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CAR T-cells to treat brain tumors

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Author Contributions

GG: conceptualization, data curation, writing – original draft, review, and editing. KP: writing, data curation – original draft. MRR: figure construction and editing. AR: conceptualization, supervision, and writing – review and editing. All authors contributed to the article and approved the submitted version.

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Declaration of Competing Interest

None.

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Abstract

Tremendous success using CAR T therapy in hematological malignancies has garnered significant interest in developing such treatments for solid tumors, including brain tumors. This success, however, has yet to be mirrored in solid organ neoplasms. CAR T function has shown limited efficacy against brain tumors due to several factors including the immunosuppressive tumor microenvironment, blood-brain barrier, and tumor-antigen heterogeneity. Despite these considerations, CAR T-cell therapy has the potential to be implemented as a treatment modality for brain tumors. Here, we review adult and pediatric brain tumors, including glioblastoma, diffuse midline gliomas, and medulloblastomas that continue to portend a grim prognosis. We describe insights gained from different preclinical models using CAR T therapy against various brain tumors and results gathered from ongoing clinical trials. Furthermore, we outline the challenges limiting CAR T therapy success against brain tumors and summarize advancements made to overcome these obstacles.

Keywords: chimeric antigen receptor, T-cell, glioblastoma, pediatric brain tumor, pediatric glioma, preclinical brain tumor models

1. Introduction

Although tremendous advancements in traditional cancer treatment, including surgery, radiation, and chemotherapy, have contributed to improved outcomes, cancer remains a difficult and grim disease to treat. More recently, treatment focus has shifted to targeted therapies that interfere with specific proteins involved in tumorigenesis as well as immunotherapies that promote the host's immune system to kill cancer cells. One cancer immunotherapy of increasing interest entails genetically modifying a patient's T-cells to express chimeric antigen receptors (CARs). CAR T-cells are a form of adoptive cell therapy that has demonstrated remarkable success in treating hematological malignancies. While preclinical and clinical models provide encouraging results, treatment of solid cancers using CAR T-cell therapies remain limited. Solid tumors present a unique set of challenges, such as an immunosuppressive tumor microenvironment, that hinder the success of CAR T treatment. The blood-brain barrier (BBB) and treatment-induced neuroinflammation present further obstacles in application of this therapy to brain tumors (Daneman and Prat, 2015). Despite these considerations, CAR T-cell therapy has the potential to be implemented in the treatment of brain tumors in adult and pediatric populations. In this review, we discuss preclinical models of CAR T-cell development for adult and pediatric brain tumors, as well as progress in clinical translation and challenges specific to brain tumors and their microenvironment.

2. CAR T-Cell Engineering: A Brief Overview

Since conceptualization of the CAR over 25 years ago, the goal has been to create genetically-engineered cells encompassing the antigen-specificity of antibodies combined with the potency of immune cells (Zhang et al., 2016; Singh and Mcguirk, 2020). In its simplest form, CAR structure consists of an antibody or ligand-derived ectodomain fused with a hinge, transmembrane domain, and an intracellular T-cell signaling domain (Mirzaei et al., 2017; Newick et al., 2017). When expressed by T-cells, CAR allows recognition of a target antigen without presentation by the major histocompatibility complex (MHC), bypassing a immune requirement required by physiological T cells (Sadelain et al., 2013). The ability of CAR T-cells to activate in an MHC-independent fashion makes this a promising immunotherapy, as MHC downregulation is a hallmark tumors use to mediate immune escape (Reiniš, 2010; Rodriguez et al., 2017).

CAR T-cells have undergone four generations of evolution, which are defined by the incorporation of different intracellular signaling domains (Figure 1A). First-generation CARs contain a single intracellular domain, most commonly CD3ζ. After the development of first-generation CARs, studies demonstrated the activation and anti-tumor potential of CAR T-cells but showed limited efficacy in clinical trials due to a lack of T-cell proliferation and persistence (Jackson et al., 2016; Roselli et al., 2021; Wang et al., 2017). To enhance T-cell expansion and persistence, advances in CAR design introduced one or two co-stimulatory domains to develop second and third-generation CAR-T cells, respectively. Common co-stimulatory domains incorporated in the CAR molecule include CD28, 4-1BB, CD27, ICOS, and/or OX40 (Sadelain et al., 2013). Most recently, CAR design has been optimized to include antitumor cytokines and ligands, such as IL-2 and 4-1BB, respectively, generating "armored" fourth-generation CAR T-cells (Yeku and Brentjens, 2016).

Theoretically, the MHC-independent fashion of CARs allows the receptor to redirect the effector functions of a T-cell to structures other than protein epitopes, like carbohydrates and glycolipids, broadening its applicability (Chmielewski et al., 2013; Patterson et al., 2020; Rodriguez et al., 2017). For the application of CARs in cancer treatment, however, the engineered target antigen should ideally only be expressed on cancer cells and not on normal cells to limit potential off-target therapeutic effects. CAR T-cell manufacturing can take place once a target is chosen and involves the collection, selection, transduction, expansion, and reinfusion of patient-derived lymphocytes, although this process is beyond the scope of the review (Feins et al., 2019; Frigault and Maus, 2016). To date, the most clinically studied target is CD19, a B-cell receptor-associated protein, present in most B-cell hematological malignancies. Exciting progress using CD19 CAR T-cell therapy for the treatment of B-cell leukemia and lymphomas led to the first two FDA approved CAR T-cell therapies in 2017: tisagenlecleucel (Kymriah, Novartis Pharmaceutical Corp) and axicabtagene ciloleucel (Yescarta, Kita Pharma, Inc.) (Maude et al., 2014a; Meng et al., 2021; Vairy et al., 2018).

While progress in CAR T-cell therapy for the treatment of hematological cancers has led to breakthrough therapeutics, CAR T-cell application in the treatment of solid tumors is limited. This limitation is due to differences in physical and physiological characteristics of solid and blood cancers. For example, tumor cells in the blood are easily infiltrated by CAR T-cells in contrast to tumor cells in organs that can exist deep within the body and are relatively concealed for access by T-cells (Liu et al., 2021). Additionally, as mentioned above, cancers such as B-cell leukemia and lymphoma universally express CD19, but solid tumors rarely express *one* unique tumor-specific antigen (Martinez and Moon, 2019). CAR T-cell trafficking and persistence, tumor antigen heterogeneity, and the immunosuppressive tumor microenvironment (TME) all present major obstacles that hinder the success of CAR T therapy in solid tumors (Thomas et al., 2021). Currently, the brain is the most common solid tumor undergoing clinical trials and presents further difficulties due to the semipermeable properties of the blood-brain barrier (BBB) (Patterson et al., 2020). Nonetheless, studies have shown early promise of CAR T-cell efficacy in the treatment of glioblastoma multiforme (GBM), providing a foundation for further investigation using brain cancer models.

In this review, we focus on CAR T-cell therapy for the treatment of adult and pediatric brain tumors. We will also discuss preclinical models and their clinical translation as well as the challenges of CAR T-cell therapy for brain cancer.

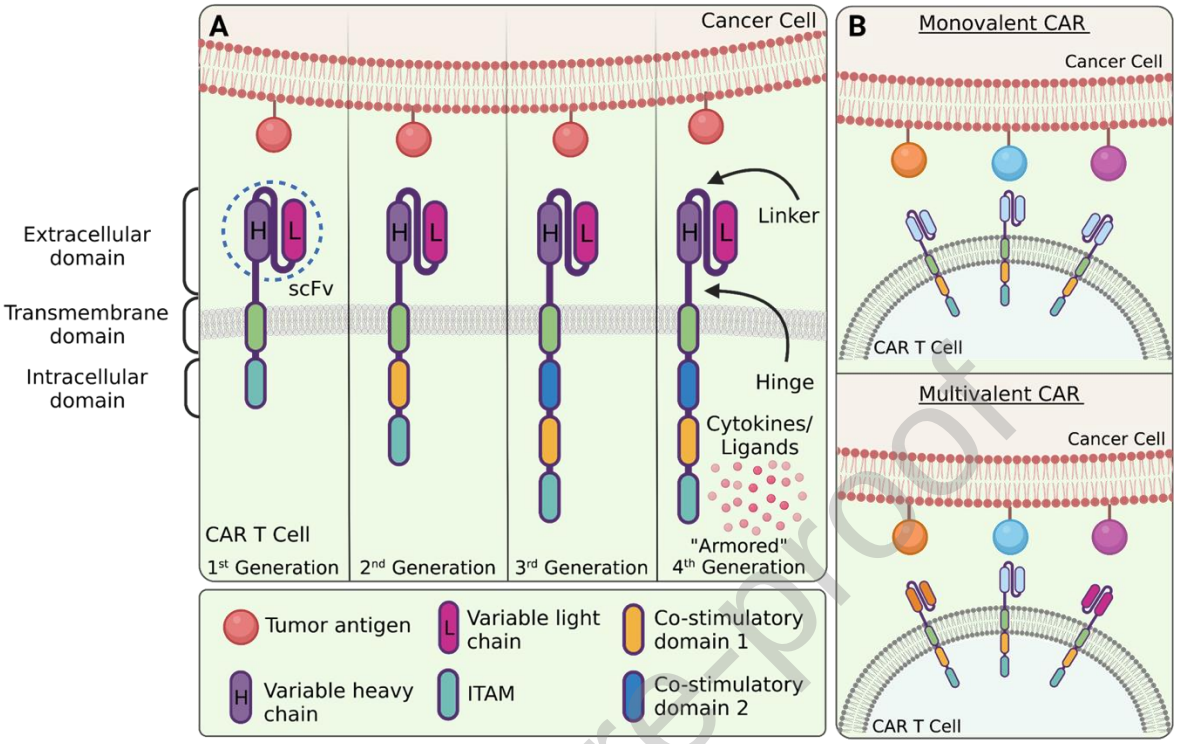

Figure 1. Evolution of chimeric antigen receptor (CAR) design. (A) Chimeric antigen receptor (CAR) constructs consist of an extracellular ligand-binding domain, most often a single-chain variable fragment (scFv) as the antigen-recognition domain, a transmembrane domain, and one or more intracellular domains compromised of immunoreceptor tyrosine-based activation motifs (ITAM). First-generation constructs contain a single signal activation domain, most commonly derived from a CD3ζ chain. Over the past two decades, CAR design has evolved into second-generation and third-generation CARs that incorporated one or two co-stimulatory domains, respectively. Inclusion of additional signaling domains, such as CD28 and 4-1BB, promote persistence and anti-tumor activity of CAR-T therapy and are necessary to avoid exhaustion observed with first-generation CAR cells. Most recently, fourth-generation known as “armored” CARs have been engineered to express a cell-surface or secreted immunomodulatory molecules, such as cytokines and/or ligands, to enhance T-cell function and favorably modify the tumor microenvironment. (B) Monovalent CARs can be generated to universally target one specific tumor antigen, whereas multivalent CARs can target several tumor antigens at once. Multivalent CARs are of increasing interest since multi-antigen targeting can overcome tumor heterogeneity. Figure made in BioRender.com.

3. Adult Brain Tumors

3.1 Glioblastomas

GBM is the most prevalent and lethal primary brain tumor in adults, with an incidence of 3.19 per 100,000 individuals (Dapash et al., 2021). While GBMs can occur at any age, the incidence increases significantly with age and the median age at diagnosis is 65 years old (Chen et al., 2021). The standard therapeutic regimen, notably known as the Stupp Protocol, involves safe maximal surgical resection followed by adjuvant chemoradiation with temozolomide (TMZ) (Dapash et al., 2021; Stupp et al, 2005). Despite aggressive treatment, the 5-year survival is only 7.2% in the United States (Wu et al., 2021).

While prognosis remains poor, GBMs display specific genetic alterations during their progression that can be predictive of survival and allow for the creation of therapeutic interventions based on molecular targeting. Alterations in molecular markers such as isocitrate

dehydrogenase (IDH) mutations, epidermal growth factor receptor (EGFR) amplification/overexpression, and O6-methylguanine DNA methyltransferase (MGMT) promoter hypermethylation are used to determine survival prognosis (Janjua et al., 2021; Lima et al., 2012). For example, patients diagnosed with IDH-mutant GBM, a mutation representing the malignant transformation of a low-grade pre-existing glioma to GBM, typically indicates a longer survival outcome when compared to patients with IDH-wildtype GBM (Eckel-Passow et al., 2015; Kim, 2021; Louis et al., 2016).

3.1.1 Antigenic Targets for GBMs

IL-13Rα2 is a monomeric high-affinity interleukin-13 (IL-13) receptor expressed in over 75% of GBM tumors (Bagley et al., 2018; Marei et al., 2021; Xu et al., 2022a). IL-13Rα2 is highly expressed in normal testis tissue but not elsewhere in the body, making it a favorable target antigen for CAR T-cell therapy (Sharma and Debinski, 2018). IL-13Rα2 is one of the two binding chains of cytokine receptor IL-13. Secreted by activated T-cells, IL-13 plays a significant role in eliciting pro- and anti-inflammatory immune response (Wadajkar et al., 2017). In most cells, IL-13 binds to IL-13Rα1, the low-affinity chain, and joins with IL-4Rα. The heterodimer complex formed results in downstream signal activation of the STAT6 transcription factor, which can promote apoptosis (Thaci et al., 2014). However, in cancer cells (and in some normal cells), free IL-13 binds strongly with IL-13Rα2, and in turn, fails to activate signaling. Consequently, IL-13Rα2 is often referred to as a “decoy” receptor since it essentially sequesters IL-13, thus providing an apoptosis escape mechanism for GBM cells (Junttila, 2018; Rodriguez et al., 2017). As a result, overexpression of IL-13Rα2 promotes tumor progression and is a prognostic marker for poor patient survival (Brown et al., 2013).

Commonly, *in vitro* assays are relatively less labor-intensive methods performed during the initial steps of CAR T-cell evaluation to determine CAR activation and activation-signaling pathways involved. In vitro models, such as 2D cell cultures, allow researchers to assess cytokine production through harvested cultures and harvested target cells are often subject to flow cytometry for killing potency evaluation (Si et al., 2022). While *in vitro* cultures and assays are useful for establishing CAR T specificity and killing kinetics, they often do not provide information on the interaction between engineered T-cells and the tumor microenvironment or on possible systemic side effects (Si et al., 2022; Vávrová et al., 2011) (Figure 2). Thus, after confirmation of CAR activation and potency through *in vitro* assays, many preclinical models move to *in vivo* animal models, most commonly using immune-comprised mice, which lack functional T, B, Natural Killer (NK), and dendritic cells allowing for the establishment of human tumors and analysis of human CAR T-cells (Ito et al., 2002)(Table 1). Table 1 summarizes brain tumor preclinical models used in the development of CAR T cell therapy. Several preclinical studies have evaluated IL-13Rα2 as a target for CAR T-cell therapy in GBM tumor models. IL-13Rα2-specific CAR T-cells termed IL-13-zetakine have been developed. City of Hope researchers expanded low-passage Glioblastoma stem cell (GSC), tumorsphere and serum-differentiated glioma lines from patient GBM specimens and assessed the expression of IL-13Rα2 positive cells (Brown et al., 2012). While IL-13Ra2 expression varied, IL-13Ra2-positive cells were observed in both GSCs and differentiated tumor cell populations. The sensitivity of IL-13-zetakine-mediated killing was compared in both IL-13Rα2+ GSC and differentiated tumor cell lines *in vitro* and in non-obese diabetic with severe compromised immunodeficiency (NOD-*scid*) mice which showed that both cell lines were killed with comparable potency. Importantly, this demonstrated that stem/progenitor-like properties were not intrinsically resistant to IL-13-

zetakine-mediated killing, providing evidence that IL-13Rα2-targeting could potentially eliminate the refractory GSC component of GBMs.

These findings led to the first clinical trial of IL-13Rα2-targeted CAR T therapy to establish the safety and efficacy of engineering, expanding, and delivering IL-13Rα2-specific CAR cells in three patients with recurrent GBM (Brown et al., 2015). First-generation IL-13-zetakine CD8 T-cell clones were safely administered intracranially and demonstrated transient anti-glioma activity in 2 of 3 patients. A subsequent preclinical model by the same group aimed to optimize IL-13-zetakine CARs by including a 4-1BB co-stimulatory domain to enhance anti-tumor potency and persistence for the treatment of GBM. The potency of second-generation IL-13Rα2-targeted CAR, termed IL-13BBζ T-cells, were compared to previously constructed first-generation IL-13-zetakine CARs in a ffLuc+ PBT030-2 GBM xenograft model (Brown et al., 2018). Mice treated with a single injection of IL-13-zetakine CD8 clones exhibited some tumor growth control and improved survival only at the highest dose (1 X 10^6), whereas a single administration of IL-13BBζ T-cells at lower doses (0.3 x10^6 and 0.1x10^6) were significantly more effective. Additionally, the impact of dexamethasone on the anti-tumor potency of IL-13BBζ was assessed, as this corticosteroid is most commonly used for the management of vasogenic edema and intracranial pressure in patients with brain tumors (Kostaras et al., 2014). IL-13BBζ CAR Tanti-tumor potency and cell-mediated effects were completely eliminated only in mice that received the highest dose of dexamethasone (5mg/kg), suggesting that *in vivo* anti-tumor effects of IL-13BBζ CAR T-cells could be maintained in the presence of low-dose dexamethasone. *In vivo* models also showed that local intracranial delivery of IL-13BBζ CAR T-cells were superior to intraventricular administration. The encouraging evidence and lack of dose-limiting toxicities of this preclinical model provided a rationale for clinical translation, in which one patient with recurrent, multifocal GBM. This patient received six weekly intracavitary infusions of IL-13BBζ CAR T-cells which resulted in complete tumor regression of lesions in the brain and spine (Brown et al., 2016). Unfortunately, the patient developed new tumors, likely due to target antigen immunity, which will be discussed in more detail later in the review. Although the patient had a recurrence, this remarkable case study provided data on the safety of locoregional delivery of CAR T-cells into the cerebrospinal fluid (CSF) and activation of host immune responses after CAR T delivery (Feldman et al., 2021).

More recently, researchers in Beijing generated a CAR derived from a murine antibody targeting IL-13Rα2 and humanized the sequence to form a humanized anti-IL-13Rα2 CAR (Xu et al., 2022). The group developed a third-generation CAR, incorporating both CD28 and 4-1BB as costimulatory chains for the CD3ζ domain and subsequently evaluated their expansion, anti-GBM efficacy, and cytokine release *in vitro and* xenograft mouse models using U251 and U373 cells. *In vitro* results demonstrated that humanized anti-IL-13Rα2 CAR-targeting did not secrete increased levels of IL-6, a significant driver of cytokine release syndrome (CRS), the most common neurologic toxicity associated with CAR T therapy (Santomasso et al., 2022). Furthermore, there was low expression of IL-10, an inducer of T-cell dysfunction (Giavridis et al., 2018; Rivas et al., 2021; Xu et al., 2022). Intravenous administration of IL-13Rα2 CAR T-cells inhibited tumor growth and significantly prolonged the survival of tumor-bearing mice in the first 40 days. However, tumors relapsed after day 40 in both U251 and U373 generated intracranial tumors. To further understand the origin of anti-GBM activity of humanized third-generation IL-13Rα2 CAR T-cells, investigators studied the gene expression profiles of the cells using high-throughput sequence data. Two notable genes highly expressed in the CAR T-cells were MYH9 and FLNA, necessary for T-cell motility and T-cell adhesion and trafficking, respectively (Jacobelli et al., 2004; Savinko et al., 2018). While tumor relapse occurred, the

study was the first to investigate humanized third-generation IL-13Ra2-specific CAR targeting for GBM with potential as a candidate tool for clinical application.

As mentioned in section 3.1, epidermal growth factor receptor (EGFR) is a molecular target used as a prognostic biomarker in GBM. EGFR is a tyrosine kinase receptor that is overamplified and/or mutated and contributes to tumor development and progression (Sigismund et al., 2018). The variant III mutation of the EGFR, EGFRvIII, is the most commonly found EGFR variant in GBM (Brennan et al., 2013), resulting from a deletion of exons 2 and 7, subsequently creating a junction site with a new glycine residue in the extracellular domain (Choi et al., 2019; Sugawa et al., 1990). Unlike wild-type EGFR, EGFRvIII is minimally expressed in normal tissue and is a strong tumor-restricted antigen expressed in approximately 50% of GBM tumors (Akhavan et al., 2019; Choi et al, 2019; Marei et al., 2021). Thus, the EGFRvIII epitope is an ideal candidate for CAR T targeting.

Third-generation EGFR vIII-specific CAR designed with 4-1BBζ and CD28 co-stimulatory domains have been constructed. A preclinical model evaluated the potency and efficacy of these CAR constructs alone and when co-transduced with miR-17-92, the most significantly upregulated microRNA cluster in interferon gamma (IFN- γ)-producing T helper type-1 (Th1) cells (Ohno et al., 2013; Xiao et al., 2008). Mice with intracranial U87-EGFRvIII tumors received a single intravenous infusion of miR-17-92 co-transduced CAR T-cells, EGFRvIII-specific CAR T-cells alone, or mock-transduced T-cells (n =10) and were administrated daily injections of TMZ for 5 days beginning on the day of CAR T-cell treatment. All mice in the control group died within 3 weeks, whereas 1 mouse died in the EGFRvIII-specific CAR T group and 2 died miR-17-92 co-transduced CAR T-cells. Forty-nine days post-T-cell infusion, 4 mice receiving EGFRvIII-specific CAR T-cells alone and 3 mice treated with co-transduced miR-17-92 survived and were re-challenged with U87-EGFRvIII cells. Tumor cells grew in all four mice treated with EGFRvII-specific CAR T alone, whereas none of the three mice treated experienced tumor growth. This study demonstrated the safety and efficacy of EGFRvIII-specific CAR T-cells alone and provided evidence that durability could be enhanced with co-expression of miR-17-92 in CAR T-cells. Other studies that determined to enhance the potency of the EGFRvIII CAR construct focused on the humanization of the scFV to promote persistence of the modified T-cells while avoiding immediate rejection or allergic responses caused by murine scFV-based CARs (Johnson et al., 2015).

In 2017, a first-in-human study involving 10 patients with recurrent EGFRvIII-positive GBM who each received a single dose of intravenously delivered second generation (4-1BB, CD3ζ) humanized anti-EGFRvIII CAR T-cells was published (NCT02209376) (O'Rourke et al., 2017). CAR T-EGFRvIII cell infiltration induced immunosuppressive effects on the GBM microenvironment, including significant upregulation of immunosuppressive molecules such as indoleamine-2,3-dioxygenase (IDO) 1, PD-L1, transforming growth factor (TGF)-β and IL-10, creating a barrier to the efficacy of this therapy. While a clinical benefit could not be clearly determined, this study provided the feasibility of and safety of CAR T-EGFRvIII as there was no cross-reactivity with wild-type EGFR, and demonstrated that the constructs could traffic to the brain and lead to decreased antigen expression in GBM.

In a pilot dose-escalation trial, third-generation EGFRvIII CAR T-cells were administered intravenously to 18 patients with recurrent EGFRvIII+ GBM after lymphodepleting chemotherapy (Goff et al., 2019). EGFRvIII CAR T-cells were supported post-infusion with low-dose intravenous interleukin-2 (IL-2), a multifaceted cytokine involved in CD8+ T-cell differentiation that is associated with systemic toxicities at high doses (Jiang et al., 2016; Rosenberg et al., 1994; Skrombolas and Frelinger, 2014). All patients developed transient leukopenia and

thrombocytopenia and, unfortunately, one patient died four hours after the highest dose (6 x 10^10 cells) was administered. While the persistence of CAR cells correlated with cell dose, treatment failed to induce objective tumor regression or prolong survival. More recently, Agliardi *et al* used an orthotopic GBM mouse model to demonstrate that when combined with a single intra-tumoral dose of IL-12, EGFRvIII CAR T targeting could achieve durable anti-tumor responses in contrast to EGFRvIII CAR T infiltration alone (Agliardi et al., 2021). IL-12, a proinflammatory cytokine that is responsible for the induction and enhancement of cell-mediated immunity has been shown to enhance anti-tumor response in mouse models of ovarian cancer (K.G. Nguyen et al., 2020; Koneru et al., 2015). Similar to high doses of IL-2, systemic IL-12 is poorly tolerated, therefore, the study aimed to observe if local delivery to the tumor site would achieve an anti-tumor response while limiting systemic toxicity. Within the GBM tumor microenvironment (TME), the addition of IL-12 led to a decreased proportion of CAR –T-cells expressing high levels of programmed cell death protein 1 (PD-1) and lymphocyte activation gene 3 protein (LAG3), inhibitory receptors that have been extensively associated with dysregulation of antigen-specific T-cells in patients with chronic infection and cancer (Barber et al., 2006; L. T. Nguyen and Ohashi, 2015; Petrovas et al., 2006; Sakuishi et al., 2010). Furthermore, local injection to the tumor caused a transient upregulation of IL-12-induced cytokines (ie IFN- γ and CXCL9) that resolved 11 days post-injection, demonstrating that benefits could be observed while limiting systemic toxicity.

Ephrin type-A receptor (Epha2) is a 130 kDa, 976 amino acid transmembrane glycoprotein belonging to the Eph family of receptor tyrosine kinases (Wykosky and Debinski, 2008). EphA2 is overexpressed in most cancers, promoting tumorigenesis through its involvement in cell proliferation, invasion, and migration (Baharuddin et al., 2018). EphA2 is activated by ephrin ligands and has a role in angiogenesis and tumor neovascularization when bound to endogenous ephrin 1 ligand, its most common ligand (Rodriguez et al., 2017; Wykosky et al., 2005). Except for some epithelial cells, EphA2 has limited expression in normal tissue (Kilian et al., 2021). However, EphA2 is strongly overexpressed in GBM tumors and associated with astrocytoma grade, making it another ideal target antigen used for developing GBM therapies (Wykosky et al., 2008.).

Development of second-generation EphA2-specific CAR T-cells with a CD28 costimulatory domain produced immunostimulatory cytokines IFN- γ and IL-2 that effectively caused tumor lysis *in vitro* and regressed EphA2+ GBM tumors *in vivo* (Chow et al., 2013). A subsequent study compared the anti-GBM efficacy of three CARs with either a CD28, 4-1BB or CD28.4-1BB signaling domain demonstrated no significant difference in phenotype and effector function of CAR T-cells *in vitro* (Yi et al., 2018). Similarly, no significant differences in CAR T-cell persistence *in vivo* were observed. This finding added to the debate whether CARs constructed with two costimulatory domains enriched T-cells with superior effector functions than with a CAR construct containing only one costimulatory intracellular signaling domain (Milone et al., 2009; Zhao et al., 2015). The lack of optimized potency missing in CD28.4-1BB EphA2-CAR T-cells highlighted the potential need for cytokine signaling in addition to the required activation and co-stimulation signaling of CAR constructs (Yeku and Brentjens, 2016). While this strategy enhances CAR T-cell potency and persistence, it could increase the risk of unwanted side effects. Recently, Lin *et al* published preliminary results of the first-in-human trial of EphA2-specific CAR T-cells to study the feasibility, safety, and clinical response of infusion in three patients with EphA2-positive recurrent GBM (NCT 03423992)(Q. Lin et al., 2021). Although CAR T proliferation in the brain could not be measured due to the unavailability of tumor tissue, it was observed in the CSF of one patient which exhibited robust proliferation of

CAR T-cells. This observation indicated that EphA2-specific CAR T-cells were able to cross the BBB and expand in the host TME. MRI imaging demonstrated stable disease in one patient, but the other two patients showed progressive disease post-CAR T infusion. The study showed that intravenous infusion of EphA2 CAR T-cells was tolerable with transit efficacy but future studies with adjusted doses and infusion frequencies are needed to further investigate the safety and efficacy of EphA2-CAR T-cells for the treatment of GBM. The need for further study assessing the persistence and longevity of EphA2-CAR T is also warranted.

Human epidermal growth factor receptor 2 (HER2), a receptor with tyrosine kinase activity, is a targeted antigen for GBM CAR T therapy. HER2 exists as a monomer on the cell surface and homo- or heterodimerization of this protein leads to autophosphorylation of tyrosine residues within the intracellular domain of the receptor that leads to activation of downstream pathways (Iqbal and Iqbal, 2014). The activated signaling pathways include Ras/MAPK and PI3K/Akt resulting in cell proliferation, survival, differentiation, invasiveness, and tumorigenesis (Zhu et al., 2021). HER2 is expressed in various cancers and is widely used as a prognostic and predictive marker in breast cancer. Specifically, HER2 is overexpressed in up to 80% of GBMs but has limited expression in healthy central nervous system (CNS) tissue (L. Zhang et al., 2016).

Preclinical models of medulloblastoma and GBM showed that HER2-CAR T-cells induced tumor regression and antitumor activity, respectively, becoming candidate tools for clinical application (Ahmed et al., 2015). While clinical usage of HER2 was initially challenged due to systemic toxicities, as will be discussed in the following section, second-generation (CD28 domain) anti-HER2 CAR T-cells derived from virus-specific T-cells (VST) were studied in phase 1 dose-escalation study for GBM (J. G. Zhang et al., 2007). HER2-CAR VST-cells ($1x10^6/m^2$ to $1 \times 10^8/m^2$) were intravenously administered to 17 patients with HER2+ GBM without prior lymphodepletion. Other than two patients that experienced seizures and headaches most likely related to the T-cell infusion, no dose-limiting toxicity was observed. qPCR was used to measure in vivo detection of the CAR T-cells in peripheral blood and analysis at 6 weeks post-infusion showed the presence of HER2 CARs in 7 of 15 patients. Only two patients had detectable HER-CAR cells 12 months post-infusion, and none were detected after 18 months. MRI imaging was used to assess tumor response 6 weeks after infusion, which showed a partial response in 1 patient and stable disease in 7 patients. The median overall survival was 11.1 months after the first T-cell infusion and 24.5 months after diagnosis. A difference between this study and others mentioned earlier in the review is that the trial included 7 children (median age, 14 years; range, 10-17 years). As children have a better prognosis than adults, it may have contributed to the outcome. However, no significant difference was seen in the probability of survival of children and adults. Treatment of HER+ GBM with HER2-CAR VSTs was feasible and safe, but the trial warranted the need to improve anti-GBM activity and efficacy of the CARs.

3.1.2 Multivalent CARs

Tumor heterogeneity and varying antigen expression profiles within GBM patients led researchers to develop CAR T-cells targeting multiple tumor antigens to avoid these potential immune escape mechanisms (Liang et al., 2005; J. G. Zhang et al., 2008) (Figure 1B). Hedge *et al* created a bispecific CAR molecule incorporating both HER2 and IL-13Rα2 scFv-domains to make a tandem CAR exodomain (TanCAR) tethered to a CD28 signaling endo-domain (Hegde et al., 2016). TanCAR T-cells demonstrated enhanced and sustained *ex vivo* antitumor activity

in comparison to unispecific HER2 and IL-13Rα2 CAR T-cells in U373 cells. Through observation of IFN- γ and IL-2 secretion levels, simultaneous targeting of both TAAs induced significantly higher cytokine secretion by TanCAR T-cells than did exposure to a single target. Furthermore, in an orthotopic GBM model, TanCAR T-cells extended the median overall survival to 86 days ($p = 0.0002$) in comparison to 53 days and 55 days using only HER2 and IL-13Rα2 CAR T-cells, respectively. Since HER2 and IL-13Rα2 antigen pair expression varied between patients making successful clinical translation challenging, Bielamowicz *et al* incorporated a third target antigen in an attempt to overcome GBM variability (Bielamowicz et al., 2018). CAR constructs using a single tricistronic vector encoding second-generation (CD28 ζ-signaling domain) HER2, IL-13Rα2, and EphA2 targets, termed UCAR T-cells, were developed. Analysis using primary GBM patient samples with confirmed intercellular expression variability showed that the UCAR T-cells captured nearly 100% of tumor cells and exhibited significantly higher cytokine production compared to mono- and bi-specific CAR T-cells. In established GBM patient-derived xenografts, UCAR T-cells demonstrated higher and more sustained antitumor effects and significantly prolonged the survival of treated animals. While the preclinical study demonstrated promising results of using U CAR T-cells, further work is needed to determine efficacy and safety since increasing the number of targeted antigens also increases the risk of “on-target off-tumor" effects that could result in serious damage to healthy tissue (Bader et al., 2021).

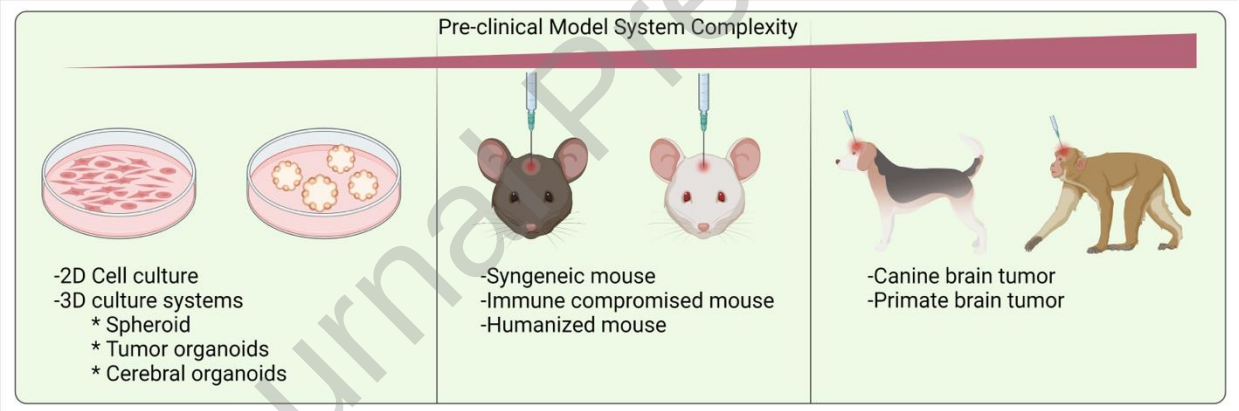

Figure 2. Preclinical CAR T brain tumor models. Schematic representation of the preclinical models used to investigate the application of CAR T-cells in brain tumors. The complexity of the models increases from left to right. Canine and primate tumor models have been used to further study the safety of CAR T-cells before transitioning to clinical trials. Figure made in BioRender.com.

3.2 Metastatic brain tumors

While GBMs are the most common *primary* brain malignancy in adults, brain metastases (BMs) account for the majority of adult intracranial tumors. Metastatic brain cancer develops as tumor cells from primary cancers present in other organs travel and spread through the blood, a phenomenon deemed as ‘hematogenous seeding’, and invade the brain (Barajas and Cha, 2016). Although less frequent than hematogenous spread, CNS metastasis can also result from direct geographical invasion (Achrol et al., 2019; Barajas and Cha, 2016). While various factors, such as underreporting of metastatic brain tumor cases, hinder a more precise prevalence and incidence, it is estimated that 20-40% of adult patients with cancer develop metastatic brain tumors (Achrol et al., 2019; Nayak et al., 2012.; Tsukada et al., 1983).

The most common site of intracranial metastases is the brain parenchyma, but it can also spread to the cranium, dura, leptomeninges, and rarer, to the pituitary, pineal gland, or the choroid plexus (Nayak et al., 2012). In adult patients, the majority of BMs originate from lung, breast, and skin cancers, with lung cancer leading as the most recurring source of metastases (Achrol et al., 2019; Nayak et al., 2012.). Most often, BMs are discovered after the diagnosis of an initial primary cancer but BMs can be identified even before the diagnosis of a primary tumor (Sacks and Rahman, 2020). The mainstay treatment of BMs involves a multimodal approach, including a combination of surgery, radiation (both stereotactic radiosurgery and whole-brain radiation therapy), chemotherapy, immunotherapy, and palliative care (Achrol et al., 2019; X. Lin and DeAngelis, 2015; Sacks and Rahman, 2020; Soffietti et al., 2020).

3.2.1 Antigenic Targets for metastatic brain tumors

Currently, systemic therapies for patients with BMs are mainly based on molecular biomarkers assessed in the primary tumor. Multiple studies have shown novel alterations in the metastatic site (Ding et al., 2010; Paik et al., 2015.; Xie et al., 2014). Sequencing analysis of 86-patient-matched brain metastases showed that 53% of BMs harbored potentially targetable mutations not detected in the matched primary tumor sample (Brastianos et al., 2015). Thus, the "branched evolution" pattern of BMs can lead to heterogeneity of the primary and metastatic tumor, posing obstacles of developing targeted therapies for BMs. Additional challenges to the creation of more precise therapies for BMs include tissue sampling, as some patients are not candidates for resection or have tumors in inaccessible sites. The brain microenvironment can also lead to spatial heterogeneity (Ali et al., 2021). Nonetheless, genomic profiling of a primary tumor and brain metastases using tumor samples, CSF fluid, and liquid biopsy have provided molecular profiles of BMs in different cancer types (Chicard et al., 2018; Pentsova et al., 2016).

Approximately 20-25% of breast cancers have overexpression of HER2 (Eroglu et al., 2014). Up to 50% of HER2+ breast cancer patients develop brain metastasis, often the final lethal consequence of many cancers (Leone and Lin, 2019). As mentioned earlier, the HER2 signaling cascade activates several pathways, including Ras-MAPK and PI3K, that when altered or enhanced, are associated with an increased risk of metastases formation (Bryan et al., 2021). Initially, HER2 as a CAR target was challenged due to the death of a patient with HER2+ metastatic colon cancer following HER2-CAR T-cell infusion (Morgan et al., 2010). Despite this, as described above, the application of lower doses of HER2 in CAR T therapy provided safety and antitumor effects in GBM and sarcoma trials (J. G. Zhang et al., 2008). The findings of these studies led City of Hope researchers to develop two second-generation HER2-CAR-4-1BB/CD28 CARs to compare antitumor activity of each co-stimulatory signaling domain in *in* vitro and *in vivo* orthotopic human xenograft models of breast cancer that metastasized to the brain (Priceman et al., 2018). In addition, they assessed the different routes of administration (intravenous, local intratumoral or regional intraventricular) to determine which resulted in the most effective CAR T trafficking. Both HER2–28ζ or HER2BBζ CAR T-cells were cocultured with various tumor targets to measure the tumor-killing abilities influenced by each costimulatory domain. Using flow cytometry to quantify tumor killing, 4-1BB-specific HER2-CAR T-cells demonstrated improved tumor-killing, with lower levels of T-cell exhaustion and greater *in vitro* antigen-dependent proliferation when compared to CD28-specific HER2-CARs. This finding aligned with prior studies of leukemia and solid tumor models that suggested further therapeutic durability with 4-1BB stimulation compared to CD28 (Long et al., 2015; Maude et al., 2014b). Equivalent antitumor responses and extended survival of mice were observed after intracranial

and intraventricular delivery of 0.5 x 10^6 CAR T-cells. In comparison, intravenous delivery of HER2 CAR T-cells resulted in only partial antitumor response even at 10-fold higher doses. The study was the first to provide preclinical evidence of using regional intraventricular delivery of HER2-CAR T-cells to effectively target breast cancer metastasis to the brain. They demonstrated potency and selective targeting of 4-1BB-specific HER2 CAR T-cells and effective intraventricular administration of the CAR T-cells established a platform for clinical translation. City of Hope researchers are currently recruiting for a phase I trial to study the dosage and side effects of intraventricular administration of HER2 CAR T-cells in patients with recurrent brain or leptomeningeal metastases (NCT03696030).

Table 1. *Summary of seven preclinical models used in CAR T-cell therapy evaluation for brain tumors.*

	Model	Advantages	Limitations	Uses	Examples
In vitro models	Cell culture assay	Less labor intensive, easier to scale up	Cannot detect systemic immune response, off-target effects	Determination of CAR activation, cytokine production, TAA density	Brown et al., 2012
	Tumor-derived organoids	Recapitulation of TME, heterogeneity, patient tumor genetics	Dependent on availability and quality of resected tumor, variable reproducibility	Examine interaction with TME, assess antigen escape	Jacob et al., 2020
In vivo models	Syngeneic mouse	Intact immune system, allowing the investigation of off-tumor effects	non-human CAR T cells, limiting clinical relevance; does not capture tumor heterogeneity	Evaluate host immune effects and safety	Kilian et al., 2021
	Immune-compromised mouse	Lack some or all adaptive immune cell population, allowing for human CAR T-cell persistence in mouse	Difficult to assess CAR T-cell interaction between host immune response	Analyzing efficacy of CAR T-cells targeting TAA	Brown et al., 2012; Santomasso et al., 2022
	Humanized mouse	Study of human CAR T-cells with intact immune system to mimic potential off-target effects;	Limited studies available, limited sources for humanization, more time-consuming	Observation of potential adverse events, such as CARS	O'Rourke et al., 2017; Norelli et al., 2018
	Canine brain tumor model	Recapitulation of TME, tumor heterogeneity, clinical and genetic	Expensive, emotive nature of treatment trials on pets, one preclinical study reported specific to	Safety validation for both human and canine clinical trials	Yin et al., 2018

	similarities to human glioma; able to mimic potential clinical side-effects	brain,		
Primate brain tumor model	Recapitulation of TME, tumor heterogeneity, clinical and genetic similarities to human glioma; able to mimic potential clinical side-effects	Expensive, small study size, limited existing studies	Safety validation for human clinical trials	Nellan et al., 2018

4. Pediatric Brain Tumors

4.1 Diffuse Midline Gliomas (DMGs)

Diffuse intrinsic pontine glioma (DIPG) is an aggressive and universally fatal tumor arising from the brainstem which accounts for the leading cause of brain tumor-related deaths in children (Pellot and De Jesus, 2022). A type of pediatric high-grade glioma, DIPGs were redefined after the 2016 World Health Organization (WHO) classification of CNS tumors in a new classification known as H3 K27M-mutant diffuse midline gliomas (DMG). The H3 K27 mutation results in the substitution of lysine with methionine in position 27 of histone 3, in isoforms H3.1 and H3.3 (Pellot and De Jesus, 2022; Srikanthan et al., 2021). DIPGs, now under the classification of DMGs are almost exclusive to the pediatric population, with an estimated 200-300 children diagnosed in the United States annually (Warren, 2012). The median age at diagnosis is 7 years of age (Misuraca et al., 2015; Pellot and De Jesus, 2022).

DIPGs arise in the brainstem, most notably infiltrating the pons, and often diffusing to adjacent locations such as the thalamus and cerebellum; metastasis to extracranial sites is rare (Gururangan et al., 2006; Yanagawa et al., 1996). Treatment includes short-term use of steroids, specifically dexamethasone (Pellot and De Jesus, 2022), radiotherapy, and targeted chemotherapy (Aziz-Bose and Monje, 2019; Jalali et al., 2010; Srikanthan et al., 2021). Of these, radiotherapy is the only treatment for prolonging progression-free survival (Hargrave, 2012; Jalali et al., 2010; Mathew and Rutka, 2018). Furthermore, due to the critical anatomical location of DIPGs, surgical resection is not feasible. Performing a stereotactic biopsy, a more advanced and minimally invasive procedure, is met with resistance and is not commonly undertaken due to tumor location (Jalali et al., 2010). Due to the limited and ineffective treatment options, the prognosis is devasting; median survival is between 8 to 12 months and less than 1% at 5 years from diagnosis (Grasso et al., 2015; Mathew and Rutka, 2018; Pellot and De Jesus, 2022; Warren, 2012).

4.1.1 Antigenic Targets for DMGs

GD2 is a glycosphingolipid containing two sialic acids (disialyl ganglioside). GD2 is a tumor-associated antigen and serves as a potential target for various cancers including DIPG,

neuroblastoma and osteosarcoma (Yuen et al., 2020). In cancer, GD2 attaches tumor cells to extracellular matrix proteins (Cheresh et al., 1986) and initiates tumor development through cell proliferation, motility, adhesion, and invasion depending on the tumor type (Nazha et al., 2020). Generally, gangliosides are widely expressed in normal tissue, making most subtypes unsuitable for targeted therapy. However, GD2 has limited expression in normal tissue, with low expression mostly restricted to neurons, skin melanocytes and peripheral nerves (Doronin et al., 2014; Nazha et al., 2020). To determine what antigens could be potential candidates for CAR T therapy for DIPG, Mount *et al* screened cell surface antigens using an antibody array in six patient-derived DIPG cultures and found high GD2 expression in all H3K27M+ DIPG cultures studied (Mount et al., 2018). This led researchers to develop second-generation human GD2BBzζ -CAR T-cells and observed GD2-dependent killing and cytokine generation after the cells were exposed to the DIPG cultures. To further confirm the specificity of GD2-CAR T-cells towards H3K27M DIPG, generated GD2 knockout DIPG cells using CRISPR-Ca9-mediated deletion of GD2 synthase. As a result of the loss of GD2 antigen expression, cytokine production was not observed. Once the specific reactivity of GD2-CAR T-cells to H3K27M+ cells was confirmed, Mount et al evaluated *in vivo* efficacy of GD2-CAR T-cells against DIPG using orthotopic mouse xenografts of DIPG cultures derived from post-mortem patient tissue. In comparison to the control group, all mice exposed to GD2-CAR T-cells demonstrated complete tumor clearance.

The findings of the preclinical model provided a rationale for expansion to a first-in-human Phase 1 clinical trial using GD2-CAR T therapy for H3K27M-mutated diffuse midline gliomas (NCT04196413) (Majzner et al., 2022). Preliminary results of four patients, 3 with intracranial DIPG and 1 with spinal cord DMG (ages 5-25 years old), showed that all patients developed CRS after day 6 or day 7 post-infusion, developing fevers between (39.4°C and 40.4°C) and symptoms consistent with tumor inflammation-associated neurotoxicity (TIAN). TIAN most often manifested as worsening of existing neurocognitive deficits but also led to increased intracranial pressure and resulted in obstructive hydrocephalus. The worsening neurocognitive symptoms were managed with intensive supportive care and treatment with immunosuppressive drugs (tocilizumab and anakinra) and/or corticosteroids. While the on-tumor neurotoxicities were observed, patients did not develop any symptoms associated with on-target, off-tumor toxicity. In the preliminary findings, three out of four patients had radiographical and clinical benefits after IV administration of GD2 CAR T-cells and all patients had additional benefits after the second dose of CAR T-cells were administrated intraventricularly. Serum and CSF samples suggested that this finding could be due to enhanced pro-inflammatory cytokines. The trial will continue to treat patients H3K27M+ DIPG patients using GD2-CAR T-cell therapy to determine safety, efficacy, and optimal dosage and administration regimen.

B7-H3 (CD276) is a transmembrane protein belonging to the B7-CD28 family, an important class of immune checkpoint molecules that regulate immune responses through co-stimulatory and co-inhibitory signaling (Collins et al., 2005; Maachani et al., 2020). The exact role of B7-H3 remains unclear. When it was first identified in 2001, B7-H3 was characterized as a T-cell stimulating protein, but increasing evidence suggests that B7-H3 may be better defined as a co-inhibitory protein, as reports show it inhibits T-cell activation, promoting tumor growth and aggressiveness (Feng et al., 2021; Lee et al., 2017; Prasad et al., 2004). B7-H3 consistently inhibits IFN- γ , IL-13, IL-10, IL-2 production, and NK cell activity. In addition, B7-H3 also has nonimmunological pro-tumorigenic abilities, such as chemoresistance, as increased levels of B7-H3 in melanoma correlated with activation of the JAK2/STAT3/survivin-dependent

pathways, known to limit success of chemotherapy and radiation therapy and thus also contributes to a poor clinical outcome (Maachani et al., 2020; Z. Zhang et al., 2020).

Although the exact role of B7-H3 has not been clearly defined, there is no doubt that its overexpression constitutes B7-H3 as an ideal candidate for immunotherapy application. In one study investigating B7-H3 protein and mRNA expression in brainstem glioma specimens, 100% of the DIPG specimens (median age of 6.5 years) showed B7-H3 immunoreactivity in comparison to the non-diffuse brainstem glioma group (median age of 12.0 years), of which only 28% stained positive for B7-H3 (Z. Zhou et al., 2013). Further analysis demonstrated that B7-H3 mRNA expression was significantly higher in DIPG samples in comparison to juvenile pilocytic astrocytoma and normal brain, providing evidence of utilizing B7-H3 as a therapeutic target in DIPG. In another preclinical model, second-generation (4-1BB) B7-H3 CAR T-cells were studied in pediatric tumors including *in vitro* and *in vivo* models of osteosarcoma, medulloblastoma, Ewing sarcoma, and DIPG. The study revealed that B7-H3 CAR T-cells in mice significantly prolonged survival in medulloblastoma and DIPG models because of significant production of IFN- γ, TNFα, and IL-2. Currently, locoregional delivery of second-generation B7-H3 CAR T-cells in pediatric patients with DMG/DIPG and recurrent or refractory CNS tumors is being investigated (NCT04185038). Researchers at Seattle Children's Hospital are establishing a treatment regimen with either 2 or 3 doses of B7-H3 CAR T-cells and evaluating CAR T distribution within the CSF, trafficking of the cells in the blood, and observing clinical response to infusion. The study was last updated in March 2022 and is in its recruitment phase.

4.2 Medulloblastomas

Medulloblastoma (MB) is the most common malignant neoplasm in children, making up 20% of all pediatric primary CNS tumors (Martin et al., 2014). Pediatric patients with MB between ages 1 and 9 years old had an incidence rate of 6.0 per million when compared to an incidence rate of 0.6 per million in adults (Smoll and Drummond, 2012). Because of the rare occurrence of MB in adults, our discussion will primarily focus on MB in the pediatric population. 2021 WHO Classification of CNS Tumors added new subgroups to the four previously established primary groups of MBs: WNT-activated, SHH-activated, group 3, and group 4 with the first two named based on distinct activation signaling pathways and the latter two involving non-WNT/non-SHH MBs (Louis et al., 2021; Taylor et al., 2012). Now, through deeper analysis involving methylation and transcriptome profiling, 4 subgroups of SHH and 8 subgroups of non-WNT/non-SHH have been created, mirroring current innovations for subgroup-specific treatment of MBs (Gershanov et al., 2021; Louis et al., 2021).

By definition, MB tumors are exclusive to the posterior fossa, developing in the cerebellar vermis (Roussel and Hatten, 2011). The molecular classification of MB can provide insight into the clinical manifestation of disease and contribute prognostic value. For example, WNT-activated tumors most often present in children between the ages of 7 and 14 years old, whereas SHH-activated tumors most often occur in infants (and later in adulthood) (Juraschka and Taylor, 2019; Orr, 2020). Furthermore, the WNT subtype had the best overall survival, while the MYCN-amplified SHH subtype had the worst (Korshunov et al., 2010; Orr, 2020). Standard treatment includes surgical resection, craniospinal irradiation, and adjuvant chemotherapy. Advances in treatment regimen have increased the 5-year survival to 70-80% in patients greater than 3-years-old (Juraschka and Taylor, 2019; Martin et al., 2014; Roussel and Hatten, 2011). Innovative treatments using molecular subtyping are now underway to reduce the increased risk

of neurocognitive impairment and long-term morbidity associated with current care (Kijima and Kanemura, 2016; Orr, 2020; Sayour and Mitchell, 2017).

4.2.1 Antigenic Targets for Medulloblastomas

As mentioned above, HER2 is known to be overexpressed in breast cancer and has served as a target antigen for CAR T therapy in both metastatic brain cancer and GBM models. Additionally, HER2 expression is observed in approximately 40% of medulloblastoma and is associated with worse overall and progression-free survival (Nellan et al., 2018). Given that HER2 is not detected in the normal brain, the use of HER2-CARs in the eradication and treatment of medulloblastoma has been examined. In 2007, the first evaluation of HER2 CAR T-cells against medulloblastoma demonstrated that CARs killed HER2+ medulloblastomas *ex vivo* but also led to tumor regression *in vivo* in an orthotopic xenogenic SCID model (Ahmed et al., 2007). HER2 CAR T-cells containing CD3ζ signaling without the inclusion of a co-stimulatory signaling domain were directly injected in established medulloblastomas in 12 mice and compared to 10 untreated mice. Tumors injected with HER2 CAR T-cells were undetectable up to 55 days post-administration and tumor-bearing mice treated with CARs had significantly higher survival than the control groups. However, tumors eventually recurred in all mice treated with the first-generation HER2-specific T-cells. The nonresistance and limited efficacy of CARs were likely due to the use of first-generation CD3 constructs since co-stimulatory domains were missing, and thus, prevented further enhancement. A recent study, which investigated the efficacy of using second generation HER2 CAR T-cells with CD3 ζ signaling domain and a 4-1BB co-stimulatory domain *in vivo* and xenograft mouse models, revealed improved response and durable regression (Ahmed et al., 2007). Nellan et al also found that intraventricular delivery of HER2-specific T-cells was feasible and safe in non-human primates (NHPs). Currently, a phase 1 clinical trial is underway investigating locoregional HER2-specific CAR T-Cell therapy for HER2-positive recurrent and refractory pediatric CNS tumors, such as gliomas, ependymomas, medulloblastomas, and germ cell tumors (NCT03500991).

Two other target antigens for CAR T therapy against medulloblastoma are B7-H3 and PRAME. The pan-cancer antigen, B7-H3 has moderate-to-high expression levels in medulloblastoma (W. T. Zhou and Jin, 2021), with a recent study showing as high as 96% of B7-H3 expression in pediatric medulloblastoma (Li et al., 2022). As mentioned earlier, second-generation B7-H3 CAR T-cells demonstrated efficacy and success by completely regressing medulloblastoma xenografts (Majzner et al, 2019). Along with DMGs, pediatric medulloblastomas are also currently studied in the clinical trials of second-generation B7-H3 CAR T-cells (NCT04185038). PRAME is a cancer-testis antigen (CTA), a family of tumor-associated antigens expressed in the testis and restricted elsewhere in healthy tissue (Steinbach et al., 2002). Growing evidence suggests that several CTAs stimulate epithelial mesenchymal transition (EMT) and generation of cancer stem-like cells, contributing to tumorigenesis, invasion, and metastasis, as well as drug resistance (Salmaninejad et al., 2016; Wei et al., 2020). Specifically, PRAME likely contributes to disease progression by functioning as a dominant repressor of retinoic acid (RA) receptor signaling. Specific to its role in antitumor activity, RAs induce growth arrest, differentiation, and apoptosis of tumor cells (Altucci and Gronemeyer, 2005; Epping and Bernards, 2006). PRAME interferes with RA signaling and thus provides a growth advantage to cancer cells. PRAME is expressed in approximately 80% of medulloblastomas and could potentially serve as an ideal target for immunotherapy. A recent study examining CAR T-cells specific for the PRAME-derived peptide SLL showed some

efficacy in orthotopic medulloblastoma models (Orlando et al., 2018). While PRAME-based CAR T therapies are in its early stages, these results as well as overexpression of PRAME in medulloblastoma, provide a promising outlook for future development.

4.3 Ependymomas

Ependymomas are the third most common brain tumor in children, comprising 8-10% of all pediatric CNS tumors (Amirian et al., 2012; Kilday et al., 2009). Ependymal tumors can manifest in the brain or spinal cord in both adult and pediatric populations. While approximately 60% of adult ependymal tumors originate in the spinal cord, almost 90% of pediatric ependymomas originate intracranially (Villano et al., 2013). For this review, we will only focus on the latter. Ependymomas are classified based on anatomical location and histopathological and molecular characteristics. The three primary classifications include supratentorial, posterior fossa (PF) and spinal ependymomas, most recently updated to include ZFTA and YAP1 fusions and groups PFA/PFB as subgroups for the first and second category, respectively (Villano et al., 2013). In addition, ependymal tumors can be classified as grade I, II, and III based on malignancy (Jünger et al., 2021).

The most frequent site of pediatric ependymomas is in the PF, followed by supratentorial sites, and rarely, the spinal cord. Like in the case of medulloblastomas, molecular classifications led to distinct clinical manifestations and outcomes (Jünger et al., 2021.; Upadhyaya et al., 2019). PFA ependymal tumors are usually only present in infants, whereas PFBs occur equally in adolescents and adults; and the latter is associated with worse overall survival (Pajtler et al., 2015; Patterson et al., 2020; Upadhyaya et al., 2019). Regardless of location and age, the standard treatment is surgery, ideally aggressive gross total resection (GTR), followed by radiation therapy, and a second surgery (if necessary) (Merchant et al., 2019). Tumor recurrence is a concern, and therefore, aggressive GTR is associated with the lowest mortality rate and longest progression-free survival (Cage et al., 2013). Overall, the 5-year survival rate for patients who undergo standard treatment range between approximately 75-85% but numbers can fall as low as 37% and 26% for patients with recurrence and subtotal resection, respectively (Pejavar et al., 2012; Thorp and Gandola, 2019; Tsang et al., 2018).

4.3.1 Antigenic Targets for Ependymomas

HER2, IL-13Rα2, EphA2, and Survivin are overexpressed in ependymomas (Donovan et al., 2020; Yeung et al., 2013). Survivin is a member of the inhibitor of apoptosis protein (IAP) family and inhibits apoptosis primarily by preventing caspase-9 activation within a functional apoptosome, but it can also operate in a caspase-independent fashion (Fukuda and Pelus, 2006; Rafatmanesh et al., 2020). Survivin blocks cancer cell death, providing an opportunity for cancer cells to proliferate. In addition to its antiapoptotic role, survivin regulates mitosis by promoting microtubule stability and regulating the spindle assembly checkpoint (Preusser et al., 2005a). Survivin is sometimes referred to as a “universal” tumor antigen (Andersen and thor, 2002) due to its overexpression in a vast majority of cancers and is commonly associated with resistance to chemotherapy and tumor recurrence (Altieri, 2003). Specifically, in ependymomas, the significance of survivin as a prognostic value has yet to be clearly defined. In a study of pediatric ependymomas and choroid plexus tumors, low levels of nuclear survivin were

associated with advanced disease and/or tumor grade (Altura et al., 2003). However, two other studies examining both adult and pediatric ependymomas found a correlation between survivin expression and increasing tumor grade (Altura et al., 2003; Preusser et al., 2005b). While these conflicting reports may be the result of the inclusion of different subtypes of ependymomas and choroid plexus tumors, the expression of survivin in ependymomas has heightened focus on its use in immunotherapies. For example, oncolytic viruses with incorporation of survivin can likely synergize with CAR T-cells and promote greater potent antitumor effects (Harrison et al., 2021).

A preclinical study investigating the route of delivery and efficacy of monovalent and trivalent (TRI) CAR T-cells for the treatment of medulloblastoma and ependymoma was recently published (Donovan et al., 2020). After identifying high expression of EPHA2, HER2, and IL-13Rα2 in three ependymoma subgroups, TRI CAR T-cells incorporating the three-target antigens were exposed to PFA ependymoma xenograft models. The TRI CARs were compared to monovalent HER2 CAR T-cells since HER2 protein expression remained consistent across the PFA ependymomas analyzed. Compared to non-transduced T-cells, both TRI and HER2 CARs showed an antitumor response, with no significant difference between the two types of CARs. The study demonstrated that CAR T therapy using either monovalent or TRI CAR T-cell is a promising therapy for ependymomas, but because recurrence is high, further work is needed to maintain durable outcomes. A comparison of completed and ongoing CAR T therapy clinical trials in both adult and pediatric populations can be seen in Figure 3.

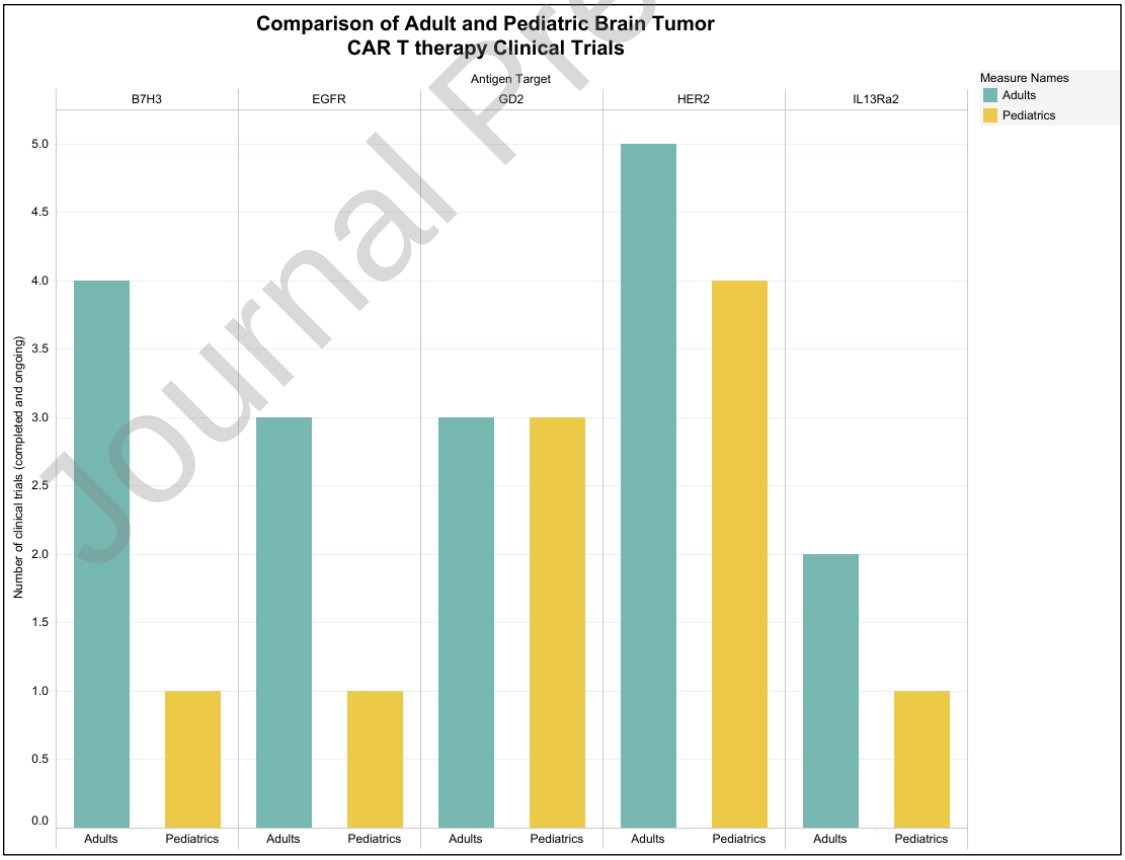

Figure 3. Comparison of Adult and Pediatric brain tumor CAR T therapy Clinical Trials. Data was curated using www.clinicaltrials.gov upon search with key phrases “CAR T brain cancer” and “CAR T brain.” Ongoing and completed trials included. Withdrawn clinical trials were excluded from data generation. The figure was created using Tableau.com (Tableau 2016).

5. Challenges of CAR T Therapy in Adult and Pediatric Brain Tumors

5.1 Difference in tumor-antigen targets

Identifying novel antigen targets for neoplasms is crucial for preventing off target recognition during CAR T-cell treatment. Research has postulated that genetic mutations can form neoantigens in tumor cells, which can be used as targets. One of the main concerns differentiating this technique between adult and pediatric brain tumors is the low mutation burden in pediatric tumors, which makes searching for unique therapy targets challenging (Patel et al., 2020). Various pediatric tumor targets have been identified, including but not limited to EGFRVIII, HER-2, B7-H3, GD2, IL-13RA2, EPHA2, survivin, PRAME, CD70, PDPN (Melcher and Kerl, 2021; Patterson et al., 2020). One of the most consistently expressed is B7-H3 and GD2, proving to be potential targets for CAR T-cell therapy (Haydar et al., 2021; Majzner et al., 2019). Research in adult brain tumors has shown a higher mutation load and, therefore, more significant potential for neoantigen targeting (Rahal et al., 2018). The specific targets for adult brain tumors have been described in clinical trials and research studies and include EGFR/EGFRvIII, IL-13Ra2, B7-H3, and HER2 (Y. J. Lin et al., 2022). Current CAR T-cell therapy proposes a steppingstone in potential therapy against brain tumors.

Several clinical trials are underway that utilize these molecular targets for CAR T-cell therapy directed against brain tumors—focusing specifically on GD2, EGFRvIII, HER2, B7-H3, CD147, and IL-13Ra2. Recent preclinical studies have found other potential molecular targets for CAR T-cell therapy in GBM, such as CAIX, EphA2, CD70, TROP2, and CSPG4 (Y. J. Lin et al., 2022). EphA2 is a receptor tyrosine kinase that binds to ephrin-A family ligands. EphA2 is highly expressed in glioblastoma and only expressed at low levels in normal brain tissue (Chow et al., 2013). EphA2 overexpression in GBM is associated with poor prognosis and aids in tumor cell migration, invasion, angiogenesis, and metastasis (Chow et al., 2013; Yi et al., 2018). Another candidate antigen target- carbonic anhydrase IX (CAIX) was examined using immunohistochemistry in 59 glioblastoma patients. CAIX is a molecular target induced by hypoxia that is highly expressed in GBMs and rarely found in normal tissue (Y. J. Lin et al., 2022; Proescholdt et al., 2012). Levels of CAIX expression were also shown to be associated with overall survival (Proescholdt et al., 2012). CD70, a type 2 transmembrane protein-ligand for CD27, has shown to be involved in a mechanism by which some GBM cell lines can kill T-cells (Chahlavi et al., 2005; Y. J. Lin et al., 2022). This key- mediator in T-cell death could be linked to the dysfunction of such cells in the TME. CSPG4 (also known as NG2) is a chondroitin sulfate proteoglycan highly involved in brain development and malignancy transformation (Y. J. Lin et al., 2022; Tsidulko et al., 2017). Reduced tumor growth was observed in GBM xerographs treated with lentiviral encoded shRNAs targeted toward CSPG4 (Y. J. Lin et al., 2022; Tsidulko et al., 2017). TROP2 is a glycoprotein and an epithelial cell adhesion molecule expressed in gliomas and glioblastomas (Lenárt et al., 2020; Y. J. Lin et al., 2022). TROP2 in glioblastomas promotes growth and dissemination and upregulation of VEGF levels (Lenárt et al., 2020).

In pediatric brain tumors, major discrepancies were identified based on tumor type and anatomical location.HER2 was expressed in 40% of medulloblastomas and no expression in normal brain tissue. This further highlights its use for CAR-T cell therapy, and recent studies have shown that first-generation CAR T-cells targeting HER2 led to regression of medulloblastoma in orthotopic xenogeneic murine models (Ahmed et al., 2007). B7-H3 CAR T-cells were also shown to be effective in anti-tumor activity, with regression of solid tumors in xenograft models of medulloblastoma (Majzner et al., 2019). Other CAR T-cell target antigens found to have positive results in preclinical trials for medulloblastomas and GBM are EPHA2, HER2, and IL-13Rα2 (Bielamowicz et al., 2018; Donovan et al., 2020). In pediatric

ependymomas, EphA2, Survivin, IL-13Rα2, and HER2 were highly expressed (Y. J. Lin et al., 2022; Yeung et al., 2012; J. G. Zhang et al., 2008). For pediatric high-grade gliomas, one study found that systemic administration of GD2-targeted CAR T-cells in patient-derived H3-K27M+ DMG orthotopic xenograft models was able to clear engrafted tumors (Mount et al., 2018). While these preclinical findings are promising, future clinical trials will be needed to identify if these antigen targets are actually conducive to CAR T treatment of brain tumors.

5.2 Route of CAR T delivery

Most trials and current treatments with CAR T-cells are delivered intravenously (IV) (Figure 4A). With IV administration, significant questions arise regarding the amount and distribution of treatment when directly targeting brain tissue. Techniques such as laser thermotherapy, electroporation, and transcranial ultrasound have been studied to alter the BBB, which has been postulated as a possible route for CAR T-cell therapy delivery, as well as other techniques such as direct delivery to the brain or intraventricular system for a targeted approach to therapy (Rodriguez et al., 2017). Current therapy delivery for brain tumors includes IV, Intraventricular, and intratumoral. The difficulty surrounding CAR T-cell therapy for brain tumors is the properties of the BBB that make it challenging to deliver treatment. Because of the difficulties posed by the BBB, many investigators have focused on regional and direct CAR T-cell delivery for treating brain tumors.

Various studies have shown the efficacy of regional administration of CAR T-cell therapy. When mesothelin-targeted CAR T-cells were administered directly into the lung, it resulted in better efficacy and persistence of treatment for lung cancer (Adusumilli et al., 2014). This form of delivery for therapy is more appealing because there was also efficacy in tumors outside the regional space where the CAR T-cells were delivered. With the results obtained from this study, phase 1 clinical trials were begun for safety evaluation of such treatment in primary or secondary pleural malignancies. When discussing treatment for brain tumors, the major question arises about the chosen delivery method that can have both the most potent effect on the tumor and minor side effects or toxicities in surrounding structures. This becomes increasingly important when delivering CAR T-cell treatment and overcoming the BBB. IV delivery of CAR T-cells is one of the most common delivery modes for this treatment. Because of its systemic approach, it has been used widely for treating hematologic malignancies. Many studies have debated this treatment method because of potential toxicities to other parts of the body. This mode of therapy has been proven to have poor outcomes against solid malignancies due to the challenges of both the systemic effects of the treatment and the obstacles posed by the TME (Grosskopf et al., 2022).

Because of these difficulties in the systemic delivery of CAR T-cells, new delivery methods are being engineered to benefit the patient and decrease toxicity. Some of these new delivery methods exploit biopolymer scaffolds that contain stimulatory molecules that can improve systemic delivery for solid tumor treatment (Grosskopf et al., 2022). The authors showed that biopolymer implants could potentially enhance CAR T-cell therapy. Other studies also demonstrated the capability of biodegradable scaffolds to surpass the TME and disperse anti-tumor T-cells (Stephan et al., 2014). Smith *et al* tested biopolymer implants to deliver CAR T-cells for treating solid tumors directly. This method exposed solid tumors to a high number of immune cells for a more extended period. This demonstrates that CAR T-cells can migrate from the biopolymer scaffolds and eradicate solid tumors more effectively compared to a systemic delivery approach.

The authors showed superior responses in mouse models of pancreatic cancer and melanoma treated with the regional delivery compared to systemic approaches. This regional approach has various advantages, one being the ability to overcome the challenges of the TME. The downside to the biopolymer scaffolds is their complexity in manufacturing and application to different tumor types (Grosskopf et al., 2022). Effective CAR T-cell therapy aims to deliver high treatment concentrations to the specific sites where the tumor is located. Systemic approaches face significant disadvantages when it comes to both delivery and extra tumoral toxicities in the patient. For this reason, locoregional treatment options have become a new area of interest for direct treatment of these solid malignancies while decreasing extra tumoral toxicities. Momin *et al* demonstrated that intratumoral administration of fused anti-tumor cytokines to collagen-binding protein lumican in cancer mouse models could prolong anti-tumor effects in the region with little systemic toxicity.

Although intravenous/systemic approaches have been the mainstream therapy delivery for various tumor types, overcoming the CNS barrier has been the goal to potentiate better brain tumor therapies while decreasing systemic toxicities. Given the challenges of TME and systemic delivery, locoregional delivery strategies have been used to bypass these hurdles. Locoregional delivery methods include both intratumoral and intraventricular approaches. Intratumoral approaches include the delivery of CAR T-cells directly into the tumor. At the same time, intraventricular relates to the delivery of the CAR T-cells into the CSF through the ventricular system. Intraventricular therapy delivery can bypass most hurdles in the brain parenchyma except for the glia limitans (Akhavan et al., 2019). Many studies have found that locoregional delivery is far superior to systemic delivery of CAR T-cell treatment (Agliardi et al., 2021). With an orthotopic GBM mouse model, Agliardi *et al* showed that CAR T-cells targeting tumor-specific epidermal growth factor receptor variant III (EGFRvIII) fail to control tumors but, when coupled with regionally delivered IL-12, attain durable anti-tumor responses. IL-12 increases CAR T-cell cytotoxic effects and modifies the TME with few systemic effects (Agliardi et al., 2021). Compared to IV treatment, regional injection of CAR T-cells improved T-cell tumor infiltration in many preclinical models of brain malignancies (Mulazzani et al., 2019; Priceman et al., 2018; Theruvath et al., 2020).

Intraventricular delivery methods have been well-tolerated in both pediatric and adult populations (Cohen-Pfeffer et al., 2017). Locoregional delivery requires that a catheter be placed to access the tumor or the CSF cavity. Although these devices have been used previously for numerous chemotherapeutic approaches, they have the potential for severe infection if not properly monitored. Priceman *et al* reported that intracranial delivery of CAR-T cells directed against HER2 showed high anti-tumor activity and effective treatment of brain metastases. This study compared regional and systemic delivery of HER2-CAR T-cells in a preclinical BBM1 model. They showed that HER2-CAR T-cells delivered intracerebroventricular (ICV) had complete tumor regression and proved more effective than a 10-fold higher dose delivered via IV, which only showed partial tumor regression (Priceman et al., 2018).

Similarly, Brown *et al* found that intracranial delivery of CAR T-cells has an anti-tumor effect that supersedes IV administration. Because the IV route has been shown to be less effective when utilizing CAR T-cell therapy for GBM, a comparison of the IV and intracranial route of IL-13BBζ T-cell delivery in the orthotopic tumor xenograft model showed that IV administered CAR T-cells had no therapeutic benefit over intracranial delivery (Brown et al., 2018). When comparing locoregional deliveries (intratumoral and intraventricular), studies have shown better tumor targeting and overall clinical response in intraventricular delivery (Weist et al., 2018) (Figure 4B, 4C). CAR T-cells, labeled with the radionuclide 89Zr, showed that intertumoral

delivery remained localized, while intraventricular delivery distributed throughout the CNS and was found in the spine, spleen, blood, and liver of mice when followed by PET imaging (Weist et al., 2018). Intraventricular delivery of CAR T-cells showed an overall better clinical response, and locoregional delivery showed a decreased risk of systemic toxicities. A study focusing on medulloblastomas showed that CAR T-cells directed against HER2 effectively cleared medulloblastoma with regional and IV delivery. However, IV delivery required a higher dose to prove effective and intraventricular delivery had no systemic toxicity (Nellan et al., 2018). Researchers focusing on CNS lymphomas in mice showed that IV administration of anti-CD19 CAR T-cells had poor tumor infiltration compared to intracerebral administration.

Intracerebral delivery of anti-CD19 CAR T-cells demonstrated deep invasion into the tumor, reduced growth, and stimulated regression (Mulazzani et al., 2019). Locoregional delivery of CAR T-cell therapy appears to be a safe and effective treatment option for brain tumors. This delivery method bypasses the limitations and barriers posed by the BBB and TME compared to systemic therapeutic approaches (Sridhar and Petrocca, 2017). Overall, intertumoral delivery of CAR T-cell therapy holds promise because it can bypass the challenges of the BBB and has the potential for higher therapeutic dosing concentrations within the brain target without the expected systemic toxicities that IV administration possesses (Loya et al., 2019). A study that looked at one patient with recurrent multifocal glioblastoma treated with anti IL-13Rα2 CAR T-cells looked to compare both intracavitary and intraventricular delivery routes (Brown et al., 2016). In this patient, both delivery methods showed low toxicity but varied when comparing effectiveness in distant tumor sites. Intracavitary delivery of CAR T-cells showed a decrease in local tumor recurrence but showed little effect in detaining the progression of the tumor in distant locations. Intraventricular therapy delivery showed regression of all CNS tumors (Brown et al., 2016). Although various factors intervene in the outcome of both therapeutic delivery routes in this patient, the data provides an insight into the mechanisms of both delivery methods in brain tumor therapy; more investigation is needed to assess the differences in clinical and therapeutic effects. This topic has been wildly studied in adult tumors, but research has also supported similar outcomes of delivery routes in pediatric patients. Studies focusing on the regional treatment of CNS tumors using anti-HER2-CAR T-cells in a pediatric population showed that intra-CNS delivery of this therapy is well tolerated (Vitanza et al., 2021). Further research is needed to compare and determine the best treatment delivery option in adult and pediatric populations.

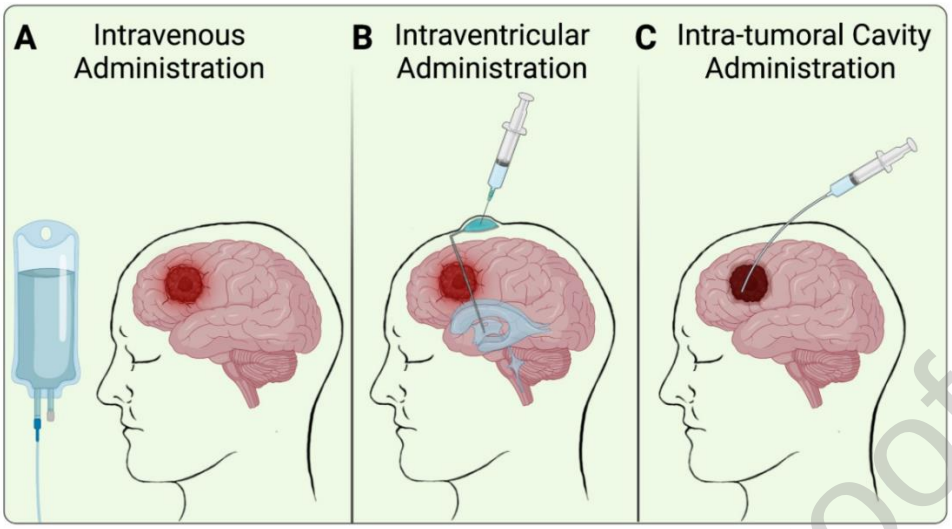

Figure 4. Types of treatment mechanisms for brain tumors. A) Intravenous (IV) administration of CAR T-cells is the most common route of delivery in current treatments and clinical trials. CAR T-cells intravenously delivered is challenging for treatment against solid tumors, as it is difficult for the cells to locate, infiltrate, and expand within the tumor microenvironment. (B) Regional intraventricular (ICV) delivery involves injecting the CAR T-cells into the CSF through the ventricular system. In CAR T treatment for brain tumors, ICV delivery was first used in a case of recurrent glioblastoma and resulted in the regression of all CNS tumors. (C) CAR T-cells can also be injected directly in the intra-tumoral cavity, negating the need of CAR T-cells migrating across the blood brain barrier. Figure made in BioRender.com.

5.3 Blood-brain barrier

The blood brain barrier (BBB) is a major component in protecting the brain from harmful toxins and chemicals. The blood vessels found in the CNS have distinct characteristics that allow for tight regulation of movement between particles in the blood and particles in the brain. This tight control allows for proper function and protection of neural tissue and plays a key role in many brain pathologies. The BBB is the distinct name given to the blood vessels in the central nervous system that possess the characteristics for tight regulation of movement in and out of the brain. Unlike other blood vessels in the body, the CNS vasculature is not fenestrated. This limits the amount and type of molecules that can make it to the brain. This type of control that the BBB provides serves as protection for the brain but presents an obstacle to therapeutic interventions (Daneman and Prat, 2015). The BBB is located at the interface between the brain tissue in the capillary walls; it consists of endothelial cells with tight junctions that line the walls of the capillaries, astrocytes with the end-feet process, and the basement membrane. The primary component of the BBB is the endothelial cells of the microvasculature, which possess unique properties not found in other tissues. These endothelial cells contain tight junctions that limit the influx and efflux of molecules (Daneman and Prat, 2015). The low levels of transcytosis that occurs in CNS endothelial cells allow for the restriction of solutes (Coomber and Stewart, 1985). The cellular component of the BBB also includes mural cells, which include the pericytes and the vascular smooth muscle cells of the microvasculature (Daneman and Prat, 2015). Pericytes are found in higher numbers in the BBB; they contain contractile proteins that control the diameter of the vasculature, which alters blood flow (Hall et al., 2014; Peppiatt et al., 2006). Pericytes have important roles in regulating blood flow, angiogenesis, and immune cell infiltration (Armulik et al., 2011; Daneman and Prat, 2015; Shepro and Morel, 1993). Another component is the basement membrane divided into inner and outer, also referred to as vascular

glia limitans perivascularis (Daneman and Prat, 2015; del Zoppo and Milner, 2006; Sorokin, 2010). Astrocytes are also an important component of the BBB. Astrocytes have perivascular end-feet that can secrete effector molecules that aid in regulating and interacting between endothelial cells and pericytes in the BBB (Cabezas et al., 2014). Astrocytes also play a major role in brain homeostasis; they provide a link between the cells of the BBB and serve as a transport and metabolic barrier controlling water, neurotransmitter, and ionic levels (Abbott et al., 2006; Cabezas et al., 2014; Kadry et al., 2020).

Not much is known about the specific differences between the adult and the pediatric BBB. Studies have suggested that age does influence the structure and permeability of the BBB. As one ages, the BBB experiences an increase in disruptions in locations inside the brain parenchyma that are most vulnerable to age-related changes (Verheggen et al., 2020). It is widely accepted that when the brain is developing, it is more vulnerable to infection and toxins than the adult brain (Costa et al., 2004; Schmitt et al., 2017). Systemic administration of drugs has been found to reach a higher concentration in the neonatal brain when compared to adults; this vulnerability is postulated to be due to the sensitivity of the brain to these toxins (Costa et al., 2004; Schmitt et al., 2017). The continuing development, differentiation, proliferation, and migration of cells in the developing brain is believed to aid in this heightened sensitivity. As these developmental changes decline with age, so does the sensitivity and vulnerability of the CNS (Saunders et al., 2012; Schmitt et al., 2017). Higher brain concentrations of toxins and drugs in immature brains are not entirely due to the increased permeability of the BBB; other factors such as the immaturity of metabolic and physiologic processes can influence the increased vulnerability of immature brains (Schmitt et al., 2017). These aspects become more crucial when treating brain malignancies, as dosing regimens for adults have differing effects on the pediatric population. When it comes to brain tumors, research has shown that there is an increase in vascular permeability due to a compromised BBB as well as disruption of tight junctions (Kadry et al., 2020; Long, 1970) (Figure 5B). Liebner *et al* conducted a study indicated that in gliomas, there is an increase in vascular permeability that contributes to developing symptoms and junctional protein dysregulation. This study found that expression of claudin-1 is lost, and claudin-5 is downregulated in the microvessels of glioblastoma multiforme (Liebner et al., 2000). Other reports state that tight junction openings in astrocytoma and metastatic adenocarcinoma are due to loss of occludin (Papadopoulos et al., 2001). The secretion of VEGF by brain tumors may be also involved in the down-regulation of tight junctions and the increase of the vascular permeability (Kadry et al., 2020; Papadopoulos et al., 2001). Saadoun *et al* concluded that there was a significant correlation between an increased expression of aquaporin-4 (AQP4) and BBB opening in high-grade astrocytoma and adenocarcinomas. With the results observed in these studies, it appears that brain tumors can dysregulate the BBB to some extent, although the difficulties encountered for therapeutic intervention are still present. Although this increase in permeability is responsible for the development of cerebral edema and possible exposure to some substances, the permeability has not been shown to ease the delivery of drug therapy to brain neoplasms. This is partly due to P-glycoprotein (P-gp), a major protein in the BBB that pumps products out of the CNS. The expression of this protein can help determine the amount and effect of the chemotherapeutic drug in the brain. It has been hypothesized that low expression of the P-gp provides a more permeable environment for chemotherapy drugs (Abdallah et al., 2015).

Treatments for brain tumors must take into consideration the gross anatomy as well as the microscopic components that enable the BBB to detain from letting any substance pass. P-gp overexpression in tumor cells reduces intracellular drug levels, which affects the cytotoxicity

of a wide range of anticancer medicines (Abdallah et al., 2015). The BBB modulates the rate of T-cell mobilization by limiting leukocyte movement into the CNS (Engelhardt, 2010). T-cell recruitment is frequently inhibited in the presence of malignancy. This decrease may give cancer cells an immunological escape mechanism (Sackstein et al., 2017). Therapeutic modalities must adapt to provide the proper molecular structure to surpass the hurdles encountered by the BBB. Crossing the BBB is an important step when dealing with systemic CAR T therapy for CNS malignancies. Side effects such as brain swelling due to the BBB disruption have been observed after CAR T-cell therapy (Gust et al., 2017; Huang et al., 2022). The disruption of the BBB due to malignancy has also been put into question. Because GBM has previously been shown to disrupt the BBB, medication, antibody, and immune cell accessibility should not be an issue. However, it has been shown that the BBB can remain intact in the presence of GBM (Maggs et al., 2021; Sarkaria et al., 2018). Further investigation is needed to assess BBB disruption in the presence of malignancy.

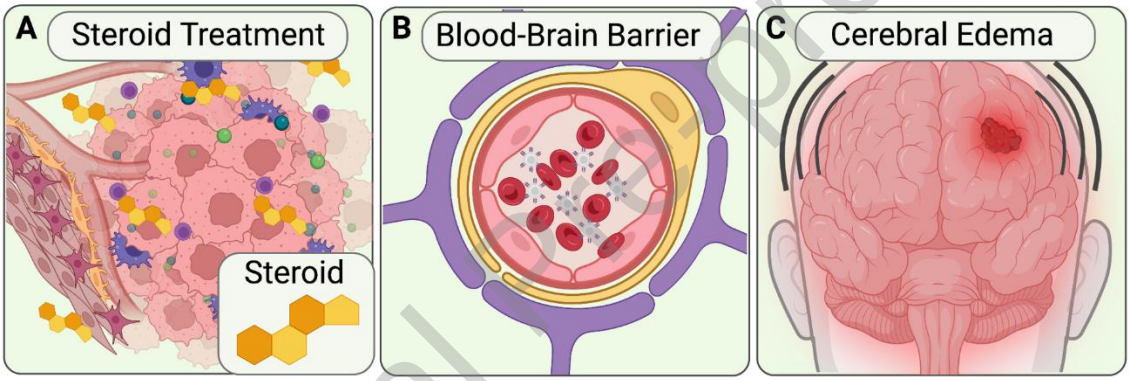

Figure 5. Barriers to CAR T-cell brain tumor treatment. (A) The use of steroids (shown in orange) is common in patients with brain tumors to manage cerebral edema but has many systemic immunosuppressive effects. Some patients remain on dexamethasone, a common steroid used in the treatment for brain tumors, indefinitely. Studies have found that dexamethasone prevents immune infiltration of CAR T-cells, limiting the effectiveness of CAR T therapy in brain tumor patients. (B) The BBB is a unique physical barrier that creates challenges for CAR T delivery. Direct infiltration of CAR T cells into the brain tumor, surgical resection cavity, or cerebrospinal fluid are methods to circumvent this obstacle. (C) Cerebral edema is a neurological adverse event that can arise from CAR T treatment. Malignant cerebral edema that is not managed well can lead to significant morbidity and even mortality. Figure made in BioRender.com.

5.4 Tumor microenvironment

Although the fundamental biological concept of pediatric and adult brain tumors is the same, pediatric brain tumors have a lower mutational load and a TME with decreased immunosuppression compared to adult brain tumors (Abedalthagafi et al., 2021; Patel et al., 2020; Wang et al., 2019). These differences make treatment very difficult. Studies determining appropriate treatment for adult brain tumors may not be as effective as their pediatric counterpart and may lead to differing adverse effects (Abedalthagafi et al., 2021). Although the interaction between the tumors and their microenvironment is highly dependent on the location and molecular component of the malignant cells, a significant amount of effort and research has found essential elements that both populations share regarding the TME. Interaction between the tumor cells and their microenvironment determines the progression or regression of the

malignancy in the brain. Some of the most studied aspects of the tumor microenvironment have been the interaction between the cancer cells and the surrounding immune milieu, while others research the impact of hypoxia in the TME (Abou-Antoun et al., 2017).

The local TME can affect the efficacy and persistence of CAR T-cells anti-tumor activity. Adult and pediatric brain tumors have heterogeneous TMEs with grade and molecular subtype specific immune compositions. Research has found that this immunosuppression is highly accentuated in solid tumors (Lindo et al., 2021). The TME comprises multiple cellular components that can aid in the tumorigenesis of cancer cells. The TME greatly depends on tumor type, anatomical location, and its cellular components, consisting of macrophages, dendritic cells, monocytes, neutrophils, mast cells, stromal cells, NK cells, and B and T-cells (Quail and Joyce, 2013). These heterogeneous microenvironment components respond to nearby signals, and aid in both immunosuppression or tumor development and growth. While CAR T-cell therapy has shown remarkable results in other malignancies, different compositions of TME raise concern for adverse events when applying similar mechanisms to brain tumor therapy. Once CAR T-cells bind to their associated antigen, they can extend their function and release signaling molecules that alter the TME and activate immune cells and inflammatory modulators (Lindo et al., 2021).

Macrophages

As mentioned above, the tumor microenvironment comprises a complex and interactive system that establishes the conditions for potential malignancy (Figure 6). Macrophages, a significant component of solid cancers, are one of the most abundant immune cells in this interactive environment. Macrophages were classically divided into M1 and M2 subgroups. Th1 cells induce M1, primarily through lipopolysaccharides (LPS) and IFN-γ, to secrete cytokines that have a pro-inflammatory and tumoricidal role (Øynebråten et al., 2019; Pasquereau et al., 2017; Quail and Joyce, 2013; Saqib et al., 2018). Th2 cells, with the aid of mast cells and basophils, release mainly IL-13 and IL-4 to induce M2 cell release of anti-inflammatory and tumor progression mediators (Pasquereau et al., 2017; Saqib et al., 2018). However, research has found that the plasticity and function of macrophages go beyond anti/pro-inflammation and immune response roles; they also serve as a critical component in the TME (Lindo et al., 2021). When circulating macrophages enter the TME, they are denoted as tumor-associated macrophages (TAMs), a subset of macrophages that play an essential role in the TME and serve as regulators of tumorigenesis (Kaina et al., 2020; Quail and Joyce, 2013). Although macrophages were previously recognized for their role in possible immunity against tumors, recent studies have found evidence to support the claim that TAMs indeed support malignancy progression (Quail and Joyce, 2013). TAMs aid in tumor progression by promoting angiogenesis, migration, invasion, intravasation, and overall mitigation of the immune responses against tumor cells, as well as playing a significant role in metastasis (Condeelis and Pollard, 2006; Qian and Pollard, 2010).

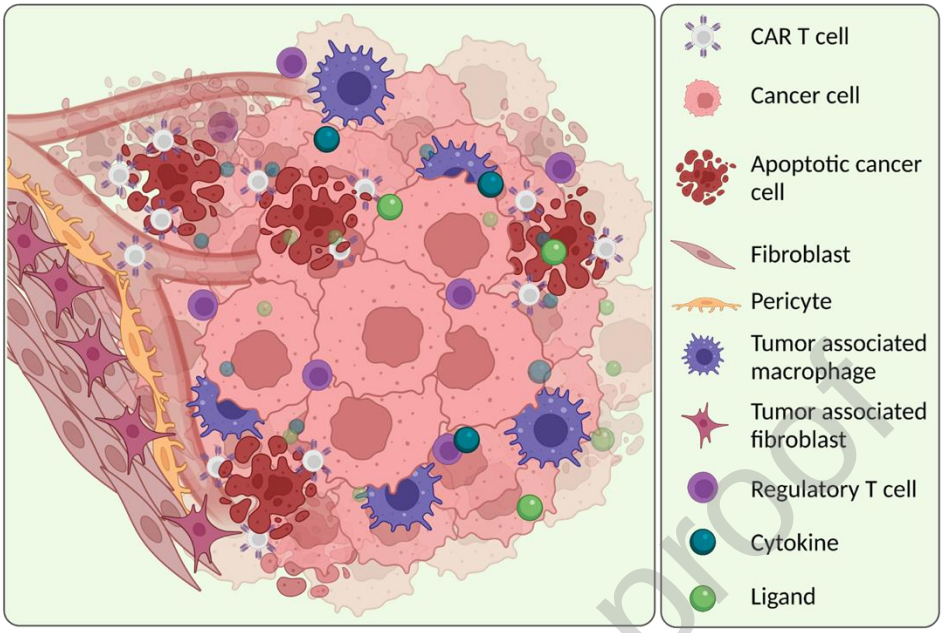

Figure 6. Theoretical targets of CAR T-cells. CAR molecules target tumor cell surface antigens. CAR T-cells could target other cancer expressed molecules such as carbohydrates and glycolipids. The cytotoxicity of CAR T-cells is dependent on interplay with these targets. Figure made in BioRender.com.

Macrophages are one of the most abundant cells found in this environment. TAMs are called to the environment in response to tumor cells; these execute a pro-tumorigenic function to aid tumor survival (Hambardzumyan et al., 2015). Further studies showed that while macrophages are the most abundant cell population in the TME, they lack T-cell co-stimulatory factors, preventing T-cell expansion in the TME (Hussain et al., 2006). Overall, we can interpret that TAMs significantly interact with brain tumor cells, stimulating these to release cytokines to recruit more TAMs, releasing pro-tumorigenic factors into the TME (Quail and Joyce, 2017). Another study focusing on the microglia population of glioblastomas found that in the TME, colony-stimulating factor 1 receptor (CSF-1R) plays a vital role in tumor invasion. The researchers showed that by blocking the CSF-1R, there was a decrease in glioblastoma invasion due to inhibition of microglial cell signaling (Coniglio et al., 2012). This indicates that the interaction between microglial cells and glioblastoma leads to possible tumor invasion and progression. The different roles that macrophages play in both normal tissue and the TME could be explained by the differing phenotypes, as macrophages have plasticity and can become polarized to fit better the physiological context of the environment (Quail and Joyce, 2013).

TAMs have also been found in significant quantities in hypoxic tumor regions (Quail and Joyce, 2013). The recruitment of these TAMs is controlled by endothelin-2 and VEGF upregulation (Lewis and Murdoch, 2005). Accumulating TAMs in these hypoxic regions has therefore been correlated to the increase in angiogenesis, change in polarization of the macrophages, and development of an invasive tumor composition (Escribese et al., 2012; Lewis and Murdoch, 2005). Macrophages appear to have a crucial role in tumor progression and metastasis. An example is breast cancer mouse models; removal through homozygous null mutation in colony-stimulating factor 1 (CSF-1) showed decreased tumor progression and metastasis (Condeelis and Pollard, 2006; E. Y. Lin et al., 2001).

Dendritic cells and Astrocytes

Dendritic cells are another essential component of the TME. These cells primarily play a role in tumor antigen presentation that can elicit an anti-tumor response in T-cells. This function is currently being considered with the development of vaccines for brain tumors (Quail and Joyce, 2017). Astrocytes play a significant role in TME. Recent studies have found that astrocytes may exert a protective role in cancer cells in the brain. Astrocytes can down-regulate pro-inflammatory factors such as TNFα and downstream suppress MHCII and CD80, impairing the antigen-presenting ability of monocytes and microglial cells, interrupting T-cell activation (Lorger, 2012; I. Yang et al., 2010).

The role of immune cells in brain TME

Neutrophils, like macrophages, have a differing role in tumor evolution; they can express a pro-tumoral or an antitumoral effect (Coffelt et al., 2016). Tumor-associated neutrophils (TANs) have exhibited plasticity in their ability to undergo different roles depending on the exposure to various signaling molecules in the TME (Y. J. Lin et al., 2021). Friedlender et al. showed that TGFβ signals TANs to take on a pro-tumorigenic role, while the presence of IFN-β or the inhibition of TGFβ signals an anti-tumor role of TANs. Research has even found that neutrophils are of prognostic value in brain cancers. Utilizing a cohort of patients that were treated with bevacizumab after radio chemotherapy or chemotherapy at recurrence, found that a high number of neutrophils found before therapy initiation is related to a positive response to bevacizumab therapy (Bertaut et al., 2016). Fossati *et al* analyzed gliomas for neutrophil infiltration and found that 70% of the samples had significant infiltration of neutrophils, correlated with tumor grade. The circulating number of neutrophils and their infiltration in tumors could be directed by specific factors released by gliomas (Fossati et al., 1999).

Although scattered information is available about the activity of immunosuppressive or immunostimulatory T-lymphocytes in the TME, it has been described that T-lymphocytes can be reprogramed in the TME and lead to an immunosuppressive state that aids in tumor growth. Studies with gliomas showed an overall decrease in the number of circulating Th cells but an increased number of T regulatory (Treg) cells, with a prominent population of them infiltrating the tumor (Fecci et al., 2014). Treg cells are usually involved in activating other B and T-cells and have a vital role in the regulation of cytotoxic lymphocytes (Gasteiger et al., 2013). Treg cells have a variety of impacts on tumorigenesis, given their complicated regulatory activities in response to various triggers (von Boehmer and Daniel, 2012). Treg cells, like myeloid-derived suppressor cells (MDSCs), a heterogenous population of early myeloid progenitors and immature myeloid cells, limit tumor-associated antigen presentation and impair cytotoxic T-cell activity by blocking cytolytic granule release (Quail and Joyce, 2013; von Boehmer and Daniel, 2012).

Fibroblasts are a multipurpose cell type that deposit extracellular matrix, help maintain structure, regulate immunological responses, and maintain homeostasis (Quail and Joyce, 2013). Cancer-associated fibroblasts (CAFs) are found in large numbers in the TME, exhibited in breast CAFs which can promote metastasis and dissemination in malignant and premalignant mammary epithelial cells by imparting a mesenchymal-like morphology (Dumont et al., 2013; Quail and Joyce, 2013).

The differences between adult and pediatric brain tumors vary in location and microscopic composition, as well as the distinct interactions with the TME. Primary studies have shown that adult brain tumors tend to have a more immunosuppressive TME than pediatric brain tumors (Haberthur et al., 2016). In the previous research, Haberthur *et al* wanted to see if

the immune escape found in adult tumors could also be observed in the pediatric population. They evaluated the expression of NKG2D and NK and myeloid cell infiltration in pediatric brain tumors. Results showed that compared to normal brain tissue, high-grade gliomas had no remarkable NK or myeloid cell infiltration and did not show upregulation of NKG2 ligand expression (Haberthur et al., 2016). This suggests a less immunosuppressive TME than adult brain tumors.

In medulloblastomas, the TME is still being investigated to characterize the distinct aspects of its composition. Medulloblastomas are categorized into four subgroups: Group 3, Group 4, WNT, and sonic hedgehog (SHH) (Margol et al., 2015). Human medulloblastoma with active SHH signaling (SHH-MB) has considerably more TAMs than other MB subtypes, according to a study (Dang et al., 2021; Patterson et al., 2020). Another study demonstrated the immune components of the microenvironment in SHH and group 3 medulloblastomas (Pham et al., 2016). Pham *et al* created two SHH-driven and group 3 medulloblastoma animal models for preclinical testing in immunocompetent C57BL/6 mice. Compared to group 3, SHH murine model tumors had considerably more dendritic cells, infiltrating lymphocytes, myeloid-derived suppressor cells, and TAMs (Pham et al., 2016). Group 3 tumors, on the other hand, had more significant numbers of CD8+ PD-1 + T-cells among the CD3 group. In animals with intracranial tumors of this group, PD-1 inhibition had more significant anti-tumor activity, and peripheral PD-1 inhibition also led to a substantial rise in CD3+ T-cells in the TME. This study showed higher frequencies of lymphocytes and myeloid cells in the subgroup SHH compared with group 3. There was a higher expression of PD-1 on lymphocytes in group 3 and not the SHH subgroup. Blocking the expression of the PD-1 was related to a higher anti-tumor effect and overall survival benefit due to tumor regression after treatment with anti-PD-1 therapy (Pham et al., 2016). Pharm *et al* also showed that Ptch1 medulloblastoma tumors contain higher numbers of MDSCs and TAMs. Medulloblastoma cells emit Shh ligands, which cause granule cells to release placental growth factor (PlGF); despite being an angiogenic factor, inhibiting PlGF has a very mild antivascular effect in these tumors (Batista et al., 2015). Instead, PlGF communicates with cancer cells via the neuropilin 1 (NRP1) receptor, activating downstream survival cues. Stating that medulloblastomas are dependent on PIGF secretion in the microenvironment and secretion of SHH ligands by cancer cells stimulates PlGF, which has a downstream effect on cell survival (Batista et al., 2015).

Other studies that have focused on high-grade gliomas have shown differences in the genetic composition of these tumors between the adult and pediatric populations. The development of these tumors occurs in distinct spatial and temporal designs that concur with myelination in pediatric and adolescent brains (Jones et al., 2017). Overall, immune cells are essential in both adult and pediatric gliomas. However, functioning roles in pediatric brain tumors are less understood than in their adult counterpart; studies have shown the involvement of TAMs as a promoter of tumor progression, correlating the presence and possibly the number of immune cells with the progression of the malignancy (Patterson et al., 2020). Other studies have shown the differences between DIPG and adult glioblastomas, demonstrating the non-inflammatory microenvironment of DIPG and the lack of a prominent immunosuppressive system (Lieberman et al., 2019; G. L. Lin et al., 2018). While both pediatric low-grade glioma (pLGG) and high-grade pediatric glioma (pHGG) had CD163+ macrophages and CD8+ T-cells in significant quantities, DIPG had no increase in immune cells (Lieberman et al., 2019). Although several studies have shown that the TME of adult GBM is highly immunosuppressive, a recent investigation of adolescent brain tumors found low expression of PD-L1 in tumor-infiltrating cells and a low amount of NKG2D ligands in serum (Lieberman et al., 2019).

Examination of the inflammatory features of DIPG and adult GBM found that in primary DIPG tissue, the leukocyte (CD45+) compartment is mainly constituted of CD11b+ macrophages and small amounts of CD3+ T-lymphocytes (G. L. Lin et al., 2018). Adult GBMs had a more prevalent population of T-lymphocytes in comparison with DIPG. RNA sequencing of macrophages isolated from primary tumor tissues showed that although both tumors (DIPG and GBM) had macrophages that played significant roles in ECM remodeling and angiogenesis, DIPG-associated macrophages expressed fewer inflammatory markers (G. L. Lin et al., 2018). Overall, high-grade lesions in pediatric tumors are depleted of lymphocyte cells.

In contrast, low-grade lesions were found to have a mixed composition of cells and a higher proportion of inflammation subtypes (Abdel-Khaleq et al., 2021). HGGs, which contained a more significant number of lymphocytes, showed a decrease in survival. Recent studies have revealed the effects of age on TME and tumor susceptibility. A study that used time-of-flight mass cytometry to compare cellular components in pediatric medulloblastomas, metastatic Ewing sarcoma, pHGG, and atypical teratoid/rhabdoid tumors to adult glioblastoma tumor samples found that glioblastomas in adults and HGG in pediatric patients contained low amounts of T-cells and similar amounts of intratumor immune populations. In contrast, medulloblastomas had a higher amount of CD4+ and CD8+ T-cells when compared to adult glioblastoma (Mochizuki et al., 2019). It has also been stated that pHGG TME cells expressed higher levels of PD-L1, B7-H3, and TGF β 1 than pLGG or DIPG (Lieberman et al., 2019).

Another component of the heterogeneous population of cells found in TME is the MDSCs. Suppression of MDSCs serves as an essential mechanism by which evasion of the immune system can be achieved, and it occurs primarily due to abnormal myelopoiesis in cancer (Almand et al., 2001). MDSCs can mitigate immune responses, aiding in the progression of different diseases (Lindo et al., 2021; Lv et al., 2019). MDSCs have been shown to support the progression of infections, inflammatory diseases, and cancer (Lv et al., 2019). Clusters of these cells have been found in tumors and have shown to suppress T-cell function and increase immunosuppression in response to environmental factors surrounding them (Kumar et al., 2016; Lindo et al., 2021). These studies have shown that the efficacy of CAR T-cells is reduced by MDSCs and restored once MDSCs are depleted from the TME (Lindo et al., 2021). Using specific antibody targets that can decrease the amount of MDSCs can enhance CAR T-cell function (Fultang et al., 2019). Fultang *et al* showed the identification of CD33 surface markers in MDSCs. With the applied use of an antibody-drug (Gemtuzumab ozogamicin) that targeted the specific surface marker identified CD33, the study showed increased cell death and restoration of the function of CAR T-cells with targets against GD2, mesothelin, or EGFRvIII. VEGFR2-positive MDSCs decreased when subjected to targeted therapy of IL-12 + VEGFR2 CAR T-cells (Chinnasamy et al., 2012). Noman et al showed that MDSCs in tumors express PD-L1 differently from peripheral MDSCs. In tumor-bearing animals, hypoxia triggered the overexpression of PD-L1, which was dependent on HIF-1α (Noman et al., 2014). This upregulation was observed on splenic MDSCs and macrophages, dendritic cells, and tumor cells. Results showed that TME exposed to hypoxia *in vivo* increased MDSCs expression of PD-L1 and consequently suppressed tumor-infiltrating lymphocytes (Noman et al., 2014). Overall, they display the use of methods that target specific markers on MDSCs and their potential in overcoming the TME in solid tumors and aiding in CAR T-cell therapy. MDSCs have been shown in various animal models to induce tumorigenesis, with the reduction of these cells dramatically reducing metastasis (Quail and Joyce, 2013). The observation corroborates these results that cancer patients have higher MDSCs found in the periphery, which has been found to

correspond with advanced illness and ineffective treatment (Almand et al., 2001; Diaz-Montero et al., 2009).

When discussing adult glioblastomas, hypoxic conditions were associated with increased stability and maintenance of the tumor cells in these regions (Abou-Antoun et al., 2017; Iwadate et al., 2016). Results from the studies showed that hypoxic conditions could utilize mechanisms involving TGF β and HIF-1 α to contribute to the maintenance of GMB cells (Abou-Antoun et al., 2017; Pistollato et al., 2009). The influence of a hypoxic environment has not been well studied in pediatric brain tumors. Research has found that high-grade pediatric glioma-derived precursors were able to expand when exposed to hypoxic conditions, which inhibited p53 activation and subsequent astroglial differentiation of HGG precursors (Abou-Antoun et al., 2017; Pistollato et al., 2009). This study found that HGG precursors produced bone morphogenetic protein (BMP) signaling, which under high oxygen tension arrests mitosis, subsequently finding that this signaling pathway is halted by hypoxia (Pistollato et al., 2009).

5.5 Treatment Toxicity and Risks

Efforts to implement CAR T-cell therapy in treating brain tumors have proven difficult, not just because of the complex nature of the TME but also the significant toxicities it could entail (Lindo et al., 2021). CAR T-cell therapy is associated with unique toxicities that often result from on-target effects and reverse when CAR T-cells are exhausted (Maggs et al., 2021). CRS had not been observed during the development of CD19 CAR T-cells and was only recognized after phase I clinical trials commenced (Maggs et al., 2021). In patients, activation of CAR T-cells caused an elevation of inflammatory cytokines, which subsequently resulted in symptoms such as fever, hypotension, tachycardia, cerebral edema and in some cases multiorgan failure and death (Maggs et al., 2021) (Figure 5C). While the hallmark characteristics of CRS are associated with increased cytokine levels, most typically IL-10, IL-6 and IFN-γ, now deemed as “core cytokines” of CRS, the mechanisms underlying the clinical manifestations were initially unclear. To better understand the complex pathogenesis of CRS, two murine models have been reported (Giavridis, van der Stegen, et al., 2018; Norelli et al., 2018).

One model used SCID-beige mice, a strain of double-mutant mice that lack B- and T-lymphocytes and reduced NK activity, to establish conditions whereby human CD19.28z CAR T-cells would initiate CRS within a few days of infusion, reflecting clinical manifestations (Giavridis, van der Stegen, et al., 2018; Shibata et al., 1997). Tumor cells from a Burkitt lymphoma human cell line (Raji cells) were intraperitoneally injected in mice and after vascularization of solid masses, thirty million CD19.28z cells were transferred to the mice. CRS was exhibited 2 to 3 days after infusion. Interestingly, 18 out of 19 cytokines in the serum cytokine profile elicited in mice were highly similar to those reported in clinical studies. High levels of murine IL-6, a predominantly myeloid-derived cytokine and signature cytokine of CRS, caused researchers to track the source of IL-6 released during CRS. Through RNA-seq analysis of purified dendritic cell, macrophage and monocytic populations from the peritoneum and spleen, it was discovered that the main source of IL-6 originated from macrophages and monocytes. After this discovery, the team investigated the role of inducible nitric oxide synthase (iNOS), an enzyme that is expressed by macrophages upon their activation. Macrophages showed the highest induction of NO production, which is known to cause vasodilation and hypotension, two common symptoms of CRS. It was demonstrated that this production was induced by IL-1 and IL-6 during CRS.

A second model used humanized NSG mice that were infused with ALL-CM leukemic cells and later targeted with either CD19.28z or CD44v6.28z CAR T-cells (Norelli et al., 2018). To study the pathogenesis of CRS and neurotoxicity, the second most common side effect of CAR T treatment, investigators used T-cells derived from triple transgenic mice (SGM3) transplanted with hematopoietic stem and progenitor cells (HSPCs). In comparison to NSG mice, HSPC-humanized SGM3 mice is a strain known to better support human lymphohematopoiesis. The study concluded that monocytes, rather than CAR T-cells, were responsible for systemic release of IL-6. Investigators also discovered that IL-1 preceded IL-6 production, demonstrating that IL-1 is the primary cytokine causing CRS and neurotoxicity. Both mouse models independently showed that macrophages and monocytes, not CAR T-cells, directly mediate CRS and neurotoxicity.

As mentioned above, neurotoxicity is another complication observed from CAR T therapy. This complication, now termed immune effector cell-associated neurotoxicity syndrome (ICANS), is the second most common adverse event after CAR T infusion (Santomasso et al., 2022). Early signs of ICANS include expressive aphasia, tremor, and dysgraphia with symptoms later progressing into seizures and a comatose state (Siegler and Kenderian, 2020). While the two murine models have provided insight behind the mechanisms of ICANS, it is relatively less understood than CRS, taking on a highly variable course in patients. For example, severe ICANS can develop rapidly after CRS has begun or it can take up to three- or four-weeks post-CAR T infusion for clinical symptoms to manifest (Santomasso et al., 2022) Interestingly, CSF protein levels are elevated in patients with severe ICANS, likely exhibiting increased blood-CSF barrier permeability (Neelapu et al., 2018).

Several developments have emerged to combat the toxicities associated with CAR T therapy. In patients, IL-6 blockade via tocilizumab, an anti-IL-6 receptor antibody, has resulted in dramatic reversal of CRS (Norelli et al., 2018). Tocilizumab is often used alone, but can be paired with steroids to manage fever and hypotension caused by CRS, but this treatment fails to revert severe neurotoxicity (Maude et al., 2018; Turtle et al., 2016). After determining that IL-1 preceded IL-6 production, investigators used leukemic HuSMG3 mice to test CRS responsiveness to anakinra, an IL-1 receptor antagonist, and whether it performed better than tocilizumab (Norelli et al., 2018). Both tocilizumab and anakinra were effective at preventing CRS by both CD19.28z and CD44v6.28z CAR T-cells and did not interfere with in vivo CAR T-cell expansion. However, after a median of 30 days, HuSGM3 mice that received either control or tocilizumab, occurrence of lethal neurological syndrome was observed. This phenomenon was not exhibited in mice that received anakinra, and instead, it effectively prevented meningeal thickening caused by macrophage infiltration.

6. Discussion

There have been significant developments in the last decade that have allowed for the application of CAR T-cells for adult and pediatric brain tumors. However, high grade gliomas and many pediatric brain tumors continue to carry a grim prognosis. Standard chemotherapeutic and radiotherapy treatment can cause neurological deficits in patients, especially in the pediatric population. This as well as the large success of treating hematologic neoplasms with CAR T-cells has led to a push in developing CAR T treatments which would increase survival in patients suffering from brain tumors. Current research in the field of CAR T-cell therapy for brain tumors seems to be a three-pronged approach: 1. to increase T-cell invasion and persistence at the tumor site, 2. to increase antigen recognition to adapt to the multiforme nature of these

tumors, and 3. to modify these CARs to better handle an immune evasive TME. While major progress has been made to identify targets common in both pediatric and adult brain tumors, further preclinical and clinical research is needed to address current gaps such as antigen escape mechanisms, multivalent antigen targeting, best mode of delivery and increasing persistence. Additionally, continued research is warranted to study the application of CAR T-cells in the developing brain and defining its microenvironment, especially when considering CARs for the treatment of pediatric brain tumors. Despite these challenges, the future of CAR T-cells for adult and pediatric brain tumors is promising.

Author Contributions

GG: conceptualization, data curation, writing – original draft, review, and editing. KP: writing, data curation – original draft. MRR: figure construction, editing, and writing. AR: conceptualization, supervision, and writing – review and editing. All authors contributed to the article and approved the submitted version.

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Declaration of Competing Interest

None.

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