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Journal

Current Opinion in Oncology, 34(6)

ISSN

1040-8746

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Publication Date

2022-11-01

DOI

10.1097/cco.0000000000000877

Peer reviewed

HHS Public Access

Author manuscript

Curr Opin Oncol. Author manuscript; available in PMC 2023 November 01.

Published in final edited form as:

Curr Opin Oncol. 2022 November 01; 34(6): 661–669. doi:10.1097/CCO.0000000000000877.

Future development of chimeric antigen receptor T cell therapies for patients suffering from malignant glioma

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Abstract

Purpose of review: Chimeric Antigen Receptor (CAR) T cell therapy has been successful in some hematologic malignancies, but the central nervous system (CNS) presents unique obstacles to its use against tumors arising therein. This review discusses recent improvements in the delivery and design of these cells to improve the efficacy and safety of this treatment against malignant gliomas.

Recent Findings: The immunosuppressive environment of the CNS affects the functionality of CAR T cells but recent developments using metabolic manipulation and cytokine delivery have shown that the performance of CAR T cells can be improved in this environment. Emerging techniques can improve the delivery of CAR T cells to the CNS parenchyma, which is normally well-protected from peripheral immune cells. The implementation of novel antigens and CAR-expression regulation strategies will improve the specificity and efficacy of these cells. Finally, while autologous T cells have historically been the standard, recent developments have made the use of allogeneic T cells or Natural Killer (NK) cells more clinically feasible.

Summary: The discoveries highlighted in this review will aid the development of CAR cells that are safer, more resilient against immunosuppressive signals in the CNS, and able to specifically target intracranial tumor cells.

Keywords

CAR T; glioma; central nervous system

INTRODUCTION

Chimeric antigen receptor (CAR)-T cells represent a groundbreaking therapy for many blood cancers, such as B-cell lymphoma, but thus far have failed to demonstrate the same efficacy against non-hematological malignancies, including gliomas. One of the key challenges is an immunosuppressive tumor microenvironment (TME) (1). The mechanisms

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Conflicts of Interest: Hideho Okada is an inventor of IL-13Ra2 (345–353:1A9V) peptide and EGFRvIII-CAR for which an exclusive licensing agreement has been executed with Stemline, Inc. and Novartis Pharma, respectively.

by which gliomas achieve immune evasion remain to be fully elucidated. However, it has been shown that malignant gliomas exhibit stable epitranscriptomic changes which facilitate the recruitment of tumor-associated macrophages (TAMs) and secretion of anti-inflammatory cytokines (2–4). The release of anti-inflammatory cytokines via TAMs and other pro-tumorigenic myeloid cells causes T cell dysfunction, which reduces the functionality of CAR-T cells (5). Tumor cells and TAMs also deplete metabolic fuels and release catabolic byproducts into the TME, which similarly impairs CAR-T cell persistence and survival (6). Furthermore, CAR-T cell trafficking to central nervous system (CNS) tumors is often poor owing to some extent to the presence of the blood-brain barrier (BBB) (7). In this review, we discuss strategies to overcome an immunosuppressive TME, enhance CAR-T persistence, and improve CAR-T cell delivery to gliomas.

Other obstacles pertain to the manufacturing of CAR-T cells and the design of CAR circuits for gliomas. Firstly, there is a lack of antigen targets that are both homogenously expressed by tumors and have absolute tumor-specificity. Heterogenous antigen expression can lead to CAR-T resistance via antigen escape (8, 9). However, targeting abundantly expressed antigens in gliomas can cause “on-target, off-tumor” toxicities (10). Secondly, because many glioma patients have insufficient T cells to produce therapeutic doses of autologous CAR-T cells (11), the development of allogeneic, off-the-shelf CAR-T cells would be beneficial. Proper engineering of allogeneic CAR-T cells should mitigate the issues, such as graft-versus-host disease (GvHD) (12). We describe gaps in the literature, next-generation CAR design, and how genetic editing could facilitate safer, more effective “off-the-shelf” CAR-T cells.

Programming metabolic fitness of CAR T cells

Clinical response is still very rare in patients with malignant glioma following CAR T cell therapy. The immunosuppressive microenvironment of glioma is one of the major factors that limits the functionality of CAR T cells. In addition to the presence of immunosuppressive cytokines and factors (PGE2, TGFb, IL10, VEGF), the glioma microenvironment is also hypoxic, hypoglycemic, lactic acid-rich, and depleted of amino acids such as glutamine, arginine, and tryptophan (6).

Tumor cells and CAR T cells compete for the limited amount of metabolic fuels in the tumor microenvironment (TME). To maintain the fitness of CAR T cells in metabolically altered and nutrient-deprived TME, CAR T cells can be manipulated either pharmacologically or by selective knocking out of genes using CRISPR. In recent years, several studies have focused on identifying the key regulators of metabolic pathways in T cells that can serve as therapeutic targets for enhancing the antitumor function and persistence of T cells (13–18). Hamaidi et al (19) investigated the role of Sirtuin-2(Sirt2), an NAD^+ dependent deacetylase, in T cell metabolism and anti-tumor immunity. Selective inhibition or genetic deletion of Sirt2 in T cells results in reprogramming towards a hyper-metabolic state characterized by an enhanced capacity for aerobic glycolysis and oxidative phosphorylation. At the operational level, Sirt2 suppresses T cell metabolism by targeting multiple enzymes involved in glycolysis, TCA cycle, fatty acid oxidation, and glutaminolysis. Importantly, Sirt2 deficient T cells display enhanced proliferation, survival, and effector function, thus

Sirt2 can serve as an actionable target for metabolic manipulation of CAR T cells. Inhibition of the MAPK pathway by MEK1/MEK2 inhibitors generates CD8+ stem cell-like memory cells that have enhanced mitochondrial respiration fueled by fatty acid oxidation and superior antitumor activity (20).

In addition to the engineering approaches applied at the cellular level, engineering the CAR construct itself can also significantly impact the fitness of the T cells. A dual antigen CAR T cells with split costimulatory signaling domains and shared CD3ζ chain exhibit a rapid effector function via glycolysis, which is supported by CD28 signaling (21). Additionally, 4-1BB costimulatory signaling preserves the oxidative function which is critical for long-term persistence and memory formation (22). Next-generation CAR T cells called TRUCKs (T-cell redirected for unrestricted cytokine-mediated killing) incorporate a transgenic cytokine release machinery to augment the proliferation and persistence in vivo (23, 24). The incorporated cytokines also have a metabolic effect on the T cells. For example, IL-15 treated CAR T cells have elevated levels of oxidative phosphorylation (OXPHOS) and fatty acid oxidation-related genes (25). Methods that can further boost the metabolic and phenotypic fitness of CAR T cells in the TME need to be evaluated in the glioma setting.

Advances in regulation of CAR expression

We and others have shown that spatial and temporal regulation of CAR expression protects T cells from exhaustion and favors a memory stem-like phenotype that improved their in vivo long-term persistence (26–29). Numerous approaches are being evaluated to achieve conditional expression of CAR (Figure 1), such as recognition of tumor-specific antigen, sensing of hypoxia (30, 31), small molecule drug-mediated transient inhibition of CAR expression, and by use of “degrons” (32).

A Synthetic Notch (SynNotch) receptor recognizes a tumor- or organ-specific antigen to induce and confine the expression of CAR to the tumor site. Additionally, transient expression of CAR prevents tonic signaling and exhaustion of T cells(26). Mechanistically, Synthetic Intramembrane Proteolysis Receptors (SNIPRs) are similar to SynNotch receptors, both of which can be tuned to match the antigen density on the tumor cells(27). Another approach is where CAR is fused to a ligand-induced degradation domain (LID) for reversible and tunable CAR expression (33). The addition of a small molecule ligand causes exposure of cryptic degron within the LID domain and subsequent proteasomal degradation of CAR-LID fusion protein and the loss of CAR on the T cells. Alternatively, drug-induced dimerization can serve as an ON-switch for split CAR circuit design (34). The strategies that allow CAR expression to be controlled via drug, may not work for glioma if the drug has poor BBB penetration.

Improvements in delivery of CAR T Cells

The BBB presents a unique challenge for treatment delivery to tumors that arise in the parenchyma of the CNS. While intravenous (IV) delivery has been the most frequently used method, locoregional delivery modes, such as the delivery of CAR T cells into the cerebral spinal fluid (CSF), are developing as an alternative, especially when the targeted antigens are

also expressed in non-CNS tissues. This technique was previously efficacious in a case with glioblastoma (35), and has been implemented in recent clinical trials delivering CAR T cells to pediatric CNS tumors (36, 37). Patients exhibited local inflammation and no dose-limiting toxicities, although most patients still progressed. A caveat of the intra-CSF delivery of CAR T cells is that the CSF space is still separated from the CNS parenchyma by the glia-limitans and the tight network of the astrocyte end-feet (Figure 2).

Focused ultrasound-mediated BBB disruption (FUS-BBBD) has been rapidly emerging as a way to disrupt the BBB (38). In this system, FUS induces vibrations of IV-injected microbubbles, thereby safely and transiently disrupting the BBB. In glioma-bearing mice, FUS-BBBD resulted in significantly increased CAR T cell delivery to the CNS and extended survival (39). FUS-BBBD can also have effects beyond delivery, such as activating microglia or releasing tumor antigens into the peripheral circulation (40). These effects may have therapeutic potential but require stringent tuning of the ultrasound parameters to ensure that they do not contribute to neuroinflammation.

New Antigen Targets

Identifying an antigen that is both abundantly expressed by tumor cells and not expressed in the normal brain is a challenge when treating malignant gliomas. Several antigens are currently being investigated in clinical trials (Table 1). EphA2 is one promising target as a clinical trial using EphA2-directed CAR T cells in glioblastoma demonstrated transient efficacy, although all patients experienced pulmonary edema during treatment (10). This is likely an “on-target, off-tumor” effect as EphA2 is expressed in the lungs and the intravenous delivery of these cells allowed them to traffic to the lung. This toxicity indicates a need for local delivery of EphA2-directed CAR T cells or restricted expression of the CAR.

Other new targets that have emerged in pre-clinical studies are CD70 and B7-H3. A flow cytometry panel of patient-derived intracranial tumor cells showed that B7-H3 was abundantly expressed, showing greater expression than EphA2, IL-13Rα2 and HER2. B7-H3-directed CAR T cells showed preclinical efficacy in murine glioma models. Although B7-H3 is expressed at low levels in the brain, thus far, no neurotoxicites have been observed in preclinical models or phase I clinical trials(41, 42). CD70-directed CAR T cells recently demonstrated efficacy in a murine glioblastoma model, although efficacy varied *in vivo* due to the heterogeneity of CD70 expression in patient samples (8). Such heterogeneity might be overcome by a tandem CAR. A tandem CAR against CD70 and B7-H3 has been developed and tested in several solid tumor types although remains to be tested in intracranial tumors (43).

Advancements in Manufacturing Allogeneic CAR-T Therapies

There are inherent challenges associated with the use of patient-derived T cells which are pertinent to gliomas. Firstly, the timeline of autologous CAR-T cell production from leukapheresis to autologous CAR-T administration is approximately 3 to 6 weeks (44, 45). Tumor progression is likely to occur during the course of autologous CAR-T cell manufacturing, especially aggressive tumors, such as glioblastoma (46, 47). Progression

may induce new neurological symptoms and deficits (48), which would require management with corticosteroids (49). Secondly, newly diagnosed, treatment-naïve glioma patients display a reduced number of circulating CD4+ T cells. Gliomas and other intracranial tumors cause sequestration of T cells in the bone marrow, thus limiting their availability for therapeutic purposes (11).

Although the use of allogeneic CAR-T cells may circumvent the issues of long manufacturing periods and inadequate T cells, other challenges related to their allogeneic nature may arise. Though the CNS and tumors therein used to be described as immune-privileged (50), active immune responses have been documented within the brain following immunotherapy and allogeneic transplantation (51, 52). Therefore, GvHD and allogeneic rejection due to human leukocyte antigen (HLA)-mismatch represent key obstacles to developing allogeneic CAR-T therapies for gliomas. Additionally, the large-scale expansion of off-the-shelf products for multiple patients may induce exhaustion in allogeneic CAR-T cell products (53).

One common strategy to reduce the risk of GvHD following allogeneic CAR-T cells is disrupting the endogenous T cell receptor (TCR) alpha constant (*TRAC*) with genomic editing techniques (e.g. CRISPR/Cas9, TAL effector nucleases) (54–56). Although not related to the mitigation of GvHD, Brown et al. generated steroid-resistance allogeneic CAR-T cells via glucocorticoid receptor knockout, which enabled corticosteroid administration without reducing CAR-T cell efficacy (53). Furthermore, it is feasible to combine these methods with additional genetic modifications that enhance allogeneic CAR-T cell persistence. For instance, Choi et al performed multiplexed gene disruption of *TRAC*, beta-2 microglobulin, and PD-1 to create universal CAR-T cells with enhanced antitumor activity *in vivo* (56). Lastly, induced pluripotent stem cells (iPSCs) may represent a future strategy for readily available, off-the-shelf CAR-T products. Research has shown the feasibility of producing large-scale, functional, expandable T cell-derived iPSCs (T-iPSCs) (57). Genetically editing T-iPSCs via the aforementioned strategies could facilitate safer, more effective off-the-shelf CAR-T therapies.

CAR NK Cells

Natural Killer (NK) cells are another attractive source for allogeneic cells as they lack a T cell receptor (TCR) and are less likely to cause GvHD. Several clinical trials are leveraging NK CAR cells against high-grade gliomas (58). One limitation of these cells is their limited persistence compared to T cells. Delivery of cytokines has been shown to improve the persistence of NK CAR cells, motivating the development of cytokine-delivery systems that will synergize with these cells (59).

Rudek et al developed a system for NK cells whereby activation of a GD2 CAR induces the expression of cytokines in a mechanism like TRUCKS (60). The authors found that, while NFAT is the promotor of choice for the cytokine package in TRUCKS, NFAT-driven promoters behave differently in NK cells than in T cells, leading the authors to select NFkB as their promotor instead. Thus, the principles of CAR T cells cannot always be directly applied to NK cells.

The application of CAR NK cells will benefit from more mechanistic studies on how NK cells are affected by the tumor microenvironment. Recently, Wang et al demonstrated that an autophagy inhibitor, chloroquine, increased the trafficking of NK cells to GBM, mediated by the NFkB signaling pathway and the release of CCL2 and CXCL12. A combination treatment of chloroquine and NK CAR cells synergistically diminished tumor volume in murine GBM (61). Further studies to examine the persistence of these NK cells will have translational importance.

Adverse Events of CAR- T Therapy

CAR-T cell therapies can incur acute and chronic adverse events. One notable example is cytokine release syndrome (CRS), defined as a systemic immune response to pro-inflammatory cytokines released by CAR-T cells (62). Most of the literature on CRS pertains to B-cell malignancies, as up to one-third of patients treated with CD19-directed CAR-T cells develop severe symptoms (e.g. hypotension, hypoxia requiring intubation) (62). Comparatively few clinical trials have tested CAR-T cells in gliomas, with most reporting no cases or mild symptoms that typically resolved with corticosteroids and/or anticytokines (9, 10, 35, 36). However, one severe case of CRS led to a mortality in a Phase 1 trial on EGFRvIII-CAR-T cells (63).

Immune-effector cell-associated neurotoxicity syndrome (ICANS) represents another major side effect of CAR-T therapies (51). ICANS case reports most commonly describe CD19-CAR-T cells, some of which are associated with the expression of CD19 in human brain mural cells (64). The neurological symptoms of ICANS may overlap with those of tumor inflammation-associated neurotoxicity (TIAN), a recently defined phenomenon involving peritumoral oedema and/or CSF obstruction as well as the transient worsening of existing deficits (36). A GD2- CAR-T therapy caused symptoms of TIAN (e.g. transient ataxia, sensory loss, impaired motor function) and potentially ICANS in H3K27M-mutated diffuse midline glioma patients. Further research should explore the clinical manifestations of ICANS versus TIAN in gliomas to elucidate their unique etiologies and facilitate better symptomatic management.

While the immediate risks associated with CAR-T therapies are well-characterized, the long-term effects remain poorly understood. It is possible that repeated CAR-T cell administrations could increase patients' risks of developing neurodegenerative diseases, such as Alzheimer's or Parkinson's. A growing body of evidence suggests that neuroinflammation is a hallmark feature of the pathogenesis of Alzheimer's. Inhibition of CD4+ T cells is known to ameliorate Alzheimer's and Parkinson's pathology in mouse models, while Treg depletion–a crucial step preceding leukapheresis for CAR-T generation–predicts the exacerbation of symptoms (65, 66). Furthermore, some adverse events associated with CAR-T therapies (e.g. CRS, increased production of reactive oxygen species, reduced immunosuppressive T_{reg} cells) (67, 68) have been implicated in the pathogenesis of Alzheimer's (69, 70). The potential for CAR-T therapies to increase long-term neurodegeneration warrants further attention, as brain tumors may independently predispose patients to CNS diseases (71). Indeed, GBM and Alzheimer's both involve similar patterns of expression in a significant number of genes–these genes hypothetically

dampen oxidative phosphorylation and establish a local chronic inflammatory state (71). We should gain a more in-depth understanding of the possible long-term impacts of CAR-T-associated inflammation on CNS function as these therapies continue to advance in safety and efficacy.

CONCLUSION

Many of the immunological features of the CNS and CNS tumors are not favorable for eliciting a successful anti-tumor immune responses. Most CNS tumors bear low mutations (72). While dural/meningeal lymphatic systems should allow the presentation of CSF-derived antigens (73), it is still unclear whether the antigens derived from tumors in the CNS parenchyma could be effectively presented by functional antigen-presenting cells. The unique anatomical challenges also include the presence of BBB which may limit both antigen-presentation and homing of the effector cells to the tumor. Furthermore, standard therapeutic modalities for these patients, such as the use of corticosteroids to alleviate CNS inflammation, significantly impact the immune system. Due to these challenges, immunotherapy strategies that rely on the induction of endogenous anti-tumor immune responses, such as checkpoint blockade and cancer vaccines, may have limited efficacy as monotherapy. By integrating our understanding of these unique challenges and novel technologies, the adoptive transfer of genetically engineered immune cells may hold a promise as discussed in this review. Selection of antigens remains fundamental to minimizing the tumor antigen-loss escape and off-tumor toxicity, and the use of novel engineered circuits, such as SynNotch receptors and SNIPRs, may allow us to improve both efficacy and safety. The therapeutic cells could be also engineered to mitigate metabolic challenges and immunosuppression in the tumor microenvironment. Improvement of the manufacturing process and the use of allogeneic CAR T cells can circumvent the current issues, such as the fitness and long manufacturing and release time of the cell products for patients with aggressive tumor.

ACKNOWLEDGMENT

Funding Support:

This work was supported by NIH/NINDS R35 NS105068 and NIH/NCI R01CA222965

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KEY POINTS

- Advances of cell engineering techniques will be coupled with in-depth understanding of the immunological and metabolic characteristics of malignant gliomas, thereby allowing the development of novel CAR T strategies that will overcome the unique challenges.
- The ideal selection of antigen targets and delivery routes should be based on our emerging understanding of glioma cell heterogeneity and CNS immunology.
- The development of the off-the-shelf CAR T cells, including T-iPSC cells, may allow timely and feasible delivery of the therapeutic cells in patients with rapidly growing tumors, such as glioblastoma.

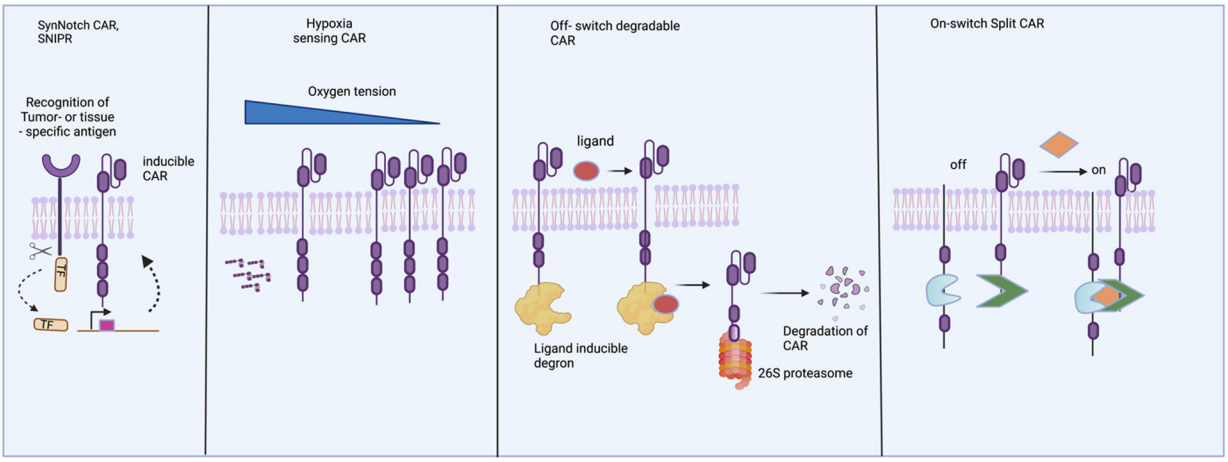

Figure 1. Schematic representation of the approaches for controlling and regulating the expression of CAR.

First panel: SynNotch receptors are engineered to sense tumor- or tissue-specific antigens and induce the expression of a CAR to a second tumor-associated antigen. *Second panel*: Dynamic on/off oxygen sensing safety switch is engineered in the CAR construct to minimize off-tumor toxicities. *Third panel*: Engineering of CAR construct to include a drug-responsive domain (degron) such that the activity of CAR T cells can be controlled by small molecule drugs such as lenalidomide. *Fourth Panel:* Drug-dependent assembly of split CAR where membrane signaling domain is separated from scFV. The split CAR is switched "on" only in the presence of the drug.

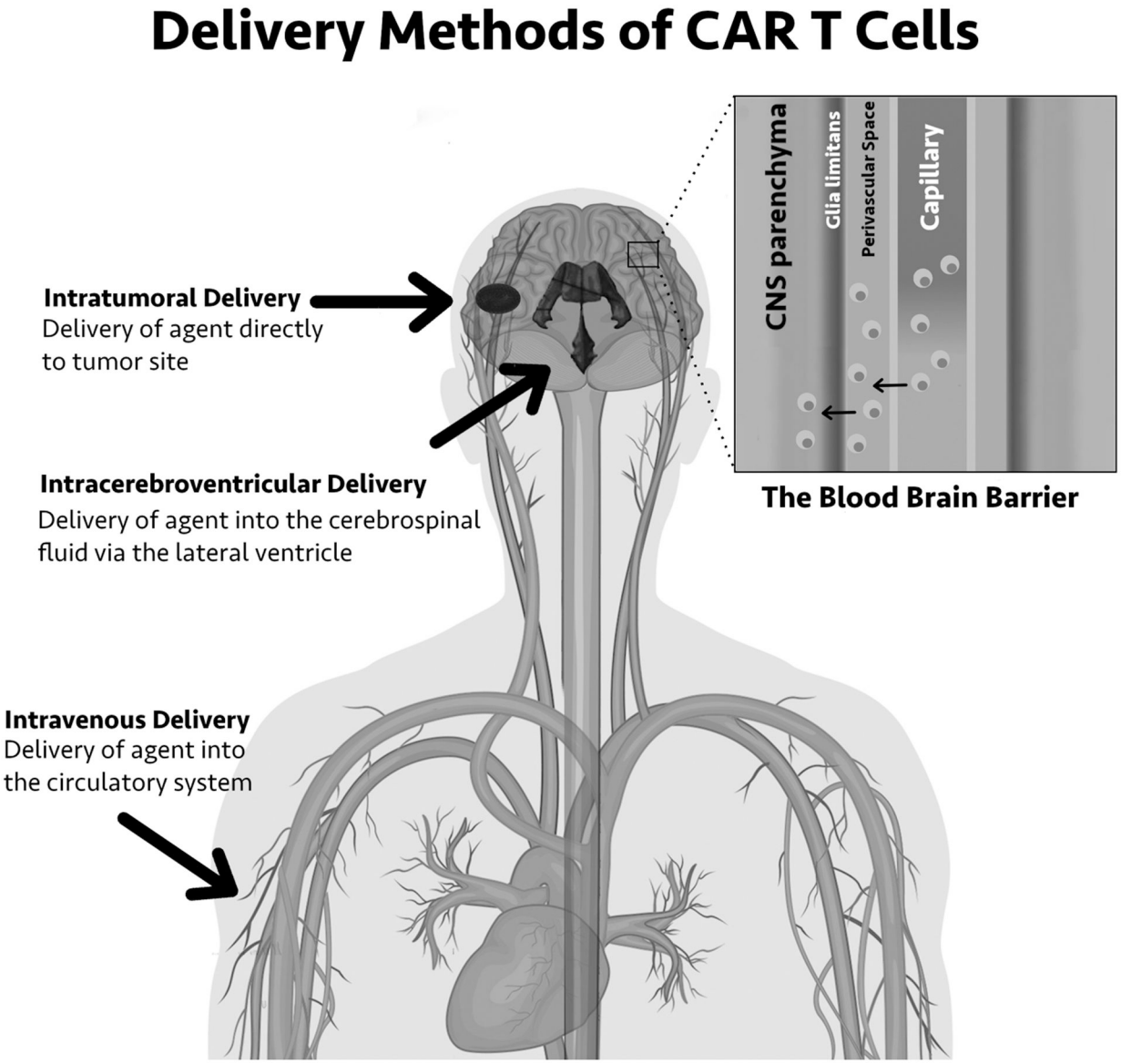

Figure 2: Delivery Methods of CAR T cells.

Intratumoral delivery completely bypasses the Blood-Brain barrier (BBB) through the delivery of the agent directly to the tumor site. Intracerebroventricular (ICV) delivery partially bypasses the BBB as cells enter the CSF, but the glia-limitans still separates the CNS parenchyma. Locoregional delivery encompasses intratumoral and intracerebroventricular delivery. Intravenous delivery is the least invasive option of the three, but CAR T cells must penetrate the endothelial cells surrounding capillaries as well as the glia-limitans to enter the CNS. Vector images from vecteezy.com

Table 1:

Antigens currently investigated for CAR T cells therapy in intracranial tumors.

Antigen	Tumor Expression	Expression in Healthy Tissues	Clinical Trials	References
EGFRvIII	Glioblastoma	None	NCT02209376 NCT01454596 NCT05063682 NCT03423992 NCT02664363 NCT03726515	Ohno et al, Cancer Sci, 2010 Sampson et al, Clin Cancer Res, 2014 O'Rourke et al, Sci Trans Med, 2017 Goff et al, J Immunother, 2019
HER2	Glioblastoma Medulloblastoma Ependymoma	Low expression on epithelial cells in gastrointestinal, respiratory, reproductive and urinary tract (Press, Cordon-Cardo and Slamon, Oncogene, 1990)	NCT01109095 NCT04903080 NCT03500991 NCT03423992	Ahmed et al, JAMA Oncol, 2017 Shen et al, *Oncol Repi*, 2019 Donovan et al, Nat Med, 2020
EphA2	Glioblastoma Medulloblastoma Ependymoma	Low expression on proliferating epithelial cells in adults (Ireton and Chen, Curr Cancer Drug Targets, 2005)	NCT03423992	Chow et al, Mol Ther, 2013 Donovan et al, Nat Med, 2020 Lin et al, Front Oncol, 2021
IL-13Rα2	Glioblastoma Medulloblastoma Ependymoma	Detectable expression in spermatocytes (Jarboe et al, Cancer Res, 2007)	NCT05168423 NCT04661384 NCT04003649 NCT04510051 NCT02208362	Kahlon et al, Cancer Res, 2004 Kong et al, Clin Cancer Res, 2012 Brown et al, Clin Cancer Res, 2015 Donovan et al, Nat Med, 2020
GD2	Glioblastoma Medulloblastoma DIPG H3K27M-mutant DMG	Detectable expression in neurons, skin melanocytes and peripheral nerves (Svennerholm et al, Biochem Biphys Acta, 1994)	NCT04196413 NCT05298995 NCT04196413 NCT03423992 NCT04539366 NCT02107963 NCT04099797	Mount et al, Nat Med, 2018 Prapa et al, NPJ Precis Oncol, 2021 Majzner et al, Nature, 2022
B7-H3	Glioblastoma Medulloblastoma DIPG H3K27M-mutant DMG	Low expression in liver, prostate, uterus and adrenal glands (Zhang et al, Mol Ther Onco, 2020)	NCT04185038 NCT04385173 NCT04077866 NCT05241392	Zhou et al, J Neurooncol, 2013 Majzner et al, Clin Cancer Res, 2019 Tang et al, Mol Ther Oncolytics, 2019