CAR T Cells

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KEYWORDS

• Chimeric antigen receptor • Chimeric antigen receptor T cell • Glioblastoma • Immunotherapy • Trial • CAR-T

KEY POINTS

- Chimeric antigen receptor T cells (CAR-T) cells are reengineered T cells that express a fusion protein targeting a specific glioblastoma (GBM) tumor antigen.
- CAR construct design and manufacture process in the context of GBM leverages many of the same development principles that were used in the development and approval process of CAR-T cells for hematologic malignancies.
- The GBM tumor microenvironment presents numerous challenges to effective immunotherapy, including a stressful metabolic environment and a markedly immunosuppressive cytokine signature.
- In-human studies of CAR-T cell therapies demonstrate reasonable safety and tolerability and preliminary evidence of antitumor activity and appropriate trafficking to tumor sites, but limited persistence of these therapeutic agents and minimal durability of clinical response.
- Ongoing and emergent trials address novel frontiers in CAR-T therapeutic design for GBM, including multiantigen targeting, lymphodepletion preconditioning, and in vivo visualization of CAR-T trafficking, to improve therapeutic efficacy, reduce antigen escape and tumor recurrence, and advance clinical development.

INTRODUCTION

Glioblastoma (GBM), the most common primary malignant brain tumor in adults, is associated with extremely poor survival outcomes and is a universally fatal disease.[1] Standard of care therapy for newly diagnosed GBM involves maximal safe resection, subsequent radiotherapy and concurrent temozolomide (TMZ; 75 mg/m^2/d for 6 weeks), followed by maintenance TMZ (150–200 mg/m^2/d for first 5 consecutive days of a 28-day cycle for six cycles),[2,3] and is associated with poor survival outcomes, especially for patients with residual or multifocal disease.[3–5] The advancing therapeutic landscape for GBM is limited in scope, with only three novel therapies receiving Food and Drug Administration approval since 2005: (1) bevacizumab, a humanized anti–vascular endothelial growth factor (VEGF) monoclonal antibody treatment; (2) TMZ, an oral chemotherapeutic agent; and (3) a tumor-treating fields device that interferes with aberrant cell proliferation. A growing evidence base implicates the host adaptive immune response in the pathogenesis of GBM and overturns the prior characterization of the central nervous system (CNS) as an immune-privileged niche.[6]

Chimeric antigen receptor T cells (CAR-T) are an innovative immunotherapy approach to GBM, in which reengineered T cells express a fusion protein that targets a specific tumor antigen. When the CAR-T cell has associated with its targeted antigen, the reengineered T cell is activated and results in cytokine release, cytolytic degranulation,

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tumor cell killing, and T-cell proliferation.[7] CAR-T therapy development has been a watershed moment in cellular therapy for relapsed or refractory hematologic malignancies. CD19-directed CAR-T cells first received approval in 2017, with two products, tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta), delivering durable clinical outcomes for patients with advanced acute lymphoblastic leukemia and large B-cell lymphoma, respectively.[8,9] Investigators are currently working to recapitulate the success of CAR-T therapies for solid tumors including GBM[10]; however, there are unique challenges that are associated with therapeutic delivery in CNS malignancies, including bioavailability, immune cell trafficking, durability of response, and a hostile tumor microenvironment.[11,12]

CHIMERIC ANTIGEN RECEPTOR T CELLS

Chimeric Antigen Receptor T Cells Design Overview for Glioblastoma

CAR-T cells involve the ex vivo reengineering of a patient's or donor's peripheral T-cell population to express a CAR tailored to a specific antigen that is expressed on the surface of tumor cells.[10,13] The CAR construct itself includes multiple structural and functional intracellular domains that confer the reengineered T-cell population desirable therapeutic attributes. These fusion proteins contain an extracellular single chain variable fragment antigen recognition domain, a transmembrane domain, and an intracellular T-cell activation domain.[13]

The intracellular domain of the CAR construct contains the T-cell coreceptor CD3ζ and its immunoreceptor tyrosine-based activation motifs. Following antigen recognition and endodomain receptor clustering, the activation signal is transmitted to the T cell.[13] CAR-T cell design has evolved from its initial iterations to incorporate novel design elements that enable more potent costimulatory signaling. Second-generation CAR constructs include a single costimulatory molecule, such as 4-1BB or CD28 that is fused to CD3ζ to deliver a more potent immunotherapy.[12,14] Third-generation CARs contain two costimulatory domains linked to CD3ζ. These costimulatory domains improve CAR-T therapeutic efficacy and durability of response compared with first-generation constructs.[15]

T-Cell Harvesting

The autologous CAR-T manufacturing process for GBM generally reflects the same common steps that apply to CAR-T design for nonsolid malignancies.[16] The patient undergoes leukapheresis to harvest the peripheral blood mononuclear cells that contain the T-cell population that serves as the backbone of the reengineered immunotherapy. After cell washing, the apheresis product can then undergo enrichment or depletion of certain subpopulations.

Activation

To mimic T-cell activation in vivo, addition of OKT3, an anti-CD3 monoclonal antibody, or interleukin (IL)-2 is a common approach to stimulate T cells.[16] Coculture with lymphoblastoid cell lines, which are Epstein-Barr virus–infected peripheral blood mononuclear cells, can also stimulate T cells in what is termed the rapid expansion protocol.[16] CD3/CD28 antibody coated beads and artificial antigen-presenting cells represent emergent stimulation protocols that can be used to reduce GBM CAR-T manufacture time and are under current investigation.[17]

Chimeric Antigen Receptor T Cells Construct Delivery

Following stimulation, the T cells are transfected using plasmids or transduced with retroviral or lentiviral vectors containing the CAR construct. Lentiviral vectors are beneficial because they can transduce nondividing cells, excluding G-0 phase.[18] In contrast, retroviruses only transduce actively dividing cells and therefore rely on robust ex vivo T-cell proliferation.[16] A plasmid-based approach, in which naked DNA is electroporated into T cells, offers cost benefits compared with viral transduction methods,[19] yet is comparatively limited by its low efficiency of stable transfection into T cells.[20] Transduction efficiencies for the viral methods vary, with GBM CAR-T trials indicating a range between 5% and 26% in a lentiviral vector approach[5] and 18% and 67% for a retroviral vector approach.[21]

Expansion

CAR-T cells are expanded using an ex vivo culture medium that often contains cytokines and other stimulating factors that encourage T-cell proliferation. This critical step can take place either before or after the transfection or transduction of the CAR-T construct and may vary by investigator. Expansion can take place in a variety of settings, including T-flasks, culture plates or bags, and rocking bioreactors.[22] The culture media contains gamma-chain cytokines that support T-cell proliferation, with IL-2, IL-7, IL-15, and IL-21 as common additions.[12,23] The addition of support cytokines and expansion methodology used is

trial-dependent and may influence the phenotypic distribution of the final infusion product.

Infusion

Following activation, transfection or transduction, and expansion, the CAR-T product is often phenotypically characterized and infused into the patient.[6] Lymphodepletion of GBM CAR-T patients before infusion is an avenue that is of particular interest. TMZ has been used as a lymphodepleting preconditioning agent in a trial setting for GBM patients.[12,24] Although it is hypothesized that lymphodepletion may yield benefits in terms of in vivo CAR-T expansion and persistence,[24] current GBM CAR-T trials demonstrate no benefit of chemotherapy preconditioning before infusion.[5,25]

CHIMERIC ANTIGEN RECEPTOR T CELLS DELIVERY IN THE CENTRAL NERVOUS SYSTEM

CAR-T delivery in the context of the CNS presents unique challenges with respect to engraftment, bioavailability, antitumor efficacy, and safety. The blood-brain barrier (BBB) is a highly selective physiologic boundary that connects brain capillary endothelial cells with the surrounding luminal and abluminal membranes[6,11] and is a critical structural and functional determinant of immune trafficking and immunotherapy delivery in the CNS.

The BBB, along with the glia limitans, formed by the fusion of astrocytes processes that line the basement membrane of the CNS, form a tightly controlled barrier.[26] The BBB specifically limits entry to activated T cells, but not to their naive counterparts. Therefore, only in settings of neuroinflammation or permissive signaling environment can T cells cross the BBB and enter the parenchymal tissue.[26–28] Given the challenges of trafficking CAR-T cells into parenchymal tissue, many GBM CAR-T trials have focused on local intracavitary and intraventricular delivery in favor of intravenous delivery.

Intravenous Delivery

Intravenous delivery of GBM CAR-T products is a viable approach even in the face of the unique challenges that the CNS poses for therapeutic delivery and bioavailability. Because the BBB and glia limitans are frequently dysregulated in the context of GBM,[29,30] systemic delivery may be a viable option. O'Rourke and colleagues[5] and Ahmed and colleagues[21] used intravenous delivery for their respective CAR-T trials. Both groups tracked engraftment of the CAR-T product in the tumor following intravenous delivery.

Intracavitary/Intratumoral Delivery

Multiple GBM CAR-T trials have successfully demonstrated intracavitary/intratumoral delivery as a means to overcome the structural and functional boundary imposed by the BBB and glia limitans. Brown and colleagues[17] and Keu and colleagues[31] provide preliminary evidence that intracavitary delivery appropriately localizes to GBM resection sites. The [^{18}F]FHBG PET-based imaging assay that was used to track CAR-T^{+} cells indicated that the intracavitary delivery of the modified cytotoxic T lymphocytes trafficked to intracranial tumor sites.[31]

Intraventricular Delivery

Intraventricular delivery represents a potentially successful approach for a subset of GBM patients with spinal involvement of disease. Brown and colleagues[32] pursued intraventricular infusions following six cycles of intracavitary delivery of the IL13BBζ–CAR T CAR in a 50-year-old patient with recurrent GBM with leptomeningeal disease because of the appearance of spinal metastatic lesions during the course of the initial intracranial infusions. Subsequent intraventricular infusions completely eliminated all metastatic lesions.[32] Throughout the infusions delivered via a catheter in the lateral ventricle, CAR-T^{+} cell numbers detected in the cerebrospinal fluid seemed to be directly associated with tumor burden and inflammatory cytokine levels.[32]

TUMOR MICROENVIRONMENT IN GLIOBLASTOMA

There are many unique considerations for CAR-T delivery, in addition to local delivery to the CNS, which are relevant to GBM patients. The GBM tumor microenvironment is an immunosuppressive and metabolically stressful niche that impairs immunotherapeutic efficacy. There are many soluble immunosuppressive factors, cytokines, and immune cells that attenuate the antitumor response.[11,33] GBM cells secrete IL-6, IL-10, transforming growth factor-β, and other anti-inflammatory cytokines that dampen cytotoxic antitumor immune responses.[33] Regulatory T cells, tumor-associated macrophages, immunosuppressive-type macrophages, microglia, and myeloid-derived suppressor cells also characterize the anti-inflammatory condition associated in GBM.[34–36]

Furthermore, the hypoxic and metabolically stressful microenvironment is a hallmark feature of GBM. Hypoxia has been shown to potentiate the immunosuppressive effects of other tumoral anti-inflammatory factors and contributes to the renewal

of glioma-like stem cell population that may confer chemotherapy and irradiation.[37] Nutrient insufficiency is also characteristic of the dysregulated metabolic state in GBM. T cells encounter a glucose supply-demand mismatch in the GBM tumor microenvironment, because the glucose-poor niche does not provide sufficient glucose supply to meet the high glycolytic activity of T cells needed to maintain proliferation and effector capacity.[11,34] In addition to dysfunctional glucose metabolism, other metabolic substrates, including tryptophan, arginine, lactate, and lysine, can have deleterious effects on protein translation and T-cell function.[38]

SPATIAL AND TEMPORAL GLIOBLASTOMA HETEROGENEITY

There are many forms of heterogeneity in the GBM tumor microenvironment, including variation in cell type, mitotic activity, vascular pattern, and necrosis.[39] Common CAR-T targets for GBM, including epidermal growth factor receptor (EGFR) variant III (EGFRvIII), IL13Rα2, and human epidermal growth factor receptor 2 (HER2), demonstrate heterogeneity at the level of the patient in spatial and temporal dimensions.[21,40,41] This intratumoral variability presents a challenge to effective CAR-T delivery. In EGFRvIII- and IL13Rα2-directed CAR-T trials, investigators noted that target antigen quantitative expression varied regionally within the tumor[5] and that CAR-T cell trafficking to distant tumoral sites away from target intracranial lesions is possible.[31] Temporal heterogeneity is also evident, with next-generation sequencing of GBM patient lesions suggesting that there is selective expansion or regression of tumor subpopulations with unique molecular signatures when treated with radiation or chemotherapy.[42]

Antigen escape is a phenomenon in which tumor cells avoid CAR-T-directed killing by expressing alternate forms of the target antigen. Loss of target antigen has been documented in GBM CAR-T trials for EGFRvIII- and IL13Rα2-directed CAR-T constructs,[5,32] which may serve as a mechanism for decreased postinfusion expansion of the CAR-T product and attenuated efficacy from a monovalent CAR-T construct. Antigen escape poses many challenges for effective CAR-T design, because single-antigen targeting may be insufficient to stimulate a durable CAR-T response postinfusion.

TARGETS OF INTEREST IN GLIOBLASTOMA

IL13Rα2

IL13Rα2, a high-affinity IL-13 receptor, is an attractive target antigen for GBM CAR-T therapy given its upregulation in high-malignancy disease, specificity for GBM cells, and limited expression in normal brain parenchyma.[11,43] Approximately 58% of World Health Organization grade IV gliomas have upregulation of this receptor, and this overexpression has been linked with poor survival outcomes.[44]

HER2

HER2 is another attractive target antigen for the purposes of CAR design for GBM patients. HER2 encodes a transmembrane glycoprotein with intracellular tyrosine kinase activity[45] and is well-characterized with respect to the pathogenesis of breast cancer. Although HER2-positive GBM is not common, initial studies suggested that 15% to 17% of GBM expressed the transmembrane protein by immunohistochemistry and that expression is linked to poor survival outcomes.[46–48] A second-generation HER2-specific CAR construct demonstrated strong antitumor activity in an orthotopic xenogeneic mouse model.[49] The same research group subsequently initiated the first GBM CAR-T study that addressed HER2-positive GBM patients with progressive disease.[21]

EGFRvIII

EGFR is a receptor tyrosine kinase that is commonly amplified or mutated in human GBM.[50] EGFRvIII is the most common variant of EGFR in human tumors and results from the in-frame deletion of exons 2 to 7 that creates a novel glycine at the junction of exons 1 and 8.[51,52] The truncated variant leads to constitutive signaling in the Ras-mitogen-activated protein kinase pathway and is associated with more malignant GBM.[53] EGFRvIII is expressed in approximately 30% of newly diagnosed patients[51] and has been associated with mixed survival outcomes. Although earlier studies suggested that EGFRvIII was a poor prognostic indicator,[53–55] more recent and larger studies have not demonstrated any significant predictive power associated with the variant.[56]

CHIMERIC ANTIGEN RECEPTOR T CELLS CLINICAL TRIALS FOR GLIOBLASTOMA PATIENTS

IL13Rα2 Trials

The first human study of first-generation IL13Rα2-directed CAR-T cells with repeated intracavitary administration in three patients with recurrent GBM provided promising results regarding the safety and efficacy of the immunotherapy

(Table 1).[17] An IL-13-zetakine construct, an MHC-independent CAR, recognizes IL13Rα2 using a unique IL-13 ligand with a point mutation (E13Y) to reduce binding affinity and attenuate off-target reactivity to the more commonly expressed IL13Ra2/IL4Ra complex. The CAR-T infusions, delivered through a catheter/reservoir system, had a favorable safety profile, with no dose-limiting toxicities recorded. However, there were two grade 3 headaches attributable to one subject, and a grade 3 neurologic event associated with another patient that were possibly related to CAR-T administration. A rapid inflammatory response after T-cell infusion followed by necrosis favored antitumor activity over progressive disease or previous treatment effect.

The City of Hope research group that oversaw the first IL13Rα2 study followed up with a subsequent trial using a second-generation IL13Rα2-directed CAR that incorporated at 4-1BB costimulatory domain in a 50-year-old GBM patient.[32] The patient presented with recurrent multifocal GBM with leptomeningeal disease with unmethylated O6-methylguanine–DNA methyltransferase (MGMT) promoter, wild-type IDH1, and IL13Rα2 H-score of 100. The patient initially received six cycles of intracavitary infusions; however, because of progression at distal sites and the emergence of spinal metastases, a catheter was placed to enable intraventricular delivery. Following 10 cycles of intraventricular infusions, all spinal metastases were completely eliminated. In contrast with the earlier study, the research group observed a more favorable safety profile with the second-generation construct, with no grade 3 or higher adverse events observed and no dose-limiting toxicities. Of note, the data indicated that IL13Rα2-directed CARs may modulate the GBM tumor microenvironment. There were significant increases in proinflammatory cytokines throughout the 7-day infusion cycle, including interferon-γ, tumor necrosis factor-α, IL-2, IL-5, IL-6, IL-8, and host immune cell populations, such as $CD19^+$ B cells and $CD11b^+CD15^+$ granulocytes in the cerebrospinal fluid. Similar to their previous trial, expansion and persistence of the second-generation IL13Rα2-directed CAR in this patient was limited in later infusions. After a substantial clinical response of 7.5 months following the initiation of the intracavitary and intraventricular infusions, GBM recurred at four novel sites. Immunohistochemistry analysis confirmed low IL13Rα2 expression, suggesting lower target antigen expression may be associated with disease recurrence at novel locations.

The localization of anti-IL13Rα2 CAR-T therapies to the appropriate compartment within the CNS is a critical therapeutic feature for antitumor activity. Keu and colleagues[31] developed a PET-based visualization methodology using [^{18}F] FHBG, a fluorine-18 radiolabeled analogue of penciclovir, to monitor in vivo trafficking of HSV1-tk expressing IL13Rα2-directed CAR-T cells. The study provided preliminary evidence of appropriate cytotoxic T lymphocytes trafficking to tumor sites; however, the investigators were not able to confirm this hypothesis given noticeable false-positive signals in preinfusion scans.

EGFRvIII Trials

Two in-human EGFRvIII-directed CAR trials have been conducted to date that provide support for further clinical advancement of CAR-T therapeutics that target this oncogenic variant. A phase I trial at the University of Pennsylvania with a single, intravenous infusion of EGFRvIII-directed CAR-T cells included 10 patients with $EGFRvIII^+$ recurrent GBM.[5] Based on a preclinical trial of an anti-EGFRvIII CAR that demonstrated antitumor activity and minimal reactivity to human skin grafts in immunodeficient mice,[57] the research group leveraged this construct for the first in-human trial of an EGFRvIII-directed CAR. Substantial tumor regression was not observed in any patients based on MRI imaging. However, one patient had residual stable disease for more than 18 months postinfusion and all seven patients reoperated on postinfusion demonstrated a decrease or complete loss of the target antigen. The poor prognostic characteristics associated with the patient sample in this trial are of interest, because 9 out of 10 patients had multifocal disease and all patients were MGMT promoter unmethylated, which has been implicated as a predictive marker of poor survival outcomes.[58] Most patients had a postinfusion resection, enabling a comparative analysis of CAR-T cell trafficking in the peripheral blood and the tumor site. For two patients, CAR-T DNA sequence copies in brain tumor specimens were 3 and 100 times greater than their pairwise peripheral blood specimens, suggesting CAR-T cell trafficking to the appropriate compartment.

In contrast, a phase I dose-escalation trial for patients with recurrent $EGFRvIII^+$ GBM using a third-generation construct incorporated lymphodepletion and systemic IL-2 administration, similar to protocols that have resulted in clinical responses for patients with melanoma and synovial sarcoma.[25] Eighteen patients ultimately received the CAR-T infusion product that included 4-1BB and CD28 costimulatory domains. There were no objective responses by MRI imaging and most

Table 1
Summary of in-human CAR-T trials for GBM patients

Study, Year	No. of Patients	Target Antigen	CAR-T	Route of Administration	TME Response	Max Dose (Cells)	Safety and Tolerability	Outcomes
Goff et al,[25] 2019	18	EGFRvIII	EGFRvIII-CD28-41BBζ (third generation)	Intravenous	N/A	2.6×10^{10}	2 DLTs. 1 patient developed acute dyspnea and severe hypotension with subsequent treatment-related mortality (grade 5). 1 patient developed dyspnea that was successfully managed with CPAP.	Median OS: 6.9 mo. Median progression-free survival: 1.3 mo. 1 patient alive at 59 mo. 2 additional patients survived >1 y.
O'Rourke et al,[5] 2017	10	EGFRvIII	EGFRvIII-4-1BB CD3ζ Bulk T cells (second generation)	Intravenous	Increased expression of IDO1, FoxP3, IL-10, PD-L1, TGF-β 5 of 10 patients with 10-fold or greater increase in IL-6 postinfusion	5×10^8	No DLTs. Grade 3–4 Possibly related adverse events: left ventricular systolic dysfunction (n = 1), left-sided muscle weakness (n = 1), facial muscle weakness (n = 1), headache (n = 1), intracranial hemorrhage (n = 1), seizure (n = 2).	Median OS: 251 d. Post-treatment EGFRvIII loss in 5 out of 7 patients.

Ahmed et al.[21] 2017	17	HER2	HER2-FRP5.CD28ζ VST (EBV-CMVpp65-AD) (second generation)	Intravenous	N/A	1×10^8	No DLTs. Grade 2–4 Possibly related adverse events: headache (n = 1), seizure (n = 2).	Median OS: 11.1 mo (24.5 mo from diagnosis), 1/16 patients partial response (>9 mo), 7/16 patients stable disease (8 wk–29 mo), 8/16 patients progressive disease. Patients with no salvage therapy before infusion had significantly longer median OS (27.2 mo) than those with previous salvage therapy (6.7 mo).
Keu et al,[31] 2017	7		IL13 (E13Y)-CD3ζ [^{18}F]FHBG-HSV1-TK-HPH- GR- deleted CD8$^+$ CTLs (second generation)	Intracerebral	N/A	1×10^8	No DLTs. No major or life-threatening events related to [^{18}F] FHBG and/or CTL infusions.	[^{18}F]FHBG gene reporter used in novel PET-based imaging approach to in vivo CTL monitoring. Survival between 4 and 59 following first infusion of CTL product.
Brown et al,[32] 2016	1	IL13Rα2	IL13(E13Y)-41BBζ-CD19 t Memory T cells (second generation)	Intracavitary, intra-ventricular	Increased CD-3$^+$ CD-14$^+$ CD-15$^+$, CD-19$^+$ immune cells and 10-fold or greater increase in inflammatory cytokines (IFN-γ, TNF-α, IL-2, IL-5, IL-6, IL-8, IL-10) and chemokines (CXCL9, CXCL10, CCR2, IL-1Rα)	10×10^6	No DLTs. No grade 3–4 possibly related adverse events related to CAR-T administration.	Case report of 50-year-old patient demonstrating 7.5 clinical response, including complete elimination of spinal metastases following intraventricular delivery of the CAR construct. Disease progression at 228 d.

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Table 1
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Study, Year	No. of Patients	Target Antigen	CAR-T	Route of Administration	TME Response	Max Dose (Cells)	Safety and Tolerability	Outcomes
Brown et al,[17] 2015	3	IL13Rα2	IL13(E13Y)-CD3ζ $CD8^+$ CTLs (first generation)	Intracavitary, intratumoral	Transient inflammatory response and increase in necrotic volume at tumor site, confirmed by elevated lactate and lipid peaks and a low choline/ creatinine ratio	1×10^8	No DLTs. Grade 3 adverse events: headache (n = 1), neurologic event shuffling gait and tongue deviation (n = 1).	Median postrelapse survival:10.3 mo. Significant decrease in IL13Rα2 expression vs pretreatment levels (1 patient). No tumor recurrence at border of resection cavity (2 out of 3 patients)

Abbreviations: CMV, cytomegalovirus; CPAP, continuous positive airway pressure; CTL, cytotoxic T lymphocytes; DLT, dose-limiting toxicity; EBV, Epstein-Barr virus; IFN, interferon; OS, overall survival; TGF, transforming growth factor; TME, tumor microenvironment; TNF, tumor necrosis factor; VST, virus-specific T cells.

patients had progressive disease at the first follow-up. With progression-free survival of 1.3 months, the investigators suggested that the anti-EGFRvIII CAR-T product provided minimal to no clinically meaningful benefit to patients, even with notable persistence of the CAR^+ cells at the 1-month postinfusion timepoint for 14 of the patients. Dose-limiting toxicities were associated with the highest dosage ($\geq 10^{10}$ cells), with one patient developing acute dyspnea and pulmonary edema and ultimately succumbing to severe hypotension and the other developing severe dyspnea that was managed successfully with continuous positive airway pressure. Refinement of EGFRvIII-directed CAR-T therapy, with respect to antitumor activity and its safety, may support ongoing clinical advancement of bispecific and trispecific CAR-T constructs that incorporate EGFRvIII targeting as a part of the therapeutic mechanism and anti-EGFRvIII antibody development.[59]

HER2 Trials

The first in-human anti-HER2 CAR-T product for GBM patients used a second-generation construct using a CD28 costimulatory domain.[21] Of note, the investigators expressed the CAR construct in virus-specific T cells (VSTs) to facilitate adoption of the infusion product. These VSTs not only provide antitumor activity, but also receive a sufficient costimulatory signal following native receptor engagement by latent virus antigens presented by endogenous professional antigen-presenting cells.[21,60] The Baylor team generated HER2-directed CAR-T cells that were specific for cytomegalovirus, Epstein-Barr virus, or adenovirus. Expansion, measured by interferon-γ Elispot assays, was not observed in vivo in GBM patients, in contrast to the significant expansion of VSTs in hematopoietic stem cell transplant recipients who are extremely lymphodepleted.[61,62] With respect to persistence, the CAR^+ cells were detectable in the peripheral blood for up to 12 months. This is a notable increase from persistence recorded in EGFRvIII- and IL13Rα2-directed CAR-T trials in GBM patients and provides additional support for the exploration of VST-based approaches to increasing CAR-T longevity in vivo.

EMERGENT CLINICAL TRIALS AND FUTURE DIRECTIONS

Currently, there are 16 trials that involve CAR-T therapy as a treatment modality for GBM on clinicaltrials.gov. Of these trials, seven are actively recruiting patients, one trial is active and not recruiting, and one trial has been terminated with results (**Table 2**).

Exploration of attractive antigen targets that can improve CAR-T engraftment, persistence, and efficacy is a prominent theme in emergent GBM CAR-T clinical trials. Targets of interest include more conventional IL-13Rα2 and HER2 and novel antigens of interest, such as B7-H3 (CD276), an antigen that is not normally expressed in CNS tissue, but has enriched expression in GBM patients (NCT04385173, NCT04077866). Erythropoietin-producing hepatocellular carcinoma A2 (EphA2), a receptor tyrosine kinase that is overexpressed in GBM and is associated with poor outcomes,[66–68] is also a promising target. A phase I/II trial explored the effectiveness and safety of an anti-EphA2 CAR-T therapy in GBM patients; however, the study was recently withdrawn (NCT02575261).

Combination therapy of CAR-T immunotherapy used in conjunction with immune checkpoint blockade and antiangiogenic therapy is an emergent area in GBM therapeutic development. Upregulation of immunosuppressive factors, including programmed death-ligand 1 (PD-L1), IDO1, FoxP3, and transforming growth factor-β, has been implicated in the GBM tumor microenvironment,[12] demonstrating a role for checkpoint blockade and other therapeutics that can potentiate the host response through reversal of T-cell exhaustion. An ongoing single-arm, open-label study at The University of Pennsylvania builds on a prior phase I study that established the safety and tumor localization profiles of an EGFRvIII-direct CAR (NCT02209376). The group is now combining 2.0×10^8 cell doses of the anti-EGFRvIII construct with 200-mg pembrolizumab, a humanized antibody directed against programmed cell death protein (PD-1) following adjuvant radiotherapy (NCT03726515). Strategies that target the abnormal vascularization of the GBM TME are also promising in the context of combination therapy.[69] CAR-T administration in combination with bevacizumab, an anti-VEGF monoclonal antibody, may counteract the immunosuppressive effects modulated by VEGF, such as the recruitment of regulatory T cells and myeloid-derived suppressor cells and disrupted dendritic cell activation[70] and has shown to strengthen the antitumor efficacy of an anti-GD2 CAR-T therapy in a preclinical study.[71]

In addition to a marked immunosuppressive signature, the GBM tumor microenvironment also presents challenges with respect to antigen escape. Loss of target antigen represents the paradox of effective CAR-T treatment; postinfusion antigen loss is indicative of effective antitumor

Table 2
Active trials of CAR-T cell therapies for glioblastoma

NCT#/Institution	Study Name	Phase	Target Antigen	ROA	Comments
NCT04385173, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China	Pilot Study of B7-H3 CAR-T in Treating Patients With Recurrent and Refractory Glioblastoma	I	B7-H3	Intratumoral/ intracerebroventricular	No lymphodepleting chemotherapy. Locoregional administration. Inclusion criteria require B7-H3-positive tumor by IHC with H-score $\geq$50.
NCT04077866, Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China	B7-H3 CAR-T for Recurrent or Refractory Glioblastoma	I/II	B7-H3	Intratumoral/ intracerebroventricular	Randomized parallel-arm study to evaluate head-to-head safety and efficacy of B7-H3 CAR-T to temozolomide alone in relapsed/refractory GBM patients.
NCT04045847, Xijing Hospital, Xi'an, Shaanxi, China	CD147-CART Cells in Patients With Recurrent Malignant Glioma	I	CD147	Intracavitary	Estimated enrollment n = 31 patients.
NCT04214392, City of Hope Medical Center, Duarte, California	Chimeric Antigen Receptor (CAR) T Cells With a Chlorotoxin Tumor-Targeting Domain for the Treatment of $MPP2^{+}$ Recurrent or Progressive Glioblastoma	I	MPP2	Dual delivery	Recognition domain of CAR derived from CTLX, a natural peptide from the nontoxic venom component of death stalker scorpion venom. Orthotopic xenograft mice models have demonstrated antitumor activity of CTLX-directed CAR therapy for $MPP2^{+}$ tumors.[63]
NCT04003649, City of Hope Medical Center, Duarte, California	IL13Ralpha2-Targeted Chimeric Antigen Receptor (CAR) T Cells With or Without Nivolumab and Ipilimumab in Treating Patients With Recurrent or Refractory Glioblastoma	I	IL13Rα2	Intratumoral/intraventricular	CAR-T combination with immune checkpoint blockade therapy (nivolumab and ipilimumab).

NCT02208362, City of Hope Medical Center, Duarte, California	Genetically Modified T-cells in Treating Patients With Recurrent or Refractory Malignant Glioma	I	IL13Rα2	Intracavitary/intratumoral, intraventricular	Published cohort of 3 patients suggests second-generation IL13Rα2 CAR has antitumor activity.[64]
NCT03389230, City of Hope Medical Center, Duarte, California	Memory-Enriched T Cells in Treating Patients With Recurrent or Refractory Grade III-IV Glioma	I	HER2	Intratumoral/intracavitary, intraventricular	Locoregional delivery of HER2-directed CAR to the brain for GBM patients.
NCT02664363, The Preston Robert Tisch Brain Tumor Center at Duke, Durham, North Carolina	EGFRvIII CAR T Cells for Newly-Diagnosed WHO Grade IV Malignant Glioma (ExCeL)	I	EGFRvIII	Intravenous	Dose-intensified lymphodepletion preconditioning to grade 3 lymphopenia with TMZ. Dose-escalation study with ^{111}In-labeled CARs.
NCT03726515, Abramson Cancer Center of the University of Pennsylvania, Philadelphia, Pennsylvania	CART-EGFRvIII + Pembrolizumab in GBM	I	EGFRvIII	Intravenous	Based on upregulation of anti-inflammatory molecules including PD-L1 in this group's previous study,[5] pembrolizumab (PD-1 inhibitor) treatment added in combination EGFRvIII-directed CAR.[65]

Abbreviations: CTLX, chlorotoxin; IHC, Immunohistochemistry; ROA, Route of administration; WHO, World Health Organization.
Data accessed from clinicaltrials.gov on November 6, 2020.

activity, but simultaneously impairs the honing mechanism of CAR-T cells and enables tumor escape, because the reengineered immune cells have lost their target on the GBM tumor cell surface that ensures appropriate localization, engagement, and activation of the T-cell construct. Bivalent and trivalent CARs that incorporate multiple well-characterized GBM antigen targets including IL13Rα2, EGFRvIII, HER2, and EphA2 are currently under investigation in preclinical animal models.[12] A preclinical trial at Baylor College of Medicine, using a trispecific CAR directed against IL13Rα2 and HER2 and EphA2 demonstrated significant antitumor activity and broader therapeutic activity[41] than a similar bivalent construct targeting IL13Rα2 and HER2 also designed by the group.[72] However, loss of target antigen was common in surviving GBM cells suggesting tumor escape.[12,41,72]

Ongoing and future trials that investigate the safety, tolerability, and activity of CAR-T cells that target novel antigens, invoke combination therapy, and address GBM tumor microenvironment considerations may provide new avenues for therapeutic development. VSTs, lymphodepletion regimens, and immune checkpoint blockade represent a few of the emergent strategies that are under investigation in active trials. Given the high unmet clinical need for relapsed/refractory GBM patients and increasingly well-characterized role of the immune system in GBM pathogenesis, clinical advancement CAR-T cell therapies from preclinical models to pivotal-stage trials is top-of-mind for clinicians and investigators because these immunotherapies may substantially improve clinical outcomes for this patient population.

CLINICS CARE POINTS

- Persistence and expansion of CAR-T cells post-infusion is limited in most patients, with lymphodepletion preconditioning and use of VSTs as potential strategies to overcome this limitation to durable therapeutic response.
- Dose-limiting toxicities with CAR-T administration, although rare, can result in potentially fatal complications including acute dyspnea and severe hypotension and patients should be closely monitored when titrating a patient to higher CAR-T cell doses.
- Preliminary evidence suggests intraventricular administration may be relevant for the treatment of leptomeningeal disease and spinal metastases and able to attenuate tumor growth at sites distant to the point of administration.
- A single study indicates that patients with no salvage therapy before CAR-T administration may have substantially longer median overall survival compared with their counterparts who did receive prior salvage therapy, suggesting that prior disease course and treatment history is relevant to a patient's course.

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DISCLOSURE

D.M. O'Rourke and Z.A. Binder are inventors on patents related to CAR-T cells that have been filed by the University of Pennsylvania.

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