Neuro-Oncology

XX(XX), 1–17, 2024 | https://doi.org/10.1093/neuonc/noae203 | Advance Access date 25 October 2024

Chimeric antigen receptor T-cell therapy in patients with malignant glioma—From neuroimmunology to clinical trial design considerations

Marco Gallus, Jacob S. Young, Sarah Cook Quackenbush, Mustafa Khasraw, John de Groot, and Hideho Okada

All author affiliations are listed at the end of the article

Corresponding Author: Hideho Okada, MD PhD, UCSF Department of Neurological Surgery,1450 3rd Street, Box 0520, San Francisco, CA 94158, USA (hideho.okada@ucsf.edu).

Abstract

Clinical trials evaluating chimeric antigen receptor (CAR) T-cell therapy in patients with malignant gliomas have shown some early promise in pediatric and adult patients. However, the long-term benefits and safety for patients remain to be established. The ultimate success of CAR T-cell therapy for malignant glioma will require the integration of an in-depth understanding of the immunology of the central nervous system (CNS) parenchyma with strategies to overcome the paucity and heterogeneous expression of glioma-specific antigens. We also need to address the cold (immunosuppressive) microenvironment, exhaustion of the CAR T-cells, as well as local and systemic immunosuppression. Here, we discuss the basics and scientific considerations for CAR T-cell therapies and highlight recent clinical trials. To help identify optimal CAR T-cell administration routes, we summarize our current understanding of CNS immunology and T-cell homing to the CNS. We also discuss challenges and opportunities related to clinical trial design and patient safety/monitoring. Finally, we provide our perspective on future prospects in CAR T-cell therapy for malignant gliomas by discussing combinations and novel engineering strategies to overcome immuno-regulatory mechanisms. We hope this review will serve as a basis for advancing the field in a multiple discipline-based and collaborative manner.

Key Points

1. We summarize basics, recent clinical trials, and our current understanding of central nervous system immunology.
2. We discuss issues related to clinical trial design and patient safety/monitoring.
3. Future developments need to integrate these considerations and novel technologies.

The promising outcomes of chimeric antigen receptor (CAR) therapy in treating hematological malignancies[1–3] have catalyzed preclinical and clinical investigations into its development for solid tumors within the central nervous system (CNS; Supplementary Table 1). While long-term benefits and safety still need to be established, recent studies have provided valuable insights into the challenges and potential solutions for enhancing the efficacy of CAR T-cell therapy for glioma in both pediatric and adult populations.

This review provides a comprehensive and critical examination of recent preclinical and clinical approaches utilizing CAR T-cell therapy for malignant glioma. We delve into inherent anatomical and biological challenges, advances in CAR T-cell bioengineering, and clinical trial design aspects. Growing interest from clinicians, scientists, and patients in this area is driven by the rapid advancements, burgeoning clinical data, and innovative strategies that promise improved efficacy, durable responses, and safety in the treatment of malignant gliomas, which is one of the areas of the greatest unmet need in oncology. This review aims to guide research and foster robust multidisciplinary collaboration and trial designs to enhance patient care and treatment outcomes.

CAR Definition and Structures

CARs represent synthetic receptors designed to confer the CAR-transduced cells with an ability to recognize cell-surface proteins on the target cells, such as tumor cells.[4] CARs typically comprise 4 main components: (1) an extracellular target antigen-binding domain, (2) a hinge region, (3) a transmembrane domain, and (4) one or more intracellular signaling domains.

The antigen-binding domains, derived from the variable heavy and light (VL) chains of monoclonal antibodies, are linked via a flexible linker, forming a single-chain variable fragment (scFv). These scFvs recognize extracellular surface antigens, leading to major histocompatibility complex (MHC)-independent T-cell activation. However, CARs with scFV capable of specifically recognizing intracellular tumor-associated antigens presented in the peptide-MHC complex (TCR-mimic CARs) represent an active area of investigation.[5]

The hinge or spacer region is the extracellular structural region that extends the antigen-binding domains from the transmembrane domain to access the targeted epitope. Differences in the length and composition of the hinge region can affect CAR functionality in terms of flexibility, CAR expression, signaling, and epitope recognition.[6] The most commonly used hinge regions are designed with amino acid sequences derived from CD8, CD28, IgG1, or IgG4.[7] Spacer length is empirically determined for each specific antigen-binding domain pair, as it has been demonstrated to be decisive for optimal activity.[8]

The transmembrane domain serves as an anchor of the CAR to the T-cell membrane. It can also impact CAR T-cell function, including expression level, stability, signaling/synapse formation, and interaction with endogenous signaling molecules. Common transmembrane domains are obtained from natural proteins such as CD3ζ, CD4, CD8α, or CD28.[9]

Intracellular signaling domains, a crucial focus in CAR engineering, have evolved over time. First-generation CARs utilized CD3ζ or FcRγ signaling domains with limited efficacy.[10] Second-generation CARs incorporated one co-stimulatory domain in addition to the CD3ζ intracellular signaling domain. The 2 most common co-stimulatory domains, CD28[11] and 4-1BB (CD137),[12] demonstrated high response rates in patients with distinct functional and metabolic features. CARs with CD28 domains caused differentiation into effector memory T-cells using aerobic glycolysis, while CARs with the 4-1BB domains caused differentiation into central memory T-cells, increasing mitochondrial biogenesis and oxidative metabolism.[13] Second-generation CAR T-cells demonstrated reproducible clinical success in patients with B-cell malignancies.[2] Third-generation CAR T-cells, aiming for enhanced efficacy, included 2 co-stimulatory domains (eg, CD28 and 4-1BB) along with CD3ζ.[14] Fourth-generation CAR T-cells (also known as T-cells redirected for antigen-unrestricted cytokine-initiated killing [TRUCKs] or armored CARs) were engineered to release a transgenic cytokine upon CAR signaling in the targeted tumor tissue, combining the direct antitumor effect of the CAR T-cell with the immune modulating features of the delivered cytokine.[15,16]

Regarding gene transfer systems, both viral and non-viral methods have been evaluated.[17] Viral vectors, such as γ-retroviruses and lentiviruses, offer efficient genomic integration and long-term expression but pose safety concerns due to insertional mutagenicity, complicating regulatory constraints.[18] Non-viral methods are being explored to mitigate these concerns. Transposons, like the reconstructed sleeping beauty, allow for larger payloads but show lower transfection efficiencies.[19] Non-integrative methods, including episomal DNA nano vectors and mRNA, offer alternatives with potentially safer profiles. Transient CAR expression via mRNA has shown a favorable safety profile, though sustained antitumor responses remain unproven.[20]

Novel Experimental CAR Circuits to Improve the Efficacy and Safety of CARs

Malignant brain tumors are characterized by genetic and molecular diversity, resulting in the expression of various antigens. This antigen heterogeneity challenges the development of CAR T-cell therapies as tumors can evade immune detection by downregulation or losing the expression of certain antigens.[21,22]

Various CAR designs have been evaluated in this context to enhance the efficacy and safety of CAR T-cells (Figure 1). Multi-specific CAR T-cells have been designed to target multiple antigens using either "AND" or "OR" gates. The "AND" gate system requires the CAR to bind all target antigens to mediate the antitumor activity, thereby reducing the cross-reactivity against non-tumor cells expressing only one target antigens. On the other hand, "OR" gate[28] is designed to maximize the efficacy against tumor cells with heterogeneous antigen expression by allowing the CAR signaling when at least one of the target antigens is present in tumor cells.[18,19] To provide more versatility of antigen-specificity, Universal CARs (Uni-CARs) have been developed whose antigen-binding domains bind to exogenous antigen-specific molecules, such as scFV, monoclonal antibodies, or tumor-specific ligands.[27]

To further improve safety and tumor-tissue specificity, antigen-dependent inducible CAR T-cells have been developed. CARs integrated with synthetic Notch Receptor (synNotch) receptors[23,24] or Synthetic Intramembrane Proteolysis Receptors (SNIPRs)[24] allow CNS tumor- or CNS tissue-specific induction of CAR. Because synNotch-or SNIPR-induced CAR expression is transient, another benefit of these systems is they prevent tonic CAR signaling, which leads to exhaustion of the T-cells.[23,24,29]

Another on-off CAR design approach is represented by split CARs characterized by their capacity to target 2 independent antigens with the co-stimulatory domains split by 2 CARs. In split CARs, CAR T-cell activation requires the presence of both antigens to assemble the functional co-stimulatory domain. Furthermore, split CAR constructs integrating drug-responsible domains (degrons) allow the on/off switch CAR signals to be controlled by small molecule drugs.[25] Alternatively, drug-induced dimerization can serve as an ON-switch for split CAR circuit design.[26]

Furthermore, split, universal, and programmable (SUPRA) CAR systems have been developed to allow to switch targets without reengineering the cells, fine-tune T-cell activation and strength, and respond to multiple antigens. Supra CARs also have a split CAR T construct,

Neuro-Oncology

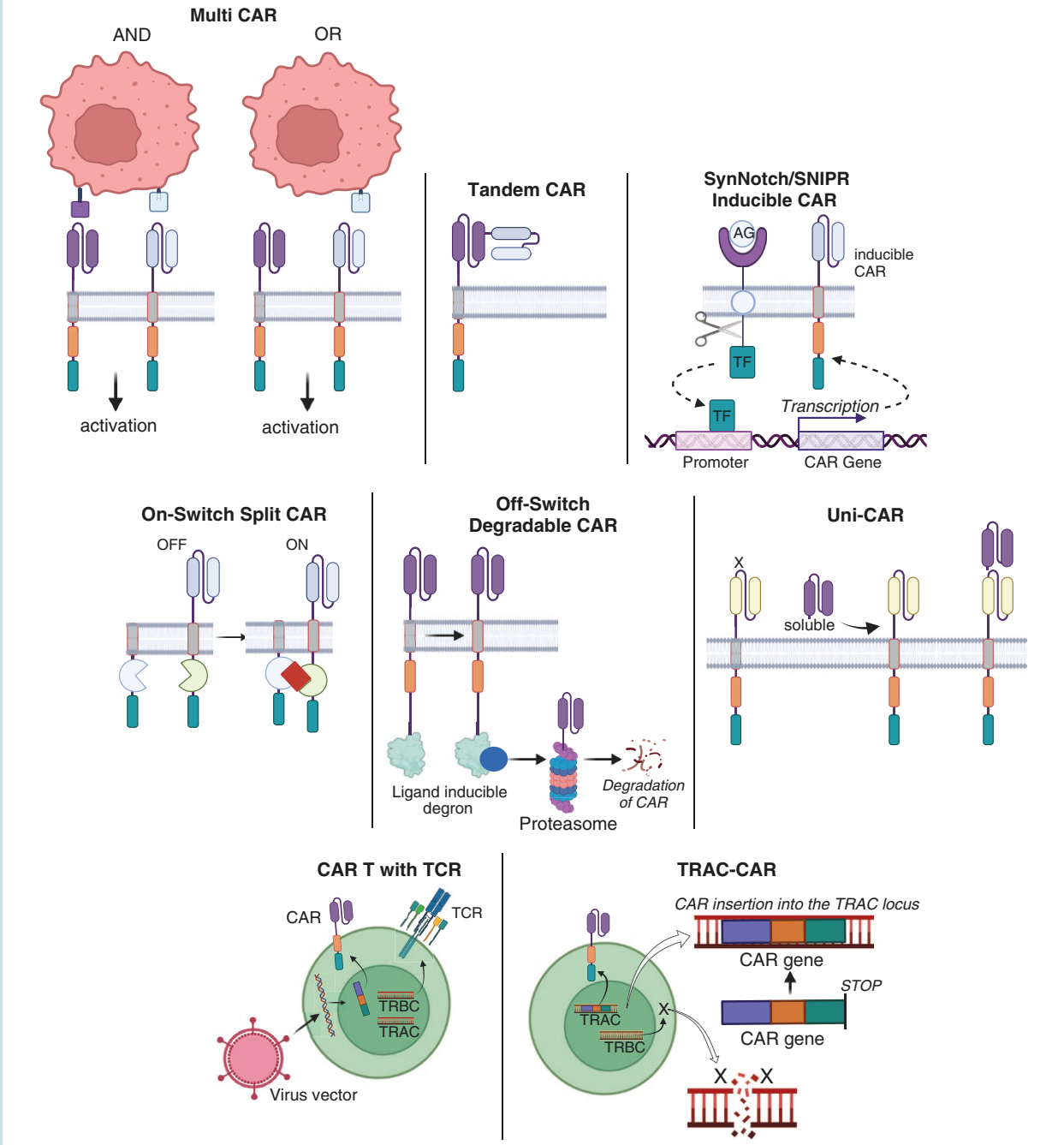


Figure 1. Novel chimeric antigen receptor (CAR) T-cell engineering concepts. Multi-specific CAR T-cells have been designed to target multiple antigens using either "AND" or "OR" gates. Tandem CAR T-cells are composed of a single CAR structure that targets 2 tumor antigens with a distinct antigen recognition domain (scFv) linked consecutively with a single intracellular domain. Furthermore, CARs integrated with synthetic Notch Receptor (synNotch) receptors[23,24] or Synthetic Intramembrane Proteolysis Receptors (SNIPRs)[24] allow central nervous system (CNS) tumor- or CNS tissue-specific induction of CAR. Another on-off CAR design approach is represented by split CARs characterized by their capacity to target 2 independent antigens with the co-stimulatory domains split by 2 CARs. In split CARs, CAR T-cell activation requires the presence of both antigens to assemble the functional co-stimulatory domain. Furthermore, split CAR constructs integrating drug-responsible domains (degrons) allow the on/off switch CAR signals to be controlled by small molecule drugs.[25] Alternatively, drug-induced dimerization can serve as an ON-switch for split CAR circuit design.[26] To provide more versatility of antigen-specificity, Universal CARs (Uni-CARs) have been developed whose antigen-binding domains bind to exogenous antigen-specific molecules, such as scFV, monoclonal antibodies, or tumor-specific ligands.[27] Recent genomic engineering approaches have reported further improved CAR functionality. For example, CRISPR/Cas9 gene screening and editing have been used to disrupt inhibitory genes or endogenous T-cell receptor expression (TRAC-CAR).

allowing for one co-stimulatory domain (B-zip) to bind several targeting domains (A-zip).[30]

Recent genomic engineering approaches have reported further improved CAR functionality. For example, CRISPR/Cas9 gene screening and editing have been used to disrupt inhibitory genes, such as RASA2[31] or to a transgene-derived expression of FOXO1.[32]

CAR T-Cell Targeting the Immune Suppressive TME

Immune suppression in the tumor microenvironment (TME) poses significant hurdles for effective cellular immunotherapy in both pediatric[33–35] and adult gliomas.[36] In particular, CNS regions controlling life-sustaining functions, such as midline structures[37] are characterized by significantly lower numbers of immune cells and chemokines compared to other CNS parts,[37,38] impacting the efficacy of immunotherapy.[33] Understanding these variations and the underlying mechanism is crucial for developing efficient therapies.

Immune suppression in the TME in malignant glioma occurs through various pathways. Malignant glioma samples have been found to express a range of immunomodulatory factors including, but not limited to, Indoleamine 2,3-dioxygenase (IDO), Transforming growth factor beta 1 (TGFβ-1), Interleukin (IL)-6, and Macrophage migration inhibitory factor (MIF).[39–41]

The overall effect of secretion of these factors is a negative cycle in which immunosuppressive cells are recruited and differentiated in the TME, leading to further secretion of immunosuppressive molecules. IL-6 secretion by endothelial cells results in immunosuppressive macrophage differentiation,[42] with tumor-associated macrophages (TAMs) and microglia having reduced expression of MHCII molecules.[43,44] The secretion of TGFβ-1 by microglia acts on glioma cells to increase glioma invasiveness and tumor growth[45] and also impedes migration of T-cells to the TME[46] and IDO, leading to the recruitment of immunosuppressive Tregs.[47,48] $CD8^+$ T-cell and NK cell-mediated cytotoxicity is further hampered by the expression of MIF, which leads to the downregulation of the NKG2D activating receptor on these cells.[40] T-cell responses in the TME are also reduced through the downregulation of MHC class I molecules by glioma.[49,50] In order to counteract these immunosuppressive molecules, CAR T-cells have been engineered to express multiple T-cell stimulating cytokines such as IL-12, IL-15, IL-18, and IL-21.[51] In an alternate approach, CAR T-cells can be engineered to (1) modify the immunosuppressive cytokine receptor to make it activating instead of suppressive,[52] (2) knock down immunosuppressive cytokine receptors,[53] (3) have CAR receptors that are stimulated by immunosuppressive factors,[54,55] or (4) block the cytokine signaling altogether,[56] the latter of which has been shown to be both safe and feasible in patients.[57]

Microglia and TAMs are the largest immune subpopulations that infiltrate gliomas,[58] and are a major contributor to the immunosuppressive environment, especially for T-cells.[59] CARs have been engineered to overcome this TAM-induced immunosuppression, such as increasing CD47-induced phagocytosis,[60] or using CAR T to target immunosuppressive TAMs themselves.[61]

Immunosuppression is not limited to the TME, and in fact, malignant glioma results in a great level of systemic immunosuppression, similar to that found in patients diagnosed with AIDS.[62] Patients with malignant glioma often present lymphopenia even prior to treatment,[62,63] and administration of dexamethasone increases this systemic lymphopenia.[64] Although standard-of-care administration of temozolomide can also exacerbate systemic lymphopenia,[62,64,65] temozolomide-induced lymphodepletion may enhance the antitumor efficacy of CAR T-cells.[66,67] Although some of these cells are sequestered in the bone marrow,[62] a reduction in T-cell proliferation occurs due to serum-associated factors.[68–70] Another factor to be considered with immunosuppression and malignant glioma is the median age of GBM diagnosis, which is 65 years.[71] During the natural aging process, there are diminished T-cell numbers and responses due to cellular senescence, thymic involution, and reduced cytotoxic T-cell functions.[67,72] Furthermore, aged T-cells may have reduced the transduction efficiency of the CAR construct, which negatively impacts antitumor efficacy.[67]

Altogether, the high amount of immunosuppression in malignant gliomas is a factor that should be taken into account when designing and administering CAR T-cell therapy to malignant patients. Therapeutic strategies to modulate the TME should be combined with CAR T-cells. While CAR T-cells can target a small number of antigens against highly heterogeneous tumors,[22] enhancing antigen-presentation may facilitate the activation of immune responses against other tumor-derived antigens not targeted by CARs.[73,74]

Clinical Trials

Clinical Trial Design

Malignant glioma clinical trials for immune and cellular therapeutics require special considerations beyond traditional drug therapy trials.[75,76] These include establishing safety, optimal methods of administration with proof of cell delivery and biodistribution throughout the tumor, optimal setting in the treatment course (newly diagnosed versus recurrent), the role of neoadjuvant cell delivery to enhance immunologic effect, and determination of ideal drug-cell combinatorial approaches.[76]

The critical first step in early-phase trials is determining the nature, frequency, and severity of adverse reactions and the relationship to cell dose, and identifying the therapeutic window and ideally the threshold or minimal effective dose.[77] These evaluations are essential because preclinical data may not adequately inform an appropriate cell therapy starting dose. In phase I first-in-human studies, simultaneous treatment of more than one patient at a time may be risky (Figure 2A), and instead, a staggered enrollment approach may be suitable with subsequent patients enrolled after the dose-limiting toxicity (DLT) period for the previous patient has lapsed (21 to 28 days minimum; Figure 2). The staggered approach can be utilized within a cohort or between cohorts to allow for a longer monitoring period and ensure safety before increasing the dose on additional patients (Figure 2B). The staggered approach

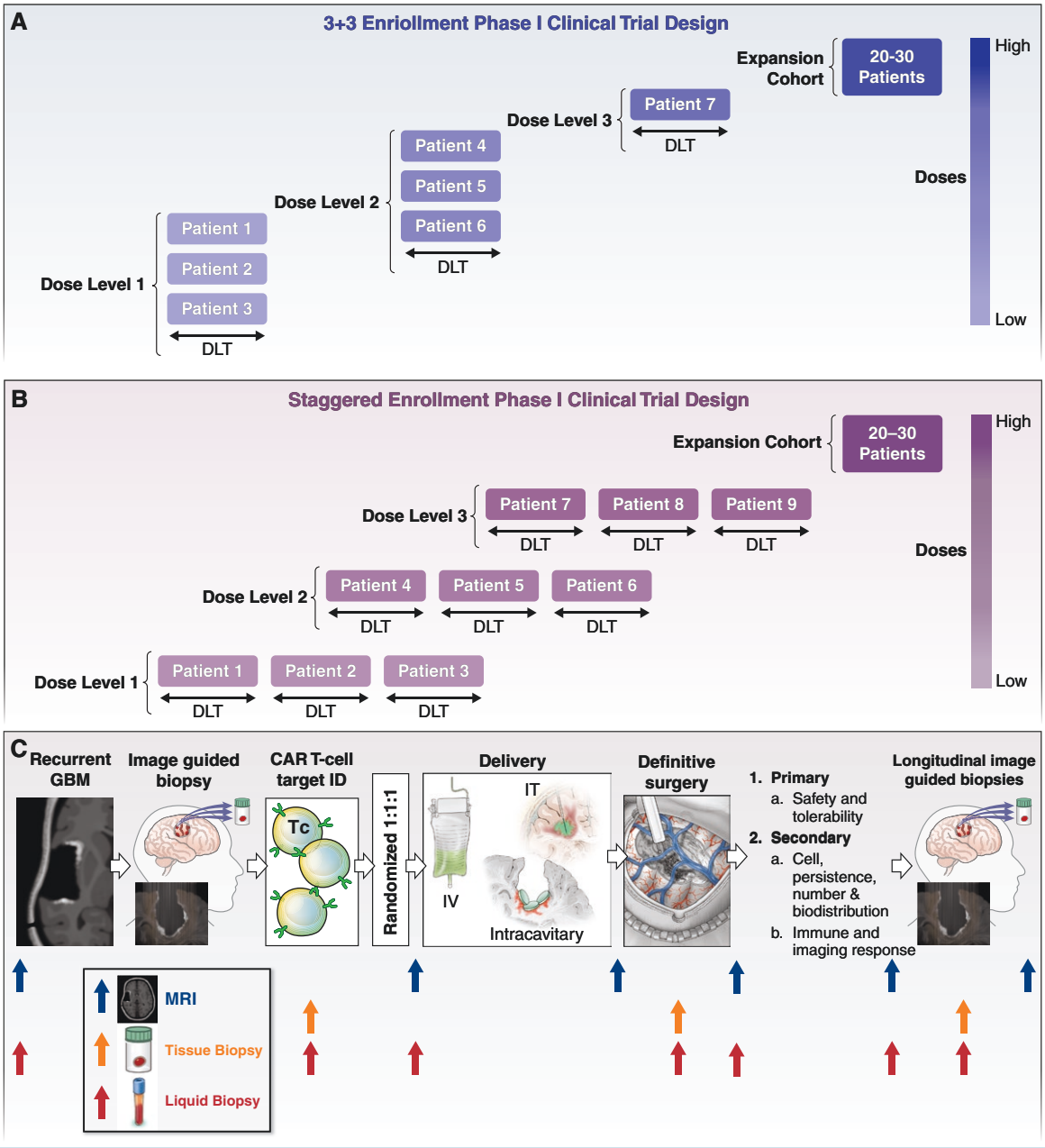


Figure 2. Clinical trial design. The critical first step in early-phase trials of autologous chimeric antigen receptor (CAR) T-cells for GBM is to determine safety, including the nature, frequency, and severity of adverse reactions and their relationship to cell dose, and to identify the therapeutic window and, ideally, the threshold or minimal effective dose. (A) Traditional 3 + 3 designs are commonly used in other malignancies, including central nervous system (CNS) tumors but simultaneous treatment of more than one patient at a time may be risky. (B) A staggered approach can be utilized within or between cohorts to allow for a longer monitoring period to assess dose-limiting toxicities (DLT) and assure systemic and neurologic safety prior to increasing the dose on additional patients. (C) An illustrative example of a theoretical trial design that allows for the evaluation of multiple delivery strategies nested within the same trial. The design also incorporates tissue collection before and after CAR T-cell administration along with serial longitudinal sampling to facilitate biological correlative studies.

is particularly appropriate since infused cells may persist for an extended period after delivery, and induce on-target/off-tissue toxicity.[78] Systemically administered cells may also distribute to systemic tissues and organs, leading to the risk of additional and unpredictable toxicities. Furthermore, local intracranial administration of cells may require invasive procedures, which may have considerable risk, particularly if delivery is required to areas with critical functions. Finally, if a study is designed with multiple doses or combined systemic and local infusions, DLT evaluation should appropriately cover the period and types of adverse events.

CAR T-cell therapies approved by the FDA for systemic malignancies are all delivered via the intravenous (IV) route after a lymphodepletion regimen.[79,80] Preconditioning with lymphodepletion prior to CAR T-cell therapy has become a critical determinant in achieving long-term therapeutic responses in hematologic malignancies,[81] however the optimal lymphodepletion method to assist cell survival in glioma patients is currently unknown. Although temozolomide is commonly used in patients with primary brain tumors and induces lymphopenia,[82] the precise effects of the cytoxan plus fludarabine regimen, which is used for lymphodepletion in other tumor types, has not been determined in neuro-oncology patients who previously received temozolomide. Collaboration with cell therapy teams to oversee the safe administration and monitoring of these regimens is required. An additional complicating factor is the prevalence of corticosteroid administration to patients with primary brain tumors to control tumor- or therapy-related vasogenic cerebral edema and associated symptoms. The impact of glucocorticoids on CAR T-cell therapy remains a topic of ongoing debate. Studies have reported no negative impact on the efficacy of CAR T-cell therapy in the context of B-cell malignancies.[83–85] However, other studies suggest that the lympholytic potential of steroids could render a cell therapy product ineffective[80,86] potentially making them a contraindication in cell therapy trials.[87] There is also the possibility that the cell therapy itself may induce cerebral edema, which creates a challenge to symptom management, although alternative methods, such as mannitol and hypertonic saline, can be used but are limited to the inpatient setting. Efforts are ongoing using genetically engineered knockout of the glucocorticoid receptor to render CAR-T-cells resistant to steroid administration.[88]

As extensively discussed in Overcoming the Immune Privilege of the CNS Parenchyma, the optimal route of cell delivery needs to be established. To this end, dedicated clinical trials are required prior to larger phase II clinical trials using neoadjuvant designs where cell therapy is delivered to recurrent glioma patients via multiple routes prior to surgical resection. This approach would allow for the intensive evaluation of CAR T-cell number and distribution throughout the tumor (Figure 2C).

A critical design component involves the selection of the ideal patient population. Multiple confounding variables create challenges unique to CAR T-cell therapies. The logistics and timeline of manufacturing cell therapies may influence the trial design, such as stage of disease (newly diagnosed vs recurrent disease), and cohort sizes. Cell products must be manufactured separately for each patient for autologous cell therapies. Monitoring the phenotype of each CAR T-cell product is important, as it may vary between patients and can influence their outcomes.[89–91] Manufacturing cells at the time of recurrence is challenging due to the timeline required to generate adequate cells for treatment, which usually takes weeks. Those patients able to tolerate a delay for this therapy may reflect a lower level of systemic immune suppression and may bias patient selection and response to treatment and outcomes. Additionally, a failure in product manufacturing would lead to an inability to treat the patient despite patient eligibility. Collecting, expanding, and release testing of cell products from patients at the time of diagnosis with the intent to use them at recurrence is fraught with practical challenges not to mention the high cost.

CAR T-cell therapy for newly diagnosed patients following the standard-of-care 6 weeks of chemoradiotherapy is more optimal. At a "healthier" disease state, lymphocytes may be less damaged, increasing the chances of manufacturing success. Although this window allows for cell manufacturing, patient eligibility post-chemoradiotherapy may adversely change. Furthermore, the impact of radiation on tumor sensitivity to cell therapy and the ability of CAR T-cells to penetrate and biodistribution in tumors is unknown. Feasibility is an important endpoint of all early-phase cell therapy trials. Early-phase studies should carefully collect data on the manufacturing failure rate and the reasons for these failures to facilitate subsequent trial design strategies for patient selection. Future approaches may include allogeneic stem cells as potential "off-the-shelf" therapeutic agents.[88]

Beyond the assessment of clinical safety, additional secondary objectives of early-phase trials are generally to assess preliminary clinical activity that could suggest potential efficacy. As mentioned, cell persistence in tumors, cerebrospinal fluid (CSF) and blood, and tumor biodistribution are critical basic components of understanding potential efficacy. Other common measures include changes in immune function, tumor response, immune-mediated toxicity, or other physiologic responses should be measured. This highlights the importance of integrating intense systemic, CSF, and immune biomarker and imaging endpoints into studies. However, there is uncertainty surrounding the value of surrogate endpoint changes, such as PFS, cytokine level changes, and transient imaging changes in early phases I and II clinical trials, to predict future benefits in randomized phase III clinical trials. The use of concurrently enrolled or carefully selected external controls for all biomarker and outcome measures should be considered for these trials. A summary of critical decisions relevant to the design of a clinical trial involving CAR T for glioma is outlined in Figure 3.

Clinical Trials for CAR T-Cell Therapy in Adult Patients With Glioma

CARs targeting several glioma-associated antigens have been investigated in pilot or phase I clinical trials, such as interleukin 13 receptor subunit alpha 2 (IL13Rα2) epidermal growth factor receptor variant III (EGFRvIII), human epidermal growth factor receptor 2 (HER2), erythropoietin-producing hepatocellular carcinoma A2 receptor (EphA2) and disialoganglioside 2 (GD2; Supplementary Table 1).

Initial efforts were focused on IL13Rα2 because of its prevalence in malignant glioma and its correlation with aggressive tumor behavior and poor prognosis.[92] Following several case reports[92–94] Brown et al. reported the results of the so-far largest CAR T-cell trial in glioma patients (NCT02208362).[95] This dose-escalation phase I trial assessed the safety of CAR-T therapy targeting IL-13Rα2 in 65 patients (including 41 glioblastomas, isocitrate dehydrogenase gene, IDH, wild type, and a variety of grades 3 and 4 gliomas including cases with confirmed IDH

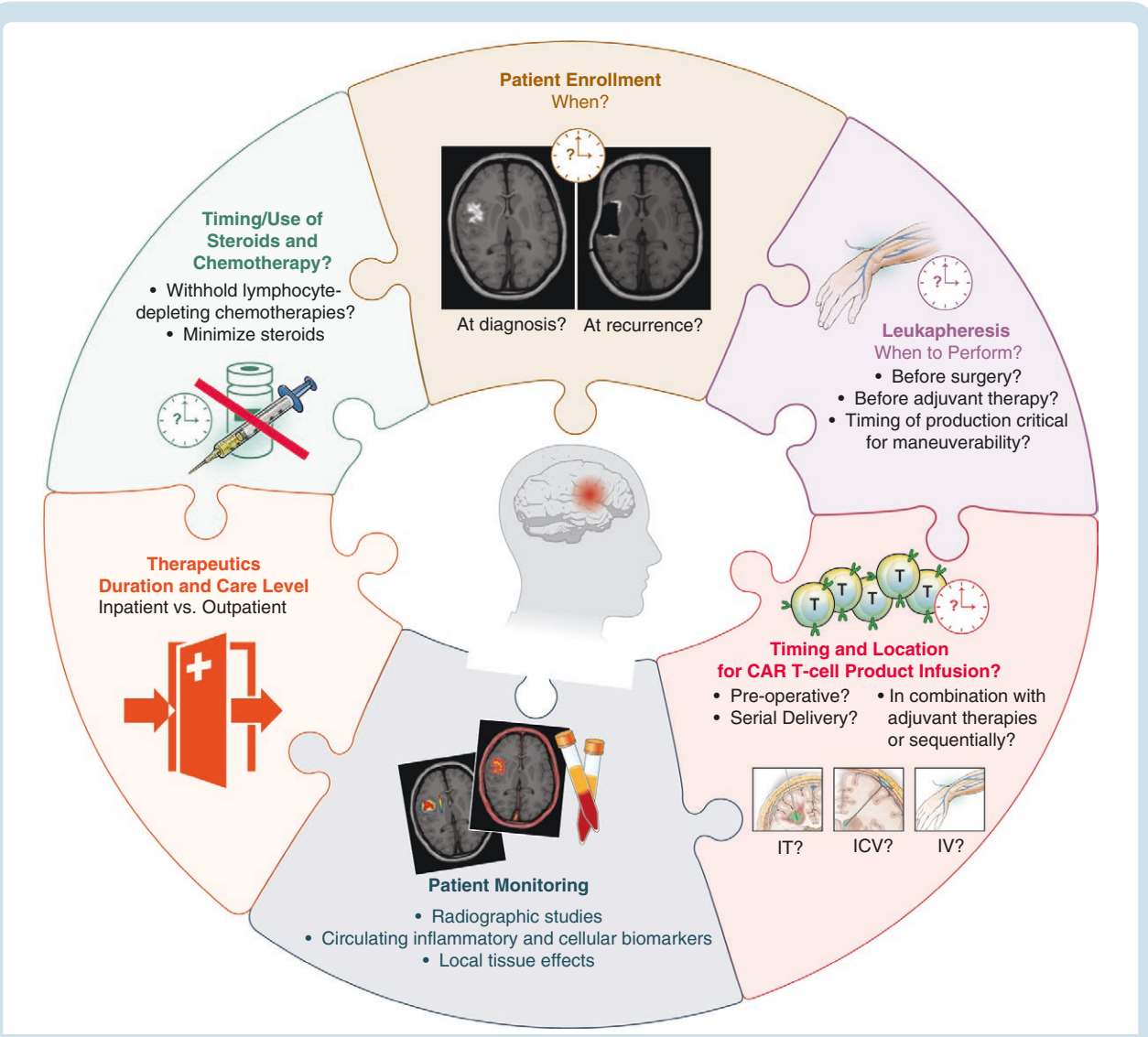


Figure 3. Outline of critical decisions relevant for the design of a clinical trial involving CAR T-cell therapy targeting glioma. Critical considerations should include the timing of enrollment, leukapheresis, and T-cell administration, duration of the treatment and surveillance, the application route, assessment of treatment response, and combination with other treatments that may enhance or suppress the efficacy of CAR T.

mutation). Eligibility for the trial included confirmation of IL-13Rα2 tumor expression. Fifty-eight patients received at least 3 CAR T-cell infusions. Around 75% of the participants had more than one prior recurrence, and most had an IDH-wild-type recurrent glioblastoma (41 of the 58). The trial investigated 3 routes of locoregional T-cell administration (intratumoral [IT], intracerebroventricular [ICV], and dual [IT/ICV]) and 2 manufacturing platforms (central memory T-cells [Tcm] and naïve, stem cell memory, and central memory T-cells [Tn/mem]), with the final arm utilizing dual intratumoral/intraventricular delivery and Tn/mem cells. While no DLTs were observed, one-third of the patients experienced grade 3 toxicities that were potentially or likely associated with the CAR T-cell therapy. Notably, there was one case each of grade 3 encephalopathy and grade 3 ataxia, and 2 patients developed grade 4 cerebral edema shortly after the first cycle of CAR T-cell treatment. A clinical maximum feasible dose of 200 million CAR T-cells per infusion cycle was achieved for the optimized (dual intratumoral/intraventricular application of the Tn/mem arm). The study reported 2 partial and one complete response, but all in patients with IDH-mutated tumors. The median overall survival for all patients with mixed diagnoses was 7.7 months, and for the optimized arm, it was 10.2 months. Despite half of the patients in this study exhibiting radiologically stable disease, the overall survival rates did not surpass those reported in recent clinical studies of recurrent glioma.[96–98]

The exploratory translational endpoints in these studies indicate that CAR T-cells administered IT or ICV were detectable in the CSF and tumor cavity fluid for more than 7 days in some patients, and interestingly, they were also found in the peripheral blood. Notably, their quantity positively correlated with the application of dual delivery.

Post-treatment, inflammatory cytokines, particularly those associated with the interferon (IFN)γ pathway, were significantly elevated in the CSF, suggesting the potential for these inflammatory cytokines as biomarkers for evaluating CAR T-cell activity.

Beyond that, a phase I trial examined off-the-shelf, healthy donor-derived, allogeneic steroid-resistant CAR T-cells coupled with recombinant human IL-2 and systemic dexamethasone in a cohort of 6 patients.[88] The IL13Rα2-targeted CAR + (IL13-zetakine+) products were generated from healthy-donor-derived T-cells and genetically engineered using zinc finger nucleases to permanently disrupt the glucocorticoid receptor (GRm13Z40-2) to endow resistance to glucocorticoids. The treatment was well tolerated, and transient tumor reduction or tumor necrosis at the site of T-cell infusion was observed in 4 of the 6 treated patients.

EGFRvIII, a mutated variant of the EGFR, is the predominant mutation of this receptor found in malignant gliomas. O'Rourke et al. evaluated a single IV delivery of CAR T-cells targeting EGFRvIII in 10 patients with recurrent GBM.[22] Seizures, weakness, and intratumoral hemorrhage were reported in 3 patients. The median overall survival was approximately 8 months, with 1 exhibiting residual stable disease lasting over 18 months. Notably, some IV-infused anti-EGFRvIII CAR T cells were observed in GBM regions, accompanied by antigen decrease in 5 of the 7 patients, while no off-tumor toxicity was reported.

Goff et al. combined post-lymphodepletion IV infusion of anti-EGFRvIII CAR T-cells with recombinant IL-2 in 18 patients with recurrent GBM.[99] Adverse events included severe hypoxia in 2 patients, with 1 resulting in treatment-related mortality, likely due to pulmonary edema resulting from congestion of the pulmonary vasculature caused by activated T-cells. The median overall survival stood at 6.9 months, with 2 patients surpassing 1 year and a third patient reaching 59 months. Most patients experienced progressive disease, with a median progression-free survival of 1.3 months.

Choi et al. recently reported an interim analysis of the first 3 patients in a first-in-human study evaluating 1 or 2 doses of intraventricularly administered CARv3-TEAM-E T-cells in EGFRvIII + rGBM patients.[100] The novel construct targets EGFRvIII through a second-generation CAR while also secreting T-cell–engaging antibody molecules (TEAMs) against wild-type EGFR, which is not expressed in the normal brain but is nearly always expressed in glioblastoma. All patients experienced fevers requiring anakinra and 2 grade 3 toxicities were observed: Encephalopathy for 3 days in 1 patient and fatigue for 8 days in another. Two patients developed cyclic fevers with transient pulmonary nodules and ground-glass opacities. None of the participants received glucocorticoids during the initial post-treatment phase or for any therapy-related indication. Despite the initial reduction in tumor contrast enhancement, which was consistent with a radiographic response within days of treatment, tumor progression was observed in 2 of the 3 participants, which correlated with limited persistence of the CARv3-TEAM-E T-cells. Interestingly, CAR T-cells were observed in the blood, with 2% or less of them showing surface-bound TEAM, while in the CSF samples surface bound TEAM varied between 17.6% and 56.2%, suggesting that TEAM-E may facilitate safe and local targeting of wild-type EGFR in the CNS.

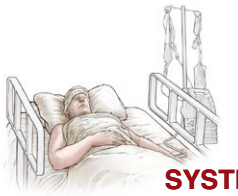
Bagley et al. reported the interim results following intraventricular delivery of a bivalent IL13Rα2 and EGFR targeting CAR T-cell in 6 patients who underwent resection of a recurrent IDH wild-type glioblastoma with evidence of EGFR amplification.[101] The presence of IL13Rα2 was not mandatory. In all 6 patients, there was early and moderate-severe neurotoxicity consistent with a combination of immune effector T-cell-associated neurotoxicity syndrome (ICANS) and tumor-inflammation-associated neurotoxicity that required management with high dose corticosteroids and the IL-1R antagonist anakinra. In 1 patient with fatigue, anorexia, and generalized muscle weakness, these side-effects were a DLT. CAR T-cells and cytokine release were observed in the CSF within the first 4 days. While early magnetic resonance imaging showed reductions in enhancement and tumor size for all patients, none met the mRANO response criteria.[86]

The HER2 tumor antigen, a receptor tyrosine kinase, is overexpressed in approximately 80% of GBMs. A study evaluated one or more IV infusions of HER2-specific CAR-modified virus-specific T-cells in patients with progressive HER2-positive GBM without prior lymphodepletion[102] (17 patients, including 7 individuals under the age of 18 years).[88] Notably, these infusions exhibited no significant toxicities. Among the 16 patients evaluated, one patient experienced a partial response lasting more than 9 months, 7 patients maintained stable disease for durations ranging from 8 weeks to 29 months, and 8 patients experienced disease progression. The median overall survival was 11.1 months from initiating the first T-cell infusion and 24.5 months from the initial diagnosis.[102]

Clinical Trials for CAR T-Cell Therapy in Pediatric Patients With Glioma

Despite genomic differences between pediatric brain tumors and their adult counterparts, most clinical trials exploring CAR T-cells for pediatric brain tumors target similar tumor antigens to those explored for adult gliomas, namely HER2, EGFR, IL13Rα2, GD2, and B7-H3[103,104] (Supplementary Table 1). The need for these immunotherapies is arguably even greater for pediatric patients with diffuse midline gliomas (DMGs), as resection of these tumors would result in devastating neurological deficits, and the low levels of MHC-I expression on non-glioma pediatric brain tumors make CAR therapies a more appealing option than other MHC-I dependent immunotherapeutic strategies like checkpoint inhibition.[103,105]

Locoregional delivery of HER2 and EGFR CAR T-cells was shown to be safe for pediatric patients with non-pontine HGGs. However, recent preclinical studies suggested GD2 and B7-H3 as more favorable targets for pediatric brain tumors, especially for DMG.[106] The BrainChild-03 study provided the first evidence that repeated intracranial administration of B7-H3 CAR T-cells is feasible and safe for pediatric HGG patients.[107] For pediatric patients with H3K27M-mutated DMGs (eg, DIPG, spinal cord gliomas), researchers investigating GD2-targeted CAR T-cells reported promising clinical and radiographic improvement following IV and subsequent ICV delivery in a subset of patients.[108] Furthermore, Wang et al. recently described the



Toxicities

- Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS)
- Tumor inflammation-associated neurotoxicity (TIAN)
- Seizures
- Aphasia
- Confusion
- Headache
- Encephalopathy
- Cytokine Release Syndrome
- Pulmonary edema
- Hypotension
- Myalgias
- Arthralgias
- Fatigue

**Potentially minimized following local delivery of CAR T-cell therapy*

Biomarkers of Response

- INF-γ
- Cell count
- IHC for antigen and CD3 from tissue
- IL6
- IL-1β
- TNF-α
- Ferritin
- IL-2

Kinetics

**Variable, ranging from acute to subacute (Day 1-10), depending on specific CAR construct and delivery route*

Treatment

- Tocilizumab/Siltuximab
- Corticosteroids
- Anakinra
- Supportive Care (e.g. Vasopressors, supplemental oxygen, acetaminophen)
- CSF diversion when indicated for obstructive hydrocephalus.

Figure 4. Local and systemic toxicities and biomarkers. Although systemic toxicities such as cytokine release syndrome (CRS) or pulmonary edema are less frequent in clinical trials for malignant gliomas, locoregional chimeric antigen receptor (CAR) T-cell administration is advocated by some to mitigate these risks.[22] Evidence indicates that inflammatory biomarkers, such as IL-6 or ferritin, are elevated following IV CAR T-cell administration.[95,101] Locoregional administration can still result in significant neurotoxicities, such as tumor-inflammation-associated neurotoxicity (TIAN), which is often accompanied by cerebral edema, and capable of causing seizures, focal neurological deficits, and obstructive hydrocephalus depending on the location. Consequently, close patient monitoring with serial neurological examinations is essential so appropriate supportive therapies can be provided if needed. Pro-inflammatory cytokines, including IFN-γ, TNF, and IL-2, detected in cerebrospinal fluid (CSF) after ICV delivery, may serve as an additional biomarker along with radiographic assessments to monitor the local effects of the CAR T-cell therapy.[95] Liquid biopsy (from blood or CSF) offers a great tool for real-time assessment of the presence and phenotype of CAR T-cells as well as tumor-derived materials and helps detect molecular changes in the tumor cells and immune environment.[110] Furthermore, if CAR T-cell therapy is administered before surgery (eg, prior to re-resection in cases of recurrent glioblastoma) tissue sampling will allow for the most accurate assessment of treatment response by analyzing the resected tumor tissue for the direct presence of activated CAR T-cells. Kinetics of local and systemic toxicities in glioma patients is variable depending on the specific agent and delivery strategy used.[111] Treatment includes the administration of Tocilizumab, Siltuximab, Anakinra, and corticosteroids for cytokine management and reduction of inflammation, supportive care such as vasopressors for hemodynamic support, supplemental oxygen, acetaminophen for fever control and if needed CSF diversion when indicated for obstructive hydrocephalus.

first experience combining lymphodepletion with ICV delivery of IL13Rα2-targeting CAR T-cells. This approach was well-tolerated and showed increased infiltration of endogenous T-cells into the CSF, and these CAR–CD8+ effector T-cells clonally expanded. These immunological changes are not observed in the peripheral blood. Critically, the authors also show that the TCRs from the tumor overlapped better with expanded TCRs in the CSF than the unexpanded population, highlighting the importance of collecting correlative samples from multiple compartments.[109] At the same time, this study also reported one case of grade 3 catheter-related infection, underscoring the need for careful consideration between patients' safety and scientific gains.

Toxicities, Monitoring, and CAR-Related Malignancy

One major concern with CAR T-cell therapy is the potential for serious neurologic and systemic toxicities, highlighting the need for close monitoring in patients receiving this immunotherapeutic strategy (Figure 4).[112] Although cytokine release syndrome (CRS) is a common adverse event following the administration of anti-CD19 CAR T-cells for B-cell malignancies, this serious systemic complication is much less common in clinical trials investigating CAR T-cells for patients with malignant gliomas.[113] Still, some groups advocate for locoregional administration of CAR

T-cells to minimize the potential or systemic side-effects, although no direct head-to-head comparison of the toxicity profiles of locoregional versus systemic CAR T-cell delivery is available. There is evidence that systemic levels of cytokines implicated in CRS, like IL-6, are higher following IV administration compared to ICV; additionally, higher increases in pro-inflammatory cytokines like IFN-γ, TNF, and IL-2 have been found to be higher in the CSF following ICV delivery compared to IV delivery.[108] While locoregional delivery may be helpful in limiting systemic adverse events, it remains true that patients receiving locoregional CAR T-cell administration still experience adverse effects and require close observation and management.

As discussed in earlier sections, recent studies with locoregional CAR administration often resulted in considerable neurotoxicities.[95,101] As reviewed previously, systematic administration of CAR T-cells, particularly high doses, has been associated with hypotension requiring vasopressor support and treatment with IL-6/IL-6R antagonists (tocilizumab or siltuximab) and corticosteroids. These adverse effects have been associated with elevated serum levels of inflammatory markers and lactate dehydrogenase.

Interestingly, toxicities appear to develop more acutely following locoregional delivery of CAR T-cells (on the order of 1–3 days) compared to IV CAR T-cell delivery (on the order of 5–8 days). This difference in the onset and duration of toxicities should be considered when designing clinical trials and determining what level of care and for how long patients require observation after CAR T-cell administration. Given most of the adverse events are the result of local effects on the nervous system, systemic inflammatory or cellular markers are lacking for assessing risk and response to treatment for these T-cell therapeutics, even in the setting of systemic CAR T-cell administration, highlighting the need for close patient monitoring during these treatments.

In November 2023, the FDA launched an investigation into cases of secondary T-cell malignancies, including CAR-positive lymphoma, in patients treated with CAR T-cell therapy. By January 2024, the FDA required drug manufacturers to include a safety label warning on CAR T-cell products. Ghilardi et al.[114] evaluated the risk of secondary cancers in patients treated with commercial CAR T-cell products by retrospectively analyzing 449 patients between January 2018 and November 2023. The study identified 16 cases of secondary cancers, 12 of which were solid tumors, indicating a low incidence of T-cell lymphoma after CAR T-cell therapy. To ensure safety, especially to minimize the risk of homologous recombination that increases oncogenesis, the number of transgene copies within a CAR T-cell product, known as vector copy numbers, must be evaluated before administering to patients.

Overcoming the Immune Privilege of the CNS Parenchyma

CNS Immunoanatomy and T-Cell Homing

Structures separating the CNS parenchyma and CSF.— Advancements in modern imaging and cellular profiling technologies have enabled a more comprehensive understanding of the different compartments of the CNS and revealed a fine-tuned communication network responsible for immune surveillance.[115]

The CNS parenchyma is surrounded by 3 meningeal layers: the outer dura mater containing venous sinuses, the arachnoid, and the pia mater. The subarachnoid space, between the arachnoid and pia mater, contains CSF. The pia mater, despite its name meaning "soft mother," acts as a tight barrier for immune cells. Superficial cerebrovasculature branches run through the perivascular space, which is continuous with the subarachnoid space and the intracerebral ventricles housing the choroid plexus, the main site of CSF production. CSF circulates from the ventricles to the subarachnoid space and can be pumped via aquaporin 4 water channels into the CNS parenchyma.[116] This allows it to mix with the interstitial fluid surrounding the parenchymal cells, which is then cleared through a convective flow into the venous system. This 'glymphatic system' is considered an alternative drainage (washout) system that may functionally replace the brain's lacking lymphatic system.[117]

Although the brain has been traditionally regarded as an immunologically privileged site, immune cells, notably T-cells, have been found in each compartment of the CNS.[118] The 2 most significant sites for T-cell entry are the choroid plexus and the meninges (Figure 5).[119] Chemokines released from nearby stromal cells stimulate the extravasation of blood T-cells circulating through the fenestrated endothelium of the choroid plexus vessel and the dural sinuses.[120] Rustenhoven et al.'s elegant research highlights a local interface where antigens carried by the CSF are captured by local antigen-presenting cells and presented to patrolling T-cells.[119] Furthermore, a recent study has identified arachnoid cuff exit points that reflect discontinuities in the arachnoid barrier created by bridging veins and allow cellular trafficking between the dura mater and the subarachnoid space.[124] Aside from these, the blood-brain barrier (BBB), consisting of capillary endothelial cells, the basement membrane, the perivascular space, and the glia limitans, limits circulating leukocytes from entering the CNS under non-inflammatory conditions.

Delivery Routes

As the efficient homing of CAR T-cells into the brain impacts the efficacy of the treatment, recent clinical trials have been investigating various delivery routes, such as IV, ICV, and IT delivery (Figure 5). Local delivery, such as ICV and IT, have often been considered more optimal than IV for patients with malignant gliomas, as they may allow the administration of lower CAR T-cell numbers, thereby mitigating the risk of off-target toxicities, especially when the CAR targets are also expressed on non-CNS cells. However, recent clinical trials in adults have found CAR T-cells in circulation within days after 'local' administration, indicating that T-cells may not persist in the CNS for a long-term but migrate into the periphery.[95,100,101] Notably, in pediatric trials involving ICV CAR T-cell infusion, CAR T cells have not been detected in the peripheral blood, despite their continued presence in the CSF for several weeks following administration.[107,125]

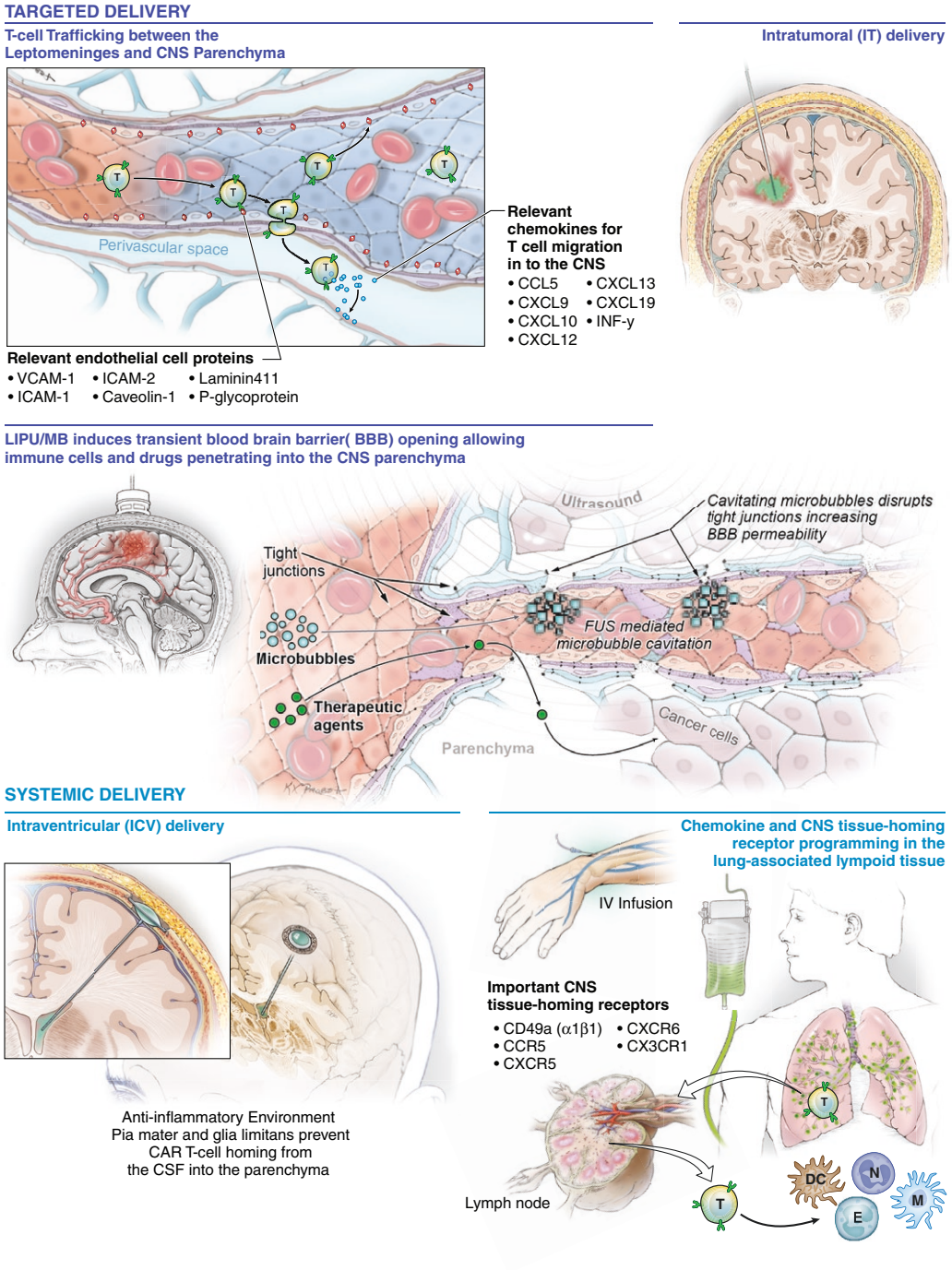


Figure 5. T-cell homing and delivery strategies: Under physiological conditions, circulating T-cells migrate into CSF through vessels located within the meninges and the choroid plexus, directed by cytokine signals and facilitated by endothelial adhesion molecules.[119,120] Subsequently, migration from the perivascular (CSF-filled) space through the glia limitans depends on T-cell reactivation. While IV-delivered T-cells may be trapped in the lungs or blocked by the BBB, preclinical studies suggest that T-cells can enter the CNS after residing transiently in the lung and associated lymphoid tissues.[121] IV-infused T-cells can then follow similar migration routes into the CSF as T-cells under immune surveillance. To overcome natural anatomical barriers of the CNS, recent clinical trials have explored various delivery routes for CAR T-cells, including IV,[22] ICV, and IT delivery[95] ICV administration, typically performed via an Ommaya reservoir, may encounter an anti-inflammatory immune environment with low adhesion molecule expression, hindering attachment to the pia mater, glia limitans, or choroid plexus epithelium. Therefore, not all of the ICV-injected T-cells may be able to migrate from the CSF into the CNS parenchyma. On the other side, cells that have successfully migrated into the CNS may persist within the CNS parenchyma or adjacent structures. IT delivery faces challenges in glioblastoma due to the tumor's highly immunosuppressive and hypoxic environment, leading to T-cell exhaustion.[21] Innovative approaches like low-intensity pulsed focused ultrasound with microbubble application (LIPU/MB) can enhance IV delivery by temporarily opening the blood–brain barrier (BBB), aiding drug[122] and cell penetration into the CNS.[123]

Although further investigations of these findings in humans are needed, preclinical studies investigating T-cell trafficking in experimental autoimmune encephalomyelitis (EAE) models may help us to understand under which conditions T-cells migrate from the CSF into the CNS parenchyma, instead of getting washed out into the periphery. Schläger et al.[126] provide an excellent insight: Effector T-cells that spontaneously detached by the flow of CSF from the surface of the leptomeninges displayed a significantly lower level of activation markers. The binding of the integrins VLA-4 and LFA-1 to their respective ligands produced by resident macrophages, chemokine signaling via CCR5/CXCR3, and antigenic stimulation of T-cells counteracted the detachment, underlying the importance of T-cell activation to prevent T-cells from washed out of the CNS.[126]

In clinical trials, ICV administration of CAR T-cells is generally performed via an implanted Omaya reservoir. Cells infused via the reservoir likely encounter an anti-inflammatory immune environment with low adhesion molecule expression, which can impede their attachment to the pia and glia limitans or choroid plexus epithelium. Consequently, not all the ICV-injected CAR T-cells may be able to migrate into the brain parenchyma. An exception to this can be tumors that are exposed to the CSF spaces by surgical resection, where T-cells may directly interact with tumor cells. On the other hand, IT delivery would bypass the glia limitans. However, IT delivery also presents challenges, particularly in glioblastoma, where the tumor core is characterized by a highly immunosuppressive and hypoxic environment, inducing T-cell exhaustion. A significant advantage of locoregional delivery over systemic administration is that locoregional administration does not necessitate lymphodepletion.[95] However, a combination could have beneficial effects and needs to be further evaluated.[109]

IV-delivered T-cells may be trapped in the lungs and blocked by the BBB. However, an innovative study by Odoardi et al.[121] using an EAE model demonstrated that T-cells acquire the ability to enter the CNS after residing transiently within the lung, its associated lymphoid tissue, and the lung-draining mediastinal lymph nodes (Figure 5). IV-infused T-cells can then enter the CSF following similar migration routes as T-cells under immune surveillance. Furthermore, preclinical BBB modeling has indicated that GBM-targeting activated CAR T-cells show excellent homing via the BBB.[127] Moreover, endothelial cell activation and BBB disruption have been observed after the adoptive transfer of CD19 CAR-T-cells.[128] Considering these, IV delivery may not be an inferior administration strategy.[129]

Innovative approaches, such as low-intensity pulsed focused ultrasound combined with microbubble application (LIPU/MB), may further enhance the efficacy of the IV route. LIPU/MB temporarily opens the BBB, thereby facilitating the penetration of drugs and cells into the CNS parenchyma. Initial preclinical and clinical studies on malignant gliomas have demonstrated enhanced penetration of immune cells and drugs into the tumor.[123] Recently, Sonabend et al. reported in a groundbreaking phase-1 trial that repeated sonication with a skull-implantable ultrasound device is safe and improves the delivery of albumin-bound paclitaxel and carboplatin chemotherapies into the brain.[122]

Future Directions for CAR T Therapies in Malignant Glioma

The development of effective CAR T-cell therapy using autologous T-cells was revolutionary when allogeneic stem cell transplantation had been the standard of care for patients with hematopoietic malignancy. CAR T-cell engineering with autologous T-cells will continue to evolve robustly, as we discussed. At the same time, recognizing inherent challenges with the use of autologous T-cells, new paradigms are being developed using cutting-edge bioengineering technologies.

Off-the-Shelf Allogeneic CAR T-Cells

While active development and evaluations are ongoing using autologous T-cells, there are inherent challenges associated with autologous cell-based approaches. Patients with malignant glioma often exhibit lymphopenia, which may limit the ex vivo production of high-quality T-cell products in adequate quantity. In addition to the high costs for manufacturing, logistic challenges, such as the timely availability of a cell manufacturing suite and the prolonged 'vein-to-vein' time, complicate the process, as discussed in Clinical Trials. Potential solutions involve the development of "off-the-shelf" allogeneic CAR T-cells or CAR-NK cells and in vivo (in situ) gene transfer in the patient's circulating blood. Recent advancements include using CRISPR to silence TRAC (T-cell receptor alpha constant) and beta-2 microglobulin (B2M) on T-cells, which can help generate more effective off-the-shelf CAR T-cells. Silencing TRAC reduces the risk of graft-versus-host disease by preventing the CAR T-cells from recognizing the host's tissues as foreign.[130] Similarly, silencing B2M helps the CAR T-cells evade the host's immune system by preventing the expression of class I MHC molecules on the surface of the CAR T-cells.[131] These genetic modifications can make allogeneic CAR T-cells safer and more universal, enhancing their availability and reducing manufacturing time and cost. These strategies offer solutions to the logistical hurdles, such as the timely cell manufacturing and the prolonged 'vein-to-vein' time, as discussed in Clinical Trials.

Allogeneic induced pluripotent stem cell (iPSC)-derived T-cells.—Allogeneic iPSCs engineered to escape allogeneic rejection[132] and differentiated into functional T-cells are being developed using scalable differentiation systems.[133] As expression of CAR in iPSC causes unwanted differentiation and antigen-nonspecific cytotoxicity,[132,134] employing strategies to reduce the tonic signals, such as synNotch,[23] may be the key to generating functional CD8αβ cells. The capacity of allogeneic iPSC cells to integrate multiple genetic edits can help overcome the unique challenges of CNS tumors.

Allogeneic NK-cells are also actively evaluated,[135] although one significant pitfall is the short persistence of allogeneic CAR-NK cells, requiring repeated infusions to sustain the presence of the therapeutic cells.

In Vivo Gene Transfer

In situ transduction may allow us to overcome the current challenges with ex vivo manufacturing of autologous

CAR T-cells.[136] For example, a Multifunctional Alginate Scaffold for T-cell Engineering and Release (MASTER) has been described as a way to cut CAR-T-cell manufacturing to 1 day. It seeds blood cells with CD19 retroviral particles, enabling gene transfer and releasing CAR-T-cells after implantation with cells persisting longer than conventional CAR-T-cells, simplifying and accelerating CAR-T therapy.[136]

Effective delivery systems with target cell-specificity still need to be developed. Nevertheless, these developments will pave the way for translating these techniques into clinical settings, potentially revolutionizing the treatment landscape for cancer, including glioblastoma, by providing rapid and accessible cellular therapies.

Conclusion

In summary, multiple approaches are actively developed for enhancing CAR T-cell specificity and persistence. Developments of iPSC-derived T-cells and direct in vivo gene transfer techniques using novel gene vectors offer practical solutions to logistical hurdles and provide scalable and potentially more effective treatment options.

While this review focused on CAR T-cell therapy, other types of cellular therapy, such as CAR macrophages,[137,138] CAR-natural killer cells,[139] dendritic cell vaccines, T-cell receptor (TCR)-transduced T-cell therapy, and tumor-infiltrating lymphocytes, are actively being developed.

Just as monoclonal antibodies were once considered novel but are now mainstream in oncology, cell therapy has the potential to follow the same path and become a widely used treatment for cancer, including CNS malignancies.

Supplementary material

Supplementary material is available online at *Neuro-Oncology* (https://academic.oup.com/neuro-oncology).

Keywords:

chimeric antigen receptor (CAR) | clinical trial | geneTherapy | malignant glioma |T lymphocytes

Funding

National Institutes of Health [R35NS105068 (HO)] German Research Foundation ([DFG, GA 3535/1-1, [MG]). List of any unpublished papers cited: Singh K, Hotchkiss KM, Cook SL, et al. IL-7 mediated upregulation of VLA-4 increases accumulation of adoptively transferred T lymphocytes in murine glioma. *bioRxiv*. Published online April 1, 2024:2024.04.01.587634.

Acknowledgments

The authors thank Kenneth X. Probst and Noel Sirivansanti in the Medical Illustration service at the Department of Neurological Surgery, University of California, San Francisco, for their assistance in creating schemas and figures. Figure 1 was partially created in BioRender.

Conflict of interest statement

Hideho Okada: Consultant for Cellula Therapeutics and Chimeric Therapeutics and a US patent application US20210023138A1.

Authorship statement

Conceptualization: M.G. and H.O. Supervision: H.O. Literature Review: M.G., J.S.Y., S.C.Q., M.K., J.G., and H.O. Figures/Table: M.G., J.S.Y., S.C.Q., M.K., J.G., and H.O. Writing- original draft: M.G., J.S.Y., S.C.Q., M.K., J.G., and H.O. Writing- review and editing: M.G., J.S.Y., S.C.Q., M.K., J.G., and H.O. All authors approved the final manuscript.

Affiliations

Department of Neurological Surgery, Unversity of California San Fracisco, San Francisco, California, USA (M.G., J.S.Y., Jd.G., H.O.); Helen Diller Family Comprehensive Cancer Center, San Francisco, California, USA (Jd.G., H.O.); The Parker Institute for Cancer Immunotherapy, San Francisco, California, USA (H.O.); The Duke Cancer Institute, Duke University Medical Center, Durham, North Carolina, USA (S.C.Q., M.K.)

References

1. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013;368(16):1509–1518.
2. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365(8):725–733.
3. Kochenderfer JN, Wilson WH, Janik JE, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood*. 2010;116(20):4099–4102.
4. June CH, Sadelain M. Chimeric antigen receptor therapy. *N Engl J Med*. 2018;379(1):64–73.
5. Yarmarkovich M, Marshall QF, Warrington JM, et al. Targeting of intracellular oncoproteins with peptide-centric CARs. *Nature*. 2023;623(7988):820–827.

6. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol.* 2019;17(3):147–167.
7. Sterner RC, Sterner RM. CAR-T cell therapy: Current limitations and potential strategies. *Blood Cancer J.* 2021;11(4):1–11.
8. Hudecek M, Sommermeyer D, Kosasih PL, et al. The non-signaling extracellular spacer domain of chimeric antigen receptors is decisive for in vivo antitumor activity. *Cancer Immunol Res.* 2015;3(2):125–135.
9. Roselli E, Faramand R, Davila ML. Insight into next-generation CAR therapeutics: designing CAR T cells to improve clinical outcomes. *J Clin Invest.* 2021;131(2):e142030.
10. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci U S A.* 1989;86(24):10024–10028.
11. Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor. *Nat Biotechnol.* 2002;20(1):70–75.
12. Imai C, Mihara K, Andreansky M, et al. Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia.* 2004;18(4):676–684.
13. Kawalekar OU, O'Connor RS, Fraietta JA, et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity.* 2016;44(2):380–390.
14. Pulè MA, Straathof KC, Dotti G, et al. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Molecular Therapy.* 2005;12(5):933–941.
15. Chmielewski M, Kopecky C, Hombach AA, Abken H. IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res.* 2011;71(17):5697–5706.
16. Hu B, Ren J, Luo Y, et al. Augmentation of antitumor immunity by human and mouse CAR T Cells Secreting IL-18. *Cell Rep.* 2017;20(13):3025–3033.
17. Ayala Ceja M, Khericha M, Harris CM, Puig-Saus C, Chen YY. CAR-T cell manufacturing: Major process parameters and next-generation strategies. *J Exp Med.* 2024;221(2):1–14.
18. Chira S, Jackson CS, Oprea I, et al. Progresses towards safe and efficient gene therapy vectors. *Oncotarget.* 2015;6(31):30675–30703.
19. Aronovich EL, McIvor RS, Hackett PB. The Sleeping Beauty transposon system: A non-viral vector for gene therapy. *Hum Mol Genet.* 2011;20(R1):R14–R20.
20. Wang X, Rivière I. Clinical manufacturing of CAR T cells: Foundation of a promising therapy. *Mol Ther Oncolytics.* 2016;3(16015):1–7.
21. Montoya M, Gallus M, Phyu S, et al. A Roadmap of CAR-T-cell therapy in glioblastoma: Challenges and future perspectives. *Cells.* 2024;13(9):726.
22. O'Rourke DM, Nasrallah MP, Desai A, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med.* 2017;9(399):eaaa0984.
23. Choe JH, Watchmaker PB, Simic MS, et al. SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. *Sci Transl Med.* 2021;13(591):7378.
24. Zhu I, Liu R, Garcia JM, et al. Modular design of synthetic receptors for programmed gene regulation in cell therapies. *Cell.* 2022;185(8):1431–1443.e16.
25. Richman SA, Wang LC, Moon EK, et al. Ligand-induced degradation of a CAR Permits Reversible Remote Control of CAR T cell activity in vitro and in vivo. *Mol Ther.* 2020;28(7):1600–1613.
26. Murawska GM, Vogel C, Jan M, et al. Repurposing the damage repair protein methyl guanine methyl transferase as a ligand inducible fusion Degron. *ACS Chem Biol.* 2022;17(1):24–31.
27. Urbanska K, Lanitis E, Poussin M, et al. A universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor. *Cancer Res.* 2012;72(7):1844–1852.
28. Schmidts A, Srivastava AA, Ramapriyan R, et al. Tandem chimeric antigen receptor (CAR) T cells targeting EGFRvIII and IL-13Rα2 are effective against heterogeneous glioblastoma. *Neurooncol. Adv..* 2023;5(1):vdac185.
29. Morsut L, Roybal KT, Xiong X, et al. Engineering customized cell sensing and response behaviors using synthetic notch receptors. *Cell.* 2016;164(4):780–791.
30. Cho JH, Collins JJ, Wong WW. Universal chimeric antigen receptors for multiplexed and logical control of T Cell Responses. *Cell.* 2018;173(6):1426–1438.e11.
31. Carnevale J, Shifrut E, Kale N, et al. RASA2 ablation in T cells boosts antigen sensitivity and long-term function. *Nature.* 2022;609(7925):174–182.
32. Doan AE, Mueller KP, Chen AY, et al. FOXO1 is a master regulator of memory programming in CAR T cells. *Nature.* 2024;629(8010):211–218.
33. Aggarwal P, Luo W, Pehlivan KC, et al. Pediatric versus adult high grade glioma: Immunotherapeutic and genomic considerations. *Front Immunol.* 2022;13(1038096):1–20.
34. Crotty E, Downey K, Ferrerosa L, et al. Considerations when treating high-grade pediatric glioma patients with immunotherapy. *Expert Rev Neurother.* 2021;21(2):205–219.
35. Levine AB, Nobre L, Das A, et al. Immuno-oncologic profiling of pediatric brain tumors reveals major clinical significance of the tumor immune microenvironment. *Nat Commun.* 2024;15(1):1–17.
36. Nduom EK, Weller M, Heimberger AB. Immunosuppressive mechanisms in glioblastoma. *Neuro Oncol.* 2015;17(suppl 7):vii9–vii14.
37. Persson ML, Douglas AM, Alvaro F, et al. The intrinsic and microenvironmental features of diffuse midline glioma: Implications for the development of effective immunotherapeutic treatment strategies. *Neuro Oncol.* 2022;24(9):1408–1422.
38. Lieberman NAP, Degolier K, Kovar HM, et al. Characterization of the immune microenvironment of diffuse intrinsic pontine glioma: Implications for development of immunotherapy. *Neuro Oncol.* 2019;21(1):83–94.
39. Avril T, Saikali S, Vauleon E, et al. Distinct effects of human glioblastoma immunoregulatory molecules programmed cell death ligand-1 (PDL-1) and indoleamine 2,3-dioxygenase (IDO) on tumour-specific T cell functions. *J Neuroimmunol.* 2010;225(1-2):22–33.
40. Mittelbronn M, Platten M, Zeiner P, et al. Macrophage migration inhibitory factor (MIF) expression in human malignant gliomas contributes to immune escape and tumour progression. *Acta Neuropathol.* 2011;122(3):353–365.
41. Tchirkov A, Rolhion C, Bertrand S, et al. IL-6 gene amplification and expression in human glioblastomas. *Br J Cancer.* 2001;85(4):518–522.
42. Wang Q, He Z, Huang M, et al. Vascular niche IL-6 induces alternative macrophage activation in glioblastoma through HIF-2α. *Nat Commun.* 2018;9(1):1–15.
43. Qian J, Luo F, Yang J, et al. TLR2 promotes glioma immune evasion by downregulating MHC Class II Molecules in Microglia. *Cancer Immunol Res.* 2018;6(10):1220–1233.
44. Schartner JM, Hagar AR, Van Handel M, et al. Impaired capacity for upregulation of MHC class II in tumor-associated microglia. *Glia.* 2005;51(4):279–285.
45. Wesolowska A, Kwiatkowska A, Slomnicki L, et al. Microglia-derived TGF-beta as an important regulator of glioblastoma invasion--an inhibition of TGF-beta-dependent effects by shRNA against human TGF-beta type II receptor. *Oncogene.* 2008;27(7):918–930.
46. Lohr J, Ratliff T, Huppertz A, et al. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-β. *Clin Cancer Res.* 2011;17(13):4296–4308.

47. Wainwright DA, Balyasnikova IV, Chang AL, et al. IDO expression in brain tumors increases the recruitment of regulatory T cells and negatively impacts survival. *Clin Cancer Res.* 2012;18(22):6110–6121.
48. Miska J, Lee-Chang C, Rashidi A, et al. HIF-1α Is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of tregs in glioblastoma. *Cell Rep.* 2019;27(1):226–237.e4.
49. Yeung JT, Hamilton RL, Ohnishi K, et al. LOH in the HLA class I region at 6p21 is associated with shorter survival in newly diagnosed adult glioblastoma. *Clin Cancer Res.* 2013;19(7):1816–1826.
50. Zagzag D, Salnikow K, Chiriboga L, et al. Downregulation of major histocompatibility complex antigens in invading glioma cells: Stealth invasion of the brain. *Lab Invest.* 2005;85(3):328–341.
51. Zhang Z, Miao L, Ren Z, Tang F, Li Y. Gene-edited interleukin CAR-T cells therapy in the treatment of malignancies: Present and future. *Front Immunol.* 2021;12(718686):1–13.
52. Mohammed S, Sukumaran S, Bajgain P, et al. Improving chimeric antigen receptor-modified T cell function by reversing the immunosuppressive tumor microenvironment of pancreatic cancer. *Mol Ther.* 2017;25(1):249–258.
53. Qiao Y, Chen J, Wang X, et al. Enhancement of CAR-T cell activity against cholangiocarcinoma by simultaneous knockdown of six inhibitory membrane proteins. *Cancer Commun (Lond).* 2023;43(7):788–807.
54. Chang ZL, Lorenzini MH, Chen X, et al. Rewiring T-cell responses to soluble factors with chimeric antigen receptors. *Nat Chem Biol.* 2018;14(3):317–324.
55. Hou AJ, Shih RM, Uy BR, et al. IL-13Rα2/TGF-β bispecific CAR-T cells counter TGF-β-mediated immune suppression and potentiate anti-tumor responses in glioblastoma. *Neuro Oncol.* 2024;26(10):1850–1866.
56. Li K, Xu J, Wang J, et al. Dominant-negative transforming growth factor-β receptor-armoured mesothelin-targeted chimeric antigen receptor T cells slow tumour growth in a mouse model of ovarian cancer. *Cancer Immunol Immunother.* 2023;72(4):917–928.
57. Narayan V, Barber-Rotenberg JS, Jung IY, et al; Prostate Cancer Cellular Therapy Program Investigators. PSMA-targeting TGFβ-insensitive armored CAR T cells in metastatic castration-resistant prostate cancer: A phase 1 trial. *Nat Med.* 2022;28(4):724–734.
58. Friebel E, Kapolou K, Unger S, et al. Single-cell mapping of human brain cancer reveals tumor-specific instruction of tissue-invading leukocytes. *Cell.* 2020;181(7):1626–1642.e20.
59. Hussain SF, Yang D, Suki D, et al. The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. *Neuro Oncol.* 2006;8(3):261–279.
60. Yamada-Hunter SA, Theruvath J, McIntosh BJ, et al. Engineered CD47 protects T cells for enhanced antitumour immunity. *Nature.* 2024;630(8016):457–465.
61. Rodriguez-Garcia A, Lynn RC, Poussin M, et al. CAR-T cell-mediated depletion of immunosuppressive tumor-associated macrophages promotes endogenous antitumor immunity and augments adoptive immunotherapy. *Nat Commun.* 2021;12(1):1–17.
62. Chongsathidkiet P, Jackson C, Koyama S, et al. Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. *Nat Med.* 2018;24(9):1459–1468.
63. Kim WJ, Dho YS, Ock CY, et al. Clinical observation of lymphopenia in patients with newly diagnosed glioblastoma. *J Neurooncol.* 2019;143(2):321–328.
64. Gustafson MP, Lin Y, New KC, et al. Systemic immune suppression in glioblastoma: The interplay between CD14+HLA-DRlo/neg monocytes, tumor factors, and dexamethasone. *Neuro Oncol.* 2010;12(7):631–644.
65. Brock CS, Newlands ES, Wedge SR, et al. Phase I trial of temozolomide using an extended continuous oral schedule. *Cancer Res.* 1998;58(19):4363–4367. http://aacrjournals.org/cancerres/article-pdf/58/19/4363/2468101/cr0580194363.pdf. Accessed July 12, 2024.
66. Suryadevara CM, Desai R, Abel ML, et al. Temozolomide lymphodepletion enhances CAR abundance and correlates with antitumor efficacy against established glioblastoma. *Oncoimmunology.* 2018;7(6):1–10.
67. Guha P, Cunetta M, Somasundar P, et al. Frontline Science: Functionally impaired geriatric CAR-T cells rescued by increased α5β1 integrin expression. *J Leukoc Biol.* 2017;102(2):201–208.
68. Ayasoufi K, Pfaller CK, Evgin L, et al. Brain cancer induces systemic immunosuppression through release of non-steroid soluble mediators. *Brain.* 2020;143(12):3629–3652.
69. Elliott LH, Brooks WH, Roszman TL. Suppression of high affinity IL-2 receptors on mitogen activated lymphocytes by glioma-derived suppressor factor. *J Neurooncol.* 1992;14(1):1–7.
70. Brooks WH, Netsky MG, Normansell DE, Horwitz DA. Depressed cell-mediated immunity in patients with primary intracranial tumors. Characterization of a humoral immunosuppressive factor. *J Exp Med.* 1972;136(6):1631–1647.
71. Ostrom QT, Price M, Neff C, et al. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2015-2019. *Neuro Oncol.* 2022;24(suppl 5):v1–v95.
72. Cook SL, Al Amin M, Bari S, et al. Immune checkpoint inhibitors in geriatric oncology. *Curr Oncol Rep.* 2024;26(5):562–572.
73. Montoya M, Collins SA, Chuntova P, et al. Interferon regulatory factor 8-driven reprogramming of the immune microenvironment enhances antitumor adaptive immunity and reduces immunosuppression in murine glioblastoma. *Neuro Oncol.* 2024.
74. Alizadeh D, Wong RA, Gholamin S, et al. IFNγ is critical for CAR T cell–mediated myeloid activation and induction of endogenous immunity. *Cancer Discov.* 2021;11(9):2248–2265.
75. Zaidi SE, Moelker E, Singh K, et al. Novel immunotherapeutic approaches for the treatment of glioblastoma. *BioDrugs.* 2023;37(4):489–503.
76. Singh K, Batich KA, Wen PY, et al. Designing clinical trials for combination immunotherapy: A framework for glioblastoma. *Clin Cancer Res.* 2022;28(4):585–593.
77. Dasyam N, George P, Weinkove R. Chimeric antigen receptor T-cell therapies: Optimising the dose. *Br J Clin Pharmacol.* 2020;86(9):1678–1689.
78. Ren X, Zhang G, Li G, Wang Y. Chimeric antigen receptor T-cell therapy-induced nervous system toxicity: A real-world study based on the FDA Adverse Event Reporting System database. *BMC Cancer.* 2024;24(1):1–11.
79. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med.* 2015;7(303):303ra139–303ra139.
80. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med.* 2017;377(26):2531–2544.
81. Amini L, Silbert SK, Maude SL, et al. Preparing for CAR T cell therapy: Patient selection, bridging therapies and lymphodepletion. *Nat Rev Clin Oncol.* 2022;19(5):342–355.
82. Weathers SP, Penas-Prado M, Pei BL, et al. Glioblastoma-mediated immune dysfunction limitS CMV-specific T cells and therapeutic responses: Results from a phase I/II trial. *Clin Cancer Res.* 2020;26(14):3565–3577.
83. Lakomy T, Akhoundova D, Nilius H, et al. Early use of corticosteroids following CAR T-Cell therapy correlates with reduced risk of high-grade CRS without negative impact on neurotoxicity or treatment outcome. *Biomolecules.* 2023;13(2):382.
84. Liu S, Deng B, Yin Z, et al. Corticosteroids do not influence the efficacy and kinetics of CAR-T cells for B-cell acute lymphoblastic leukemia. *Blood Cancer J.* 2020;10(2):1–4.
85. Munoz AM, Urak R, Taus E, et al. Dexamethasone potentiates chimeric antigen receptor T cell persistence and function by enhancing IL-7Rα expression. *Mol Ther.* 2024;32(2):527–539.
86. Smith LK, Cidlowski JA. Glucocorticoid-induced apoptosis of healthy and malignant lymphocytes. *Prog Brain Res.* 2010;182(0079-6123):1–30.

87. Poiret T, Vikberg S, Schoutrop E, Mattsson J, Magalhaes I. CAR T cells and T cells phenotype and function are impacted by glucocorticoid exposure with different magnitude. *J Transl Med.* 2024;22(1):273.
88. Brown CE, Rodriguez A, Palmer J, et al. Off-the-shelf, steroid-resistant, IL13Rα2-specific CAR T cells for treatment of glioblastoma. *Neuro Oncol.* 2022;24(8):1318–1330.
89. Zhang Z, Liu X, Chen D, Yu J. Radiotherapy combined with immunotherapy: The dawn of cancer treatment. *Signal Transduct Target Ther.* 2022;7(1):1–34.
90. Gattinoni L, Klebanoff CA, Restifo NP. Paths to stemness: Building the ultimate antitumour T cell. *Nat Rev Cancer.* 2012;12(10):671–684.
91. Gattinoni L, Lugli E, Ji Y, et al. A human memory T-cell subset with stem cell-like properties. *Nat Med.* 2011;17(10):1290–1297.
92. Brown CE, Aguilar B, Starr R, et al. Optimization of IL13Rα2-targeted chimeric antigen receptor T cells for improved anti-tumor efficacy against glioblastoma. *Mol Ther.* 2018;26(1):31–44.
93. Brown CE, Badie B, Barish ME, et al. Bioactivity and safety of IL13Rα2-redirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. *Clin Cancer Res.* 2015;21(18):4062–4072.
94. Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N Engl J Med.* 2016;375(26):2561–2569.
95. Brown CE, Hibbard JC, Alizadeh D, et al. Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: A phase 1 trial. *Nat Med.* 2024;30(4):1001–1012.
96. Wick W, Gorlia T, Bendszus M, et al. Lomustine and bevacizumab in progressive glioblastoma. *N Engl J Med.* 2017;377(20):1954–1963.
97. Reardon DA, Brandes AA, Omuro A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: The CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncol.* 2020;6(7):1003–1010.
98. Lombardi G, De Salvo GL, Brandes AA, et al. Regorafenib compared with lomustine in patients with relapsed glioblastoma (REGOMA): A multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet Oncol.* 2019;20(1):110–119.
99. Goff SL, Morgan RA, Yang JC, et al. Pilot trial of adoptive transfer of chimeric antigen receptor-transduced T cells targeting EGFRvIII in Patients With Glioblastoma. *J Immunother.* 2019;42(4):126–135.
100. Choi BD, Gerstner ER, Frigault MJ, et al. Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. *N Engl J Med.* 2024;390(14):1290–1298.
101. Bagley SJ, Logun M, Fraietta JA, et al. Intrathecal bivalent CAR T cells targeting EGFR and IL13Rα2 in recurrent glioblastoma: Phase 1 trial interim results. *Nat Med.* 2024;30(5):1320–1329.
102. Ahmed N, Brawley V, Hegde M, et al. HER2-specific chimeric antigen receptor-modified virus-specific T Cells for progressive glioblastoma: A Phase 1 Dose-Escalation Trial. *JAMA Oncol.* 2017;3(8):1094–1101.
103. Antonucci L, Canciani G, Mastronuzzi A, et al. CAR-T therapy for pediatric high-grade gliomas: Peculiarities, current investigations and future strategies. *Front Immunol.* 2022;13(867154):1–10.
104. Haydar D, Houke H, Chiang J, et al. Cell-surface antigen profiling of pediatric brain tumors: B7-H3 is consistently expressed and can be targeted via local or systemic CAR T-cell delivery. *Neuro Oncol.* 2021;23(6):999–1011.
105. Guillaume J, Perzolli A, Boes M. Strategies to overcome low MHC-I expression in paediatric and adult tumours. *Immunother Adv.* 2023;4(1):1–13.
106. Mount CW, Majzner RG, Sundaresh S, et al. Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M+ diffuse midline gliomas. *Nat Med.* 2018;24(5):572–579.
107. Vitanza NA, Wilson AL, Huang W, et al. Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: Preliminary first-in-human bioactivity and safety. *Cancer Discov.* 2023;13(1):114–131.
108. Majzner RG, Ramakrishna S, Yeom KW, et al. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. *Nature.* 2022;603(7903):934–941.
109. Wang L, Oill AT, Blanchard M, et al. Expansion of endogenous T cells in CSF of pediatric CNS tumor patients undergoing locoregional delivery of IL13Rα2-targeting CAR T cells: An interim analysis. *Res Sq.* 2023.
110. Miller AM, Shah RH, Pentsova EI, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. *Nature.* 20192019;565(7741):654–658.
111. Gatto L, Ricciotti I, Tosoni A, et al. CAR-T cells neurotoxicity from consolidated practice in hematological malignancies to fledgling experience in CNS tumors: fill the gap. *Front Oncol.* 2023;13(1206983):1.
112. Bagley SJ, Desai AS, Linette GP, June CH, O'Rourke DM. CAR T-cell therapy for glioblastoma: Recent clinical advances and future challenges. *Neuro Oncol.* 2018;20(11):1429–1438.
113. Goutnik M, Iakovidis A, Still MEH, et al. Advancements in chimeric antigen receptor-expressing T-cell therapy for glioblastoma multiforme: Literature review and future directions. *Neurooncol. Adv..* 2024;6(1):vdae025.
114. Ghilardi G, Fraietta JA, Gerson JN, et al. T cell lymphoma and secondary primary malignancy risk after commercial CAR T cell therapy. *Nat Med.* 2024;30(4):984–989.
115. Castellani G, Croese T, Peralta Ramos JM, Schwartz M. Transforming the understanding of brain immunity. *Science.* 2023;380(6640):eabo7649.
116. Iliff JJ, Wang M, Liao Y, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci Transl Med.* 2012;4(147):147ra111.
117. Jessen NA, Munk ASF, Lundgaard I, Nedergaard M. The glymphatic system – a beginner's guide. *Neurochem Res.* 2015;40(12):2583–2599.
118. Kivisäkk P, Mahad DJ, Callahan MK, et al. Human cerebrospinal fluid central memory CD4+ T cells: Evidence for trafficking through choroid plexus and meninges via P-selectin. *Proc Natl Acad Sci U S A.* 2003;100(14):8389–8394.
119. Rustenhoven J, Drieu A, Mamuladze T, et al. Functional characterization of the dural sinuses as a neuroimmune interface. *Cell.* 2021;184(4):1000–1016.e27.
120. Smolders J, van Luijn MM, Hsiao CC, Hamann J. T-cell surveillance of the human brain in health and multiple sclerosis. *Semin Immunopathol.* 2022;44(6):855–867.
121. Odoardi F, Sie C, Streyl K, et al. T cells become licensed in the lung to enter the central nervous system. *Nature.* 2012;488(7413):675–679.
122. Sonabend AM, Gould A, Amidei C, et al. Repeated blood-brain barrier opening with an implantable ultrasound device for delivery of albumin-bound paclitaxel in patients with recurrent glioblastoma: A phase 1 trial. *Lancet Oncol.* 2023;24(5):509–522.
123. Sabbagh A, Beccaria K, Ling X, et al. Opening of the blood–brain barrier using low-intensity pulsed ultrasound enhances responses to immunotherapy in preclinical glioma models. *Clin Cancer Res.* 2021;27(15):4325–4337.
124. Smyth LCD, Xu D, Okar SV, et al. Identification of direct connections between the dura and the brain. *Nature.* 2024;627(8002):165–173.
125. Vitanza NA, Johnson AJ, Wilson AL, et al. Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: An interim analysis. *Nat Med.* 2021;27(9):1544–1552.
126. Schläger C, Körner H, Krueger M, et al. Effector T-cell trafficking between the leptomeninges and the cerebrospinal fluid. *Nature.* 2016;530(7590):349–353.

127. Huang J, Li YB, Charlebois C, et al. Application of blood brain barrier models in pre-clinical assessment of glioblastoma-targeting CAR-T based immunotherapies. *Fluids Barriers CNS*. 2022;19(1):38.
128. Gust J, Hay KA, Hanafi LA, et al. Endothelial activation and blood–brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. *Cancer Discov*. 2017;7(12):1404–1419.
129. Gust J, Hay KA, Hanafi LA, et al. Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T Cells. *Cancer Discov*. 2017;7(12):1404–1419.
130. Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature*. 2017;543(7643):113–117.
131. Torikai H, Reik A, Soldner F, et al. Toward eliminating HLA class I expression to generate universal cells from allogeneic donors. *Blood*. 2013;122(8):1341–1349.
132. Wang B, Iriguchi S, Waseda M, et al. Generation of hypoimmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells. *Nat Biomed Eng*. 2021;5(5):429–440.
133. Iriguchi S, Yasui Y, Kawai Y, et al. A clinically applicable and scalable method to regenerate T-cells from iPSCs for off-the-shelf T-cell immunotherapy. *Nat Commun*. 2021;12(1):430.
134. Themeli M, Kloss CC, Ciriello G, et al. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. *Nat Biotechnol*. 2013;31(10):928–933.
135. Shaim H, Shanley M, Basar R, et al. Targeting the αv integrin/TGF-β axis improves natural killer cell function against glioblastoma stem cells. *J Clin Invest*. 2021;131(14):e142116.
136. Agarwalla P, Ogunnaike EA, Ahn S, et al. Bioinstructive implantable scaffolds for rapid in vivo manufacture and release of CAR-T cells. *Nat Biotechnol*. 2022;40(8):1250–1258.
137. Kingwell K. CAR T therapies drive into new terrain. *Nat Rev Drug Discov*. 2017;16(5):301–304.
138. Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol*. 2020;38(8):947–953.
139. Laskowski TJ, Biederstädt A, Rezvani K. Natural killer cells in antitumour adoptive cell immunotherapy. *Nat Rev Cancer*. 2022;22(10):557–575.