier | Dose, route | Dose-limiting toxicity | Additional treatment | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| Her2 | 80-90% | Moderate variability | | | | | | | | [40,41,69-71] |
| | | | 2nd-generation HER2-CD28ζ virus-specific T cells | Baylor | I | NCT01109095 | 1 or up to 6, IV | No | Surgery | [32] |
| | | | 2nd-generation HER2-CD28ζ virus-specific T cells | Baylor | I | NCT02442297 | 3 or more, ICT and/or ICVent | Currently recruiting | Surgery | [72] |
| | | | 2nd-generation HER2-41BBζ memory T cells | City of Hope | I | NCT03389230 | 3 or more, ICT and/or ICVent | Currently recruiting | Surgery | [73] |
| | | | 2nd-generation HER2-41BBζ T cells | Seattle Children's Hospital | I | NCT03500991 | ICT or ICVent | Currently recruiting | Surgery | [74] |
| EGFRvIII | 24-67% | Wide regional/spatial/temporal | | | | | | | | [31,75-77] |
| | | | 2nd-generation EGFRvIII-41BBζ T cells | University of Pennsylvania + University of California San Francisco | I | NCT02209376 | 1 dose, IV | No. Study terminated | Surgery post T cell infusion | [31,78] |
| | | | 2nd-generation EGFRvIII-41BBζ T cells | University of Pennsylvania | I | NCT03726515 | Not reported | Currently recruiting | Surgery, followed by CAR T + Pembrolizumab | [79] |
| | | | 3rd-generation EGRvIII-CD28-41BBζ T cells | National Institutes of Health Clinical Center | I, II | NCT01454596 | 1 split dose, IV | Active, not recruiting | Surgery, progression after radiation therapy +/- chemo, Lymphocyte depletion with cyclophosphamide and fludarabine followed by CAR T plus aldesleukin | [80] |
| | | | 3rd-generation labeled EGRvIII-CD28-41BBζ T cells with and without 111Indium labeling | Duke | II | NCT02664363 | 1 or more, IV | Active, not recruiting | Surgery, CART then followed with radiation therapy and temozolomide | [81] |
| | | | 3rd-generation 111Indium labeled EGRvIII-CD28-41BBζ T cells | Duke | I | NCT03283631 | 1 or more, ICT | Currently recruiting | Biopsy, followed by SRS + CART | [82] |

**TABLE. Continued**

| CAR target | Freq in GBM | Homogeneity in GBM | Study | Institution | Phase | Identifier | Dose, route | Dose-limiting toxicity | Additional treatment | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| IL-13Rα2 | Up to 75% | Highly variable | | | | | | | | 33,34,69,83 |
| | | | 1st-generation IL13(E13Y)ζ CD8 T cell clone | City of Hope | I | NCT00730613 | 12 infusions, ICT | No | Surgery | 30 |
| | | | 1st-generation IL13(E13Y)ζ CD8 T cell clone; glucocorticoid receptor knock-out allogeneic | City of Hope | I | NCT01082926 | 4 infusions ICT | No | Biopsy | 84 |
| | | | 2nd-generation IL13(E13Y)41BBζ memory T cells | City of Hope | I | NCT02208362 | 6 infusions, IC, IVent | No; currently recruiting | Surgery | 29,85 |
| | | | 2nd-generation IL13(E13Y)41BBζ memory T cells | City of Hope | I | NCT04003649 | 4 cycles or more CAR T IC, ICVent | Not yet recruiting | Surgery, IV nivolumab and ipilimumab | 86 |

CAR, chimeric antigen receptor; GBM, glioblastoma multiforme; ICT, intracranial-intratumoral; IV, intravenous; IVent, intracerebroventricular; RT, radiation therapy; SRS, stereotactic radiation; U Penn, university of pennsylvania; UCSF, university of california san francisco.

toxicity associated with CD19-CAR T cells that is indicative of immunotherapeutic potency.[59] Neurotoxicity, including symptoms of encephalopathy, aphasia, delirium, and seizures, is also a common treatment-related toxicity of CD19-CAR therapy, resulting from increased inflammatory cytokine levels and endothelial dysfunction of the BBB.[60-62] Somewhat unexpectedly, CAR T-cell trials in GBM have thus far reported less severe CRS and neurotoxicity-like adverse events as compared to CAR T cells targeting hematological cancers. Our understanding of the full toxicity profile of GBM CAR T-cell therapy will continue to evolve as this therapy is further optimized for potency. For CNS brain tumors, however, avoiding any severe brain inflammation is of utmost importance, as increased intracranial pressure in patients already with increased mass effect from tumor can lead to deadly outcomes. Many groups are investigating alternative methods to reduce local endogenous inflammation seen after CAR T administration besides corticosteroids, which can impede CAR T function. One strategy to desensitize CAR T cells to steroids is to genetically disrupt the glucocorticoid receptor,[63] whereas others use anti-IL6 antibody tocilizumab[31] or anti-VEGF antibody bevacizumab[57] to reduce local inflammation. A balance is needed in treating CAR T patients with symptomatic brain edema while prioritizing for CAR T-cell therapeutic activity, and at City of Hope, we do this by limiting dexamethasone to 6 mg in a 24-h period in our patients. Other safety considerations include minimizing the risk of off-tumor targeting within the CNS as well as the peripheral tissues, which can have lethal consequences (ie, HER2[64] and MAGE[65]). Optimization of the CAR design, through affinity tuning, spacer selection, and signaling modifications (for review, see Abate-Daga et Davila[66]) can specify the CAR to differentially recognize overexpressed tumor antigens vs endogenous antigen expression. Suicide switches and regulatable CAR systems are also being explored to improve the safety of CAR T-cell therapy.[67] For brain tumors, regional administration of CAR T cells is also a strategy to limit peripheral tissue toxicities. Overall, ensuring safety of this therapy remains a critical concern, particularly as the repertoire of targets for brain tumor immunotherapy is expanded, and given the sensitivity of the CNS to inflammation and immune-based targeting.

Because second-generation CAR T cells persist and proliferate in the host's body following administration, dosing concentrations and schedules do not respect standard pharmacokinetic guidelines,[68] and to date, dosing schedules related to route of delivery have not been ironed out. Additionally, because T-cell migration and accumulation in solid tumors is challenged by interstitial pressure and the immunosuppressive tumor microenvironment, increased CAR T-cell concentrations and frequent dosing may be needed for more effective CAR T-cell response. Thus, further clinical studies evaluating optimal route of delivery, dose, and dosing schedule are necessary to optimize the administration of CAR T cells. These studies will require incorporating robust patient monitoring, and liquid biopsy of the CSF during CAR T-cell treatment will be particularly important to better understand the pharmacokinetics and pharmacodynamics in the CNS. In fact, our clinical experiences suggest that local changes in inflammatory cytokines and immune cell frequencies are more reflective of CAR bioactivity than those seen in systemic monitoring.[29] Questions also remain on the use of traditional chemotherapies and stereotactic radiosurgery (SRS) to augment CAR T efficacy; however, the timing of when to initiate these therapies can only be speculated at this time.

Lastly, the current costs associated with CAR T-cell therapy need also be recognized as a significant challenge to this strategy becoming a main-stream therapy for cancers in general. Specialized training and personnel at high expertise centers are required to deliver this therapy, and the resources needed to support such centers are substantial. Inventive methods of funding and budgeting for such costs, including partnerships between academic centers and biotech, are critical to continue to advance this therapy for GBM.

## CONCLUSION

As CAR T-cell therapy has shown exciting results in treating blood-born malignancies, there is much hope that this therapy may provide new opportunities in the treatment of CNS solid tumors. So far, early clinical trials have demonstrated safety and suggestive efficacy profiles of CAR T cells targeting 3 specific antigens. This therapy, however, continues to have significant challenges in treating GBM including a hostile immunosuppressive tumor microenvironment and tumor antigen heterogeneity. Sophisticated strategies including the identification of novel tumor-specific targets, the use of bi- and tritargeted CARs, and combination of therapies with biologics like checkpoint inhibitors should continue to improve the effectiveness of this therapy for CNS malignancies. Although CAR T therapy has been most extensively evaluated in the recurrent setting, should these additional measures render a stronger therapeutic response, perhaps this treatment may become an upfront therapy for newly diagnosed brain tumors.

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