OPEN

CURRENT OPINION

Chimeric antigen receptor adoptive immunotherapy in central nervous system tumors: state of the art on clinical trials, challenges, and emerging strategies to addressing them

Giada Del Baldo[a,b], *Andrea Carai*[c] *and Angela Mastronuzzi*[a]

Purpose of review
Central nervous system (CNS) tumors represent a significant unmet medical need due to their enduring burden of high mortality and morbidity. Chimeric antigen receptor (CAR) T-cell therapy emerges as a groundbreaking approach, offering hope for improved treatment outcomes. However, despite its successes in hematological malignancies, its efficacy in solid tumors, including CNS tumors, remains limited. Challenges such as the intricate tumor microenvironment (TME), antigenic heterogeneity, and CAR T-cell exhaustion hinder its effectiveness. This review aims to explore the current landscape of CAR T-cell therapy for CNS tumors, highlighting recent advancements and addressing challenges in achieving therapeutic efficacy.

Recent findings
Innovative strategies aim to overcome the barriers posed by the TME and antigen diversity, prevent CAR T-cell exhaustion through engineering approaches and combination therapies with immune checkpoint inhibitors to improving treatment outcomes.

Summary
Researchers have been actively working to address these challenges. Moreover, addressing the unique challenges associated with neurotoxicity in CNS tumors requires specialized management strategies. These may include the development of grading systems, monitoring devices, alternative cell platforms and incorporation of suicide genes. Continued research efforts and clinical advancements are paramount to overcoming the existing challenges and realizing the full potential of CAR T-cell therapy in treating CNS tumors.

Keywords
cell and gene therapy, central nervous system tumors, chimeric antigen receptor, chimeric antigen receptor T-cells, neurotoxicity

INTRODUCTION

Central nervous system (CNS) tumors that affect both children and adults remain an unmet medical need as they are still burdened by high mortality and morbidity. New therapeutic approaches are indispensable to address these needs. Chimeric antigen receptor (CAR) T-cells are a groundbreaking form of immunotherapy where T-cells are genetically modified to express a synthetic receptor called CAR on their surface [1] designed to recognize a specific tumor antigen expressed on cancer cells. Once the CAR T-cells are infused back into the patient, they can recognize and bind to the tumor antigen, triggering immuno-mediated death of cancer cells. CAR T-cells are engineered from patient or donor-derived

[a]Department of Pediatric Haematology and Oncology, and Cell and Gene Therapy Bambino Gesù Children's Hospital, IRCCS, [b]Department of Experimental Medicine, Sapienza University of Rome and [c]Department of Neurosciences, Neurosurgery Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Correspondence to Angela Mastronuzzi, MD, PhD, Department of Pediatric Haematology and Oncology, and Cell and Gene Therapy Bambino Gesù Children's Hospital, IRCCS, Rome 00165, Italy.
Tel: +390668594647; e-mail: angela.mastronuzzi@opbg.net

Curr Opin Oncol 2024, 36:000–000

DOI:10.1097/CCO.0000000000001076

KEY POINTS

- Brain tumors are still associated to high mortality and morbidity and CAR T-cells could be an effective strategy to improve outcome
- CAR T-cells in brain tumors possesses many challenges that need to be overcome, including immunosuppressive microenvironment, heterogeneous antigens, CAR T-cell exhaustion, cell trafficking and neurotoxicity.
- New strategies and research are being explored to overcome these obstacles.
- To date are available about 40 clinical trial to treat pediatric and adults patient affected by refractory or recurrent CNS tumors and some data have recently been published.
- Both preclinical and clinical data have shown that locoregional delivery CAR T cells is well tolerated.

T-cells and designed to target specific tumor antigen independently of the major histocompatibility complex [2]. These receptors typically comprise three key components: an extracellular or antigen-recognition domain, a transmembrane domain, and an intracellular signaling domain [3]. CAR design has undergone progressive refinement to enhance efficacy. Second- and third-generation CARs integrate one or two co-stimulatory domains, such as CD28 and/or 4-1BB, to increase T-cell proliferation, cytotoxicity, and persistence. Fourth-generation CARs also termed T-cells redirected for universal cytokine-mediated killing, build upon second-generation constructs by introducing an inducible transgenic protein, like interleukin-12 (IL-12), to amplify antitumor effect. The fifth generation of CARs is currently under development, featuring a novel design based on second-generation constructs but with the addition of a truncated cytoplasmic receptor and a binding motif for transcription factors like STAT3/5 [4].

The CAR T-cell approach has shown remarkable success in treating hemopoietic cancers, such as B-cell malignancies [5]. Improvements in survival rate for solid tumors, including CNS tumors, have been limited.

The main challenges of CAR T-cell therapy for solid tumors include the immunosuppressive and hostile tumor microenvironment (TME), heterogeneous antigen expression, and rapid CAR T-cell exhaustion. These limitations are even more evident in CNS tumors. Moreover, additional specific challenges exist in targeting CNS tumors, such as the peculiarity of the location, presence of the blood-brain barrier (BBB), and risk of neurotoxicity.

Although cellular immunotherapy represents a new potential treatment for CNS tumors, clinical experience with CAR T-cells in this setting is limited, but the field is continuously expanding, and most trials are ongoing in different contexts.

Antigen selection and target

Optimal antigen candidates should exhibit high and uniform expression in tumor cells, demonstrate minimal inter-tumor heterogeneity, and show little to no expression in normal tissue [6].

In contrast to hematological diseases, CNS tumors are characterized by antigenic heterogeneity on the cell surface as largely demonstrated in glioblastoma (GBM), the most common target of preclinical and clinical studies [7].

Epidermal growth factor receptor variant III (EGFRvIII), human epidermal growth factor receptor 2 (HER2), interleukin-13 (IL-13) receptor alpha 2 subunit (IL-13R2), B7-H3, and disganglioside-GD2 are among the main antigens expressed in GBM.

IL-13Rα2 is a single-chain, high-affinity receptor for IL-13 found in over 75% of GBMs with limited expression in health tissue. IL-13Rα2 serves as one of the binding subunits of the IL-13 receptor. Produced by activated T-cells, IL-13 plays a pivotal role in triggering both pro and anti-inflammatory immune responses [8]. Overexpression and/or mutation of tyrosine kinase receptor EGFR contribute to tumor development and progression [9]. The variant III mutation of the EGFR is the most commonly found variant in GBM and is not expressed in normal tissue, rendering it an optimal target for CAR T-cell therapy [10,11]. Moreover, EGFRvIII is a crucial oncogenic driver in GBM associated with episomal amplification and genomic instability, representing a valuable example of temporal versatility. Its role in continuous cell signaling and tumor progression makes it a significant focus for targeted therapies [12,13].

HER2, 80% expressed in GBM [14], is a receptor with tyrosine kinase activity. The activated signaling pathways result in cell proliferation, survival, differentiation, invasiveness, and tumorigenesis [15]. Ephrin type-A receptor (Epha2) is a transmembrane glycoprotein belonging to the Eph family of receptor tyrosine kinases and is overexpressed in most cancers, including GBM, promoting tumorigenesis through its involvement in cell proliferation, invasion, and migration [16]. B7-H3 is a transmembrane protein belonging to the B7-CD28 family, a class of checkpoint molecules that regulate immune responses through co-stimulatory and co-inhibitory signaling. In cancer, B7H3 expression has been associated with tumor progression and

immune evasion and it is expressed in different CNS tumors [17]. GD2 is a glycosphingolipid containing two sialic acids (disialylganglioside) and serves as a potential target for various tumors including medulloblastoma and diffuse midline glioma [18▪▪,19].

Extensive preclinical experience with these targets has enabled translation into clinical trials (Table 1) with preliminary results suggesting that targeting of single antigens in a heterogeneous disease results in limited impact in the clinical setting [19–24,25▪▪–27▪▪,28,29▪▪] (Table 2).

Therefore, various strategies to allow CAR T-cells to concomitantly engage multiple antigens are being investigated. Schmidts *et al.* [30▪▪] developed a dual-specific tandem CAR T (TanCART)-cell with the ability to target both EGFRvIII and IL-13Rα2 demonstrating high cytotoxicity *in vitro* against heterogeneous GBM populations and in multiple orthotopic preclinical models. A few years earlier, Hegde *et al.* [31] described their experience on co-targeting both HER2 and IL-13Rα2 and Bielamowicz *et al.* [32] conceived a kind of universal CAR T, which could express even trivalent CARs co-targeting HER2, IL-13Rα2, and EphA2 with promising results in Patient-Derived Xenograft (PDX) models. More recently, Bagley *et al.* [26▪▪] published interim findings from a phase 1 trial encompassing 6 patients with multifocal recurrent GBM who received intrathecal injections of bivalent CAR T-cells targeting both IL-12Rα2 and EGFR. Despite not meeting objective radiological response criteria, reduction in tumor enhancement and size was observed in all cases [26▪▪].

With the latest CAR T-cells developments, innovative targets are being evaluated. Members of the unfolded protein response (UPR) represent a promising option due to their role in regulating cancer cell survival, proliferation, and metastasis. Glucose-regulated protein 78 (GRP78), a critical UPR regulator, is frequently overexpressed to the cell surface in various cancers under increased endoplasmic reticulum stress conditions. Ibanez *et al.* [33▪] described significant cell surface expression of GRP78 in multiple solid and CNS tumors, suggesting its potential as a CAR T-cell target. They demonstrated the ability of GRP78-CAR T-cells to effectively recognize and eliminate GRP78-positive tumors both *in vitro* and *in vivo*. Also, Wang *et al.* [34▪] documented that GRP78-CAR T-cells selectively targeted and eliminated GBM tumor cells and glioma stem cells, inducing release of IFN-γ in co-culture assays. Comparable results were obtained in PDX after systemic administration, without any noticeable off-target effects [34▪].

In recent years, macrophages have surfaced as promising contenders for addressing solid tumors, owing to their natural ability to infiltrate tumors and their copious presence within the TME. The first-generation CD3ζ-based CAR macrophages could phagocytose tumor cells in an antigen-dependent manner [35]. Jin *et al.* [36▪] developed a protocol to generate macrophages from human pluripotent stem cells (hPSCs). In their study, a GBM-specific CAR was genetically incorporated into hPSCs to generate CAR hPSC-derived macrophages and a potent anticancer activity against GBM cells *in vitro* was demonstrated [36▪]. These findings open new avenues for the treatment of solid tumors, including GBM. Moreover, Lei *et al.* [35] engineered induced pluripotent stem cell-derived macrophages (iMACs) with toll-like receptor 4 intracellular toll/IL-1R (TIR) domain-containing CARs resulting in a markedly enhanced antitumor effect over first-generation CAR-macrophages. The design of a tandem CD3ζ-TIR dual signaling CAR endows iMACs with both target engulfment capacity and antigen-dependent M1 polarization and M2 resistance in a nuclear factor kappa B (NF-κB)-dependent manner conferred the capability to modulate the TME [35].

Tumor microenvironment

The TME poses numerous challenges to CAR T therapy, including the presence of a suppressive tumor stroma consisting of tumor-associated macrophages and myeloid-derived suppressor cells (MDSCs), as well as hypoxic conditions that impede its effectiveness [37]. Countless research studies have been conducted to counteract the antagonistic effect of the microenvironment on the efficacy of CAR T-cells against cancer.

The transgenic expression of IL-15 represents an appealing approach to regulate the TME. Zannikou *et al.* [38▪] found that MDSCs from both human and murine GBMs express IL-15Rα. They engineered T-cells to express an IL-13Rα2-CAR alongside secretory IL-15 or an IL-13Rα2-CAR with IL-15 directly fused to the CAR to concurrently target MDSCs and malignant GBM cells while further enhancing T-cell effector function. *In vitro*, CAR.IL15 s and CAR.IL15f T-cells effectively eliminated MDSCs and reduced their secretion of immunosuppressive molecules, with CAR.IL15f T-cells exhibiting greater efficacy. Likewise, CAR.IL15f T-cells substantially prolonged survival in two GBM mouse models. Analysis of the TME revealed that treatment with CAR.IL15f T-cells led to increased frequencies of $CD8^+$ T-cells, NK cells, and B cells while decreasing CD11b+ cells within tumors compared to therapy with CAR T-cells. Overall, targeting of MDSCs showed antitumor efficacy in murine glioma models [38▪], suggesting a

Table 1. Overview on CAR-T cell phase 1 clinical trial for pediatric and adults central nervous system tumors

Study number	Target antigen	Disease	Site	Status
ADULTS				
NCT03696030	HER-2	- Malignant brain tumor - Other solid tumors	City of Hope Medical Center, Duarte, California	Recruiting
NCT04406610	GD2	Glioma of brain	Fuda Cancer Hospital, Guangzhou, China	Withdrawn
NCT03638167	EGFR806	Central nervous system tumors	Seattle Children's Hospital, Washington	Active, not recruiting
NCT05474378	B7-H3	Central nervous system tumors	Stanford University, California	Recruiting
NCT04661384	IL13Ralpha2	- Ependymoma - GBM - Medulloblastoma - Recurrent Metastatic Malignant Neoplasm in the Leptomeninges	City of Hope Medical Center, Duarte, California	Recruiting
NCT05353530	IL-8 receptor	GBM	University of Florida, Florida	Recruiting
NCT02541370	CD133	- Brain Tumor - Other solid tumors	Chinese PLA General Hospital, China	Completed
NCT05063682	EGFRvIII	GBM	Finland India	Unknown status
NCT01454596	EGFRvIII	- GBM - Malignant glioma	National Cancer Institute (NCI)	Completed
NCT05366179	B7-H3	GBM	UNC Lineberger Comprehensive Cancer Center, North Carolina	Recruiting
NCT03423992	Personalized chimeric antigen receptor T cells	Malignant glioma	Xuanwu Hospital, Beijing, China	Unknown status
NCT05835687	B7-H3	Central nervous system tumors	St. Jude Children's Research Hospital, Memphis, Tennessee	Recruiting
NCT03726515	EGFRvIII pembrolizumab	GBM	University of Pennsylvania, Philadelphia, Pennsylvania	Completed
NCT00730613	IL13Rα2	High-grade malignant glioma	City of Hope Medical Center, Duarte, California	Completed
NCT05241392	B7-H3	GBM	Beijing Tiantan Hospital, Beijing, China	Recruiting
NCT04077866	B7-H3	GBM	Second Affiliated Hospital, School of Medicine, Zhejiang University, China	Recruiting
NCT04214392	CAR T with Chlorotoxin Tumor-Targeting Domain	GBM	City of Hope Medical Center, Duarte, California	Recruiting
NCT03389230	HER2	Grade III-IV Glioma	City of Hope Medical Center, Duarte, California	Active, not recruiting
NCT03638206	EGFRvIII	-Glioma -Other solid tumors	The First Affiliated Hospital of Zhengzhou University, China	Unknown
NCT03941626	EGFRvIII	-Glioma -Other solid tumors	Henan Provincial People's Hospital, China	Unknown
NCT05540873	IL13Rα2	Malignant glioma	National Cancer Center, Korea, Goyang-si, Gyeonggi, Republic of Korea	Recruiting
NCT04003649	IL13Rα2	GBM	City of Hope Medical Center, Duarte, California	Recruiting
NCT05131763	NKGD2	GBM Medulloblastoma	Xunyang Changchun Shihua Hospital, Jiujiang, China	Unknown
NCT05024175	CARv3-TEAM-E EGFR	GBM	Massachusetts General Hospital, Boston, Massachusetts	Not yet recruiting

Table 1 (*Continued*)

Study number	Target antigen	Disease	Site	Status
NCT05577091	IL7Ra	GBM	Beijing Tiantan Hospital, Beijing, China	Recruiting
NCT04717999	NKG2D	GBM	Not listed	Unknown
NCT04550663	NKG2D	- Glioma - Other solid tumors	The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School, Nanjing, Jiangsu, China	Unknown
NCT03383978	NK-92/5.28.z + Ezabenlimab	GBM HER2 pos	Johann Wolfgang Goethe University Hospital, Germany	Active, not recruiting
NCT05168423	EGFR-IL13Ra2	GBM	University of Pennsylvania, Philadelphia, Pennsylvania	Recruiting
NCT05660369	CARv3-TEAM-E	GBM	Massachusetts General Hospital, Boston, Massachusetts	Recruiting
CHILDREN AND YOUNG ADULTS				
NCT05298995	GD2	Recurrent and refractory pediatric and young adults brain tumors	Bambino Gesu Hospital and Research Institute, Rome, Italy	Recruiting
NCT06221553	B7H3 IL-7Ra	DMG	Chulalongkorn University, Bangkok, Thailand	Recruiting
NCT04099797	C7R-GD2	- Diffuse Intrinsic Pontine Glioma - High Grade Glioma - Embryonal Tumor - Ependymal tumor	Baylor College of Medicine, Houston, Texas	Recruiting
NCT04510051	IL13Ralpha2	Recurrent and refractory pediatric brain tumors	City of Hope Medical Center, Duarte, California	Recruiting
NCT03170141	Antigen-specific IgT cells	GBM	Shenzhen Geno-Immune Medical Institute, China	Enrolling by invitation
NCT03500991	HER2	Recurrent and refractory pediatric brain tumors	Seattle Children's Hospital, Washington	Active, not recruiting
NCT03638167	EGFR806	Recurrent and refractory pediatric brain tumors	Seattle Children's Hospital, Washington	Active not recruiting
NCT04185038	B7-H3	Recurrent and refractory pediatric brain tumors	Seattle Children's Hospital, Washington	Recruiting
NCT02442297	HER-2	Recurrent and refractory pediatric brain tumors	Baylor College of Medicine, Houston, Texas	Active, not recruiting
NCT04196413	GD2	DMG	Stanford University, California	Recruiting
NCT01109095	HER.CAR CMV-specific CTLs	GBM	Baylor College of Medicine, Houston, Texas	Completed
NCT05768880	B7-H3, EGFR806, HER2, And IL13-Zetakine (Quad)	- DMG - Recurrent and refractory CNS Tumor	Seattle Children's Hospital, Washington	Recruiting

potential advantage in co-targeting MDSCs and tumor cells for various malignancies.

Moreover, as largely demonstrated, human neutrophils possess effective capabilities to pass physiological barriers and demonstrate effector immunity against pathogens and tumor cells. However, their brief lifespan and resistance to genome editing have constrained their extensive utilization in immunotherapy. Chang *et al.* [39] generate CAR-neutrophils with optimal antitumor efficacy, designed to deliver and release tumor microenvironment-responsive nanodrugs to target GBM specifically and noninvasively, obviating the need for inducing additional inflammation at the tumor sites. They modified human pluripotent stem cells through CRISPR/Cas9-mediated gene knock-in to express diverse anti-GBM CAR constructs, incorporating either T-specific CD3ζ or neutrophil-specific γ-signaling domains. This combined chemo-immunotherapy demonstrated superior and targeted anti-

Table 2. Clinical data published on adults and pediatrics patients affected by central nervous system tumors and treated with chimeric antigen receptor T cells

Study number	NCT00730613	NCT01975701	NCT02209376	NCT01109095	NCT03423992.	NCT05660369	NCT05168423	NCT02208362	NCT03500991	NCT04196413	NCT04185038
Author Years	Brown *et al.* [20]	Brown *et al.* [21]	O'Rourke *et al.* [22]	Ahmed *et al.* [23]	Lin *et al.* [24]	Choi *et al.* [25▪▪]	Bagley *et al.* [26▪▪]	Brown *et al.* [27▪▪]	Vitanza *et al.* [28]	Majzne *et al.* [19]	Vitanza *et al.* [29▪▪]
Number of patients	3 (adult)	1 (adult)	10 (adult)	17 (10 adults, 7 children)	3 (adult)	3 (adult)	6 (adult)	65 (adult)	3 (children, young adults)	4 (children, young adults)	3 (children, young adults)
Brain tumor	GBM	GBM	GBM	GBM	GBM	GMB	GBM	GBM	1 anaplastic astrocytoma 2 ependymoma	DMG	DMG
Antigen target	IL13Rα2	IL13Rα2	EGFRvIII	HER2	EphA2	CARv3-TEAM-E	EGFR- IL13Rα2	IL13Rα2	HER2	GD2	B7H3
construct	First generation	First generation	Second generation	Second generation	Second generation	Second generation	Third generation	Second generation	Second generation	Second generation	Second generation
Mode of administration	Locoregional	Locoregional	Intravenously	Intravenously	Intravenously	Locoregional	Locoregional	Locoregional	Locoregional	Intravenously and locoregional for patients who exhibited clinical benefit	Locoregional
Response	Transient antitumor activity in 2 patients on MRI (necrosis and inflammation)	CR per RANO criteria	SD at week 4 MRI in 90% of patients	PR 1 patient, SD in 7 patients at week 6 MRI	1 SD 2 PD	2 PR, 1 near CR per RANO criteria	100% radiographic regression, none fulfilling objective response for RANO criteria	29/58 (50%) SD or better, 2 PR, 2 CR per modified RANO criteria	1 SD and 2 PD at first examination after CAR T infusion	three of four patients exhibited clinical and radiographic improvement	2 PD 1 PR through 12 months on study
Toxicity	Grade 3 headache in one individual and grade 3 neurologic event in another one	Grade 1-2 headaches, fatigue, myalgia and olfactory auras	Grade 3 toxicity in 2 patients and grade 4 in 1	No dose-limiting toxicity was observed. Grade 2 seizures and/or headaches in 2 patients	2 CRS. No dose-limiting toxicity was observed	Grade 3 encephalopathy in 1 case and grade 3 in another one	ICANS One patient in dose level 2 experienced a dose-limiting toxicity (grade 3 anorexia, generalized muscle weakness and fatigue)	Grade 3 toxicities in 35%, one grade 3 encephalopathy and one grade 3 ataxia. Transient grade 4 cerebral edema in 2 cases	No associated dose-limiting toxic effects. Mild CRS and transient worsened neurological symptoms	CRS and TIAN (reversible in all cases)	No associated dose-limiting toxic effects. Mild CRS and transient worsened neurological symptoms

CR, complete remission; CRS, cytokine release syndrome; DMG, diffuse midline glioma; GBM, glioblastoma; ICANS, immune effector cell-associated neurotoxicity syndrome; MRI, magnetic resonance images; PD, progressive disease; PR, partial response; RANO, Response Assessment in Neuro-Oncology; SD, stable disease; TIAN, tumor inflammation-associated neurotoxicity.

GBM effects, diminishes off-target drug delivery, and extends lifespan in female tumor-bearing mice. Collectively, this biomimetic CAR-neutrophil drug delivery system emerges as a secure, potent, and adaptable platform for treatment of GBM and other debilitating conditions [39].

Furthermore, Wang *et al*. [40] described their experience in combining an oncolytic adenovirus with a chemokine CXCL11 to increase the infiltration of CAR T-cells and reprogramming the immunosuppressive TME, thus improving its therapeutic efficacy.

Chimeric antigen receptor T-cell exhaustion

The mechanisms underlying CAR T-cell exhaustion are incredibly intricate and warrant thorough exploration. Inadequate CAR T-cell structure may trigger ligand-independent tonic signaling, consequently predisposing CAR T-cells to exhaustion. Additionally, both the cytokine milieu and the duration of in-vitro expansion play roles in influencing CAR T-cell exhaustion. Finally, the TME harbors immunosuppressive factors, which further contribute to this phenomenon.

Prolonged persistence of CAR T-cells is a feature of new-generation CAR T constructs. Numerous studies have delved into CAR T engineering strategies, highlighting that prioritizing a central memory phenotype could be pivotal in inhibiting exhaustion and bolstering CAR T-cells proliferation and persistence [41,42]. In addition to modifying the CAR costimulatory signals themselves, engineering approaches aimed at producing suitable cytokines are also essential for the full activation of CAR T-cells [43].

Moreover, the hypoxic tumor microenvironment can enhance tumor progression through various mechanisms, such as increasing adenosine receptor expression in immunosuppressive cells [44,45]. Inhibiting the adenosine signaling of CAR T-cells using the CRISPR/Cas9 system, shRNA, or overexpressing adenosine deaminase 1 has been shown to enhance the antitumor function and prevent CAR T-cell exhaustion *in vitro* [46–49].

A combined therapeutic approach could mitigate this issue. Zhang *et al*. [50] recently described an orthotopic NOD/SCID GBM animal model to assess the safety and efficacy of a combined treatment approach across various doses of GD2 CAR T and Nivolumab. In-vitro studies demonstrated that the addition of Nivolumab to GD2 CAR T enhanced the persistence of GD2 CAR T-cells cytotoxicity. Animal models confirmed that GD2 CAR T-cells effectively infiltrated tumor tissue. The longest survival was achieved combining moderate doses of CAR T with Nivolumab. Further examination of toxicity revealed that high doses of GD2 CAR T-cells induced tumor apoptosis via the p53/caspase-3/PARP signaling pathway [50].

Trafficking and route of administration

Activated T-cells are known to cross the BBB [51] and three pathways are described: via postcapillary venules into the perivascular space; by extravasation through the choroid plexus into the cerebrospinal fluid (CSF); and through superficial leptomeningeal vessels into the subarachnoid space [52]. These findings suggest that T-cells delivered through systemic infusion may reach tumors, challenging the notion that the brain is an immune sanctuary. Moreover, studies employing intravenously administered CD19-targeted CAR T-cells have demonstrated that they are capable of breaching the BBB, having detected them in the CSF through flow cytometry and immunofluorescence after treatment [53,54]. Local delivery of T-cells within the CNS presents an appealing strategy to mitigate systemic toxicity while enhancing CAR T-cell migration and accumulation in the tumor site. Research comparing delivery methods in preclinical models of GBM has demonstrated that local administration surpasses systemic delivery. Direct intratumoral injection of IL-13Rα2-CAR T-cells led to prolonged survival in orthotopic GBM models, while IV delivery did not yield significant benefits over control groups [55].

The clinical evidence available are summarized in Table 2. Intrathecal and intraventricular delivery of CAR-T-cells were evaluated in a patient with multifocal GBM, proving to be well tolerated, without CRS or severe neurotoxicity. Notably, intraventricular administration resulted in superior disease control [21]. Similarly, results on safety were reported by Majzner *et al.*'s [19]. The safety of the loco-regional administration of HER-2 CAR T-cells has been demonstrated also in the BrainChild-01 trial [28]. The optimal administration route could also vary depending on the molecular target; antigens with broad expression in normal tissues (e.g., HER2 and B7H3) may exhibit considerably lower toxicity following local administration [56].

The existing evidence from limited clinical experiences leans towards locoregional administration.

Neurotoxicity

In addition to the well known CRS and immune effector cell-associated neurotoxicity syndrome (ICANS), neurotoxicity can be a more challenging in patients with CNS tumors. Tumor inflammation-associated neurotoxicity (TIAN) is a brain tumor associated toxicity recently described by Madhi *et al.* [57▪▪].

A pseudo-progression can result in the increase of local mass effect, hydrocephalus, and intracranial hypertension (ICP). A ventricular access device might be inserted before the infusion of CAR T-cells and used both to directly assess intracranial pressure waves and to remove determined CSF volumes, if appropriate to improve intracranial pressure management. Moreover, implantable telemetric ICP monitoring devices are commercially available. documenting a decrease in the number of invasive procedures [58]. Other strategies can be considered to reduce the risk of severe TIAN namely multiple administration of low doses of CAR T-cells that can result in a lower tumor infiltration with CAR T-cells and a relatively slow and progressive tumor disruption. The use of different cell platforms like NK, with a lower persistence over time and a reduced inflammatory profile upon activation, could reduce the risk associated with these treatments. Lastly, the introduction of a suicide gene capable of rapidly inducing the apoptosis of CAR T-cells and, thus, mitigating the inflammation and the pseudo-progression, represents an attractive option for increasing the safety profile of the approach [59▪▪].

Regarding safety from the studies published so far in Table 2, only one case of dose-limiting-toxicity were described. In the other cases, CRS and neurotoxicity were easily managed with steroids and anti-inflammatory therapy. Neurosurgical measures for ICP were necessary in only a very few cases.

Regarding efficacy, most patients showed a response at the first reevaluation imaging, with stable disease as the most frequent occurrence. Three cases of complete response were reported according to Response Assessment in Neuro-Oncology criteria.

CONCLUSION

The advancement of CAR T-cell therapy holds promise for treating solid tumors, including CNS tumors, but challenges remain in achieving similar success as seen in blood cancers. Complexities like the TME, antigen diversity and instability/versatility, and CAR T-cell exhaustion hinder efficacy. Innovative strategies like multiantigen targeting, exploring new targets, combining therapy with drugs, and modulating the TME show potential in preclinical and early clinical studies. Preventing CAR T-cell exhaustion through engineering approaches and combining therapy with immune checkpoint inhibitors can enhance outcomes. However, neurotoxicity in CNS tumors requires specialized management, including grading systems, monitoring devices, alternative cell platforms, and suicide gene incorporation. Continued research and clinical advancements are crucial to overcome challenges and improve patient outcomes in this complex disease landscape.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. Mitra A, Barua A, Huang L, *et al.* From bench to bedside: the history and progress of CAR T cell therapy. Front Immunol 2023; 14:1188049.
2. Guzman G, Reed MR, Bielamowicz K, *et al.* CAR-T therapies in solid tumors: opportunities and challenges. Curr Oncol Rep 2023; 25:479–489.
3. Lindner SE, Johnson SM, Brown CE, Wang LD. Chimeric antigen receptor signaling: functional consequences and design implications. Sci Adv 2020; 6: eaaz3223.
4. Kagoya Y, Tanaka S, Guo T, *et al.* A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. Nat Med 2018; 24:352–359.
5. Maude SL, Laetsch TW, Buechner J, *et al.* Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018; 378:439–448.
6. Bourdeaut F. Are B7-H3 CAR-T cells the future universal treatment for pediatric brain tumors? Neuro Oncol 2021; 23:872–873.
7. Rao P, Furst L, Meyran D, *et al.* Advances in CAR T cell immunotherapy for paediatric brain tumours. Front Oncol 2022; 12:873722.
8. Bagley SJ, Desai AS, Linette GP, *et al.* CAR T-cell therapy for glioblastoma: recent clinical advances and future challenges. Neuro Oncol 2018; 20: 1429–1438.
9. Sigismund S, Avanzato D, Lanzetti L. Emerging functions of the EGFR in cancer. Mol Oncol 2018; 12:3–20.
10. Akhavan D, Alizadeh D, Wang D, *et al.* CAR T cells for brain tumors: lessons learned and road ahead. Immunol Rev 2019; 290:60–84.
11. Choi BD, Maus MV, June CH, Sampson JH. Immunotherapy for glioblastoma: adoptive T-cell strategies. Clin Cancer Res 2019; 25:2042–2048.
12. Del Vecchio CA, Giacomini CP, Vogel H, *et al.* EGFRvIII gene rearrangement is an early event in glioblastoma tumorigenesis and expression defines a hierarchy modulated by epigenetic mechanisms. Oncogene 2013; 32: 2670–2681.
13. Gan HK, Burgess AW, Clayton AHA, Scott AM. Targeting of a conformationally exposed, tumor-specific epitope of EGFR as a strategy for cancer therapy. Cancer Res 2012; 72:2924–2930.
14. Zhang L, Ren J, Zhang H, *et al.* HER2-targeted recombinant protein immunocaspase-6 effectively induces apoptosis in HER2-overexpressing GBM cells in vitro and in vivo. Oncol Rep 2016; 36:2689–2696.
15. Zhu Y, Zhu X, Wei X, *et al.* HER2-targeted therapies in gastric cancer. Biochim Biophys Acta Rev Cancer 2021; 1876:188549.
16. Kilian M, Bunse T, Wick W, *et al.* Genetically modified cellular therapies for malignant gliomas. Int J Mol Sci 2021; 22:12810.
17. Guo X, Chang M, Wang Y, *et al.* B7-H3 in brain malignancies: immunology and immunotherapy. Int J Biol Sci 2023; 19:3762–3780.
18. ▪▪ Ciccone R, Quintarelli C, Camera A, *et al.* GD2-targeting CAR T-cell therapy for patients with GD2+ medulloblastoma. Clin Cancer Res 2024; 30: 2545–2557.
The authors documented GD2 high expression in of medulloblastoma tumors (73.17%), with SHH and G4 subtypes expressing the highest levels; CAR.GD2 T-cells ability to kill GD2+MB cells in in-vitro co-culture assays and Tazemetostat upregulation of GD2 expression, sensitizing GD2dimMB cells to CAR.GD2 T-cells cytotoxic activity. In orthotopic mouse models of medulloblastoma, intravenously injected CAR.GD2 T-cells significantly controlled tumor growth, prolonging overall survival of treated mice. Moreover, the dimerizing drug AP1903 was able to cross the murine blood brain barrier and to eliminate both blood circulating and tumor infiltrating CAR.GD2 T-cells. This work supports the testing of this construct in the clinic as well with possibly encouraging results.
19. Majzner RG, Ramakrishna S, Yeom KW, *et al.* GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. Nature 2022; 603:934–941.
20. Brown CE, Badie B, Barish ME, *et al.* Bioactivity and safety of IL13Rα2-redirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. Clin Cancer Res 2015; 21:4062–4072.
21. Brown CE, Alizadeh D, Starr R, *et al.* Regression of glioblastoma after chimeric antigen receptor T-cell therapy. N Engl J Med 2016; 375: 2561–2569.

22. O'Rourke DM, Nasrallah MP, Desai A, *et al.* A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 2017; 9:eaaa0984.

23. Ahmed N, Brawley V, Hegde M, *et al.* HER2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma. JAMA Oncol 2017; 3:1094–1101.

24. Lin Q, Ba T, Ho J, *et al.* First-in-human trial of EphA2-redirected CAR T-cells in patients with recurrent glioblastoma: a preliminary report of three cases at the starting dose. Front Oncol 2021; 11:694941.

25. Choi BD, Gerstner ER, Frigault MJ, *et al.* Intraventricular CARv3-TEAM-E T ■■ cells in recurrent glioblastoma. N Engl J Med 2024; 390:1290–1298.

The importance of this report lies in the fact that it describes first-in-human three patients with recurrent glioblastoma treated with CARv3-TEAM-E T cells. Treatment with CARv3-TEAM-E T cells did not result in adverse events greater than grade 3 or dose-limiting toxic effects. Radiographic tumor regression was dramatic and rapid, occurring within days after receipt of a single intraventricular infusion, but the responses were transient in two of the three participants. This work supports the effectiveness of this CAR construct against GBM.

26. Bagley SJ, Logun M, Fraietta JA, *et al.* Intrathecal bivalent CAR T cells ■■ targeting EGFR and IL13Rα2 in recurrent glioblastoma: phase 1 trial interim results. Nat Med 2024; 30:1320–1329.

The authors report clinical data on the first six patients with GBM treated in a phase 1 trial of intrathecally delivered CAR T cells targeting both EGFR and IL13Rα2, defining sides effect, radiological response and bioactivity. Administration of CART-EGFR-IL13Rα2 cells was associated with early-onset ICANS well managed with high-dose dexamethasone and anakinra. One patient in dose level 2 experienced a dose-limiting toxicity (grade 3 anorexia, generalized muscle weakness, and fatigue). Reductions in enhancement and tumor size at early magnetic resonance imaging timepoints were observed in all six patients; however, none met criteria for ORR. CAR T cell expansion and cytokine release in the cerebrospinal fluid (CSF) were detected in all six patients. Dual targeting has certainly provided very promising results that will need to be tested and continued in the future.

27. Brown CE, Hibbard JC, Alizadeh D, *et al.* Locoregional delivery of IL-13Rα2- ■■ targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. Nat Med 2024; 30:1001–1012.

This work is the largest description of the use of phase 1 CAR T cell trial published. The authors described safety and response of IL-13Rα2-targeted CAR-T cells in 65 patients with recurrent high-grade glioma. Moreover they evaluate three routes of locoregional T cell administration [intratumoral (ICT), intraventricular (ICV), and dual ICT/ICV] and two manufacturing platforms. Locoregional CAR-T cell administration was feasible and well tolerated, and as there were no dose-limiting toxicities. Probable treatment-related grade 3 toxicities were one grade 3 encephalopathy and one grade 3 ataxia. Stable disease or better was achieved in 50% (29/58) of patients, with two partial responses, one complete response and a second complete response after additional CAR-T cycles off protocol. CNS increases in inflammatory cytokines were associated with CAR-T cell administration and bioactivity.

28. Vitanza NA, Johnson AJ, Wilson AL, *et al.* Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis. Nat Med 2021; 27:1544–1552.

29. Vitanza NA, Wilson AL, Huang W, *et al.* Intraventricular B7-H3 CAR T cells for ■■ diffuse intrinsic pontine glioma: preliminary first-in-human bioactivity and safety. Cancer Discov 2023; 13:114–131.

Vitanza *et al.* [29■■] designed B7-H3-specific chimeric antigen receptor (CAR) T cells, confirmed their preclinical efficacy, and opened BrainChild-03 (NCT04185038), a first-in-human phase I trial administering repeated locoregional B7-H3 CAR T cells to children with recurrent/refractory CNS tumors and DIPG. They report the results of the first three patients with DIPG who received 40 infusions with no dose-limiting toxicities. One patient had sustained clinical and radiographic improvement through 12 months on study. Patients exhibited correlative evidence of local immune activation and persistent CSF B7-H3 CAR T cells. Targeted mass spectrometry of CSF biospecimens revealed modulation of B7-H3 and local immune activation. The importance of this work lies in the fact that multiple infusions could better control the disease compared with single infusion and ensure a more stable level of circulating CAR T cells that can therefore act more consistently on the disease.

30. Schmidts A, Srivastava AA, Ramapriyan R, *et al.* Tandem chimeric antigen ■■ receptor (CAR) T cells targeting EGFRvIII and IL-13Rα2 are effective against heterogeneous glioblastoma. Neurooncol Adv 2023; 5:vdac185.

They developed a novel, dual-specific, tandem CAR T (TanCART) cell against both EGFRvIII and IL-13Rα2 and demonstrate efficacy of this approach against GBM. Further evidence that multiple targets appear to be promising.

31. Hegde M, Mukherjee M, Grada Z, *et al.* Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. J Clin Invest 2016; 126:3036–3052.

32. Bielamowicz K, Fousek K, Byrd TT, *et al.* Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma. Neuro Oncol 2018; 20: 506–518.

33. Ibanez J, Hebbar N, Thanekar U, *et al.* GRP78-CAR T cell effector function ■ against solid and brain tumors is controlled by GRP78 expression on T cells. Cell Rep Med 2023; 4:101297.

Given the urgent need for new therapeutic targets, these two works are good in vitro and in vivo research on GRP78-CAR T cells in brain and other solid tumors.

34. Wang S, Wei W, Yuan Y, *et al.* Chimeric antigen receptor T cells targeting cell ■ surface GRP78 efficiently kill glioblastoma and cancer stem cells. J Transl Med 2023; 21:493.

Given the urgent need for new therapeutic targets, these two works are good in-vitro and in-vivo research on GRP78-CAR T cells in brain and other solid tumors.

35. Lei A, Yu H, Lu S, *et al.* A second-generation M1-polarized CAR macrophage with antitumor efficacy. Nat Immunol 2024; 25:102–116.

36. Jin G, Chang Y, Bao X. Generation of chimeric antigen receptor macrophages ■ from human pluripotent stem cells to target glioblastoma. Immunooncol Technol 2023; 20:100409.

In this study, the authors describe innovative functional CAR-macrophages generating from hPSCs, opening new avenues for the treatment of solid tumors, particularly GBM.

37. Renner K, Singer K, Koehl GE, *et al.* Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. Front Immunol 2017; 8:248.

38. Zannikou M, Duffy JT, Levine RN, *et al.* IL15 modification enables CAR T cells ■ to act as a dual targeting agent against tumor cells and myeloid-derived suppressor cells in GBM. J Immunother Cancer 2023; 11:e006239.

Promising data are demonstrated in this recent work on IL15Ra expression on MDSC of the glioma TME and IL15Rα-targeting with CAR T cells. IL15-modified CAR T cells act as a dual targeting agent against tumor cells and MDSC in GBM, warranting their future evaluation in early-phase clinical studies.

39. Chang Y, Cai X, Syahirah R, *et al.* CAR-neutrophil mediated delivery of tumor-microenvironment responsive nanodrugs for glioblastoma chemo-immunotherapy. Nat Commun 2023; 14:2266.

40. Wang G, Zhang Z, Zhong K, *et al.* CXCL11-armed oncolytic adenoviruses enhance CAR-T cell therapeutic efficacy and reprogram tumor microenvironment in glioblastoma. Mol Ther 2023; 31:134–153.

41. Chan JD, Lai J, Slaney CY, *et al.* Cellular networks controlling T cell persistence in adoptive cell therapy. Nat Rev Immunol 2021; 21:769–784.

42. Gargett T, Truong N, Ebert LM, *et al.* Optimization of manufacturing conditions for chimeric antigen receptor T cells to favor cells with a central memory phenotype. Cytotherapy 2019; 21:593–602.

43. Zhang ZZ, Wang T, Wang XF, *et al.* Improving the ability of CAR-T cells to hit solid tumors: challenges and strategies. Pharmacol Res 2022; 175:106036.

44. Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. J Immunother Cancer 2018; 6:57.

45. Giannone G, Ghisoni E, Genta S, *et al.* Immuno-metabolism and microenvironment in cancer: key players for immunotherapy. Int J Mol Sci 2020; 21:4414.

46. Li N, Tang N, Cheng C, *et al.* Improving the antisolid tumor efficacy of CAR-T cells by inhibiting adenosine signaling pathway. Oncoimmunology 2020; 9:1824643.

47. Giuffrida L, Sek K, Henderson MA, *et al.* CRISPR/Cas9 mediated deletion of the adenosine A2A receptor enhances CAR T cell efficacy. Nat Commun 2021; 12:3236.

48. Masoumi E, Jafarzadeh L, Mirzaei HR, *et al.* Genetic and pharmacological targeting of A2a receptor improves function of antimesothelin CAR T cells. J Exp Clin Cancer Res 2020; 39:49.

49. Qu Y, Dunn ZS, Chen X, *et al.* Adenosine deaminase 1 overexpression enhances the antitumor efficacy of chimeric antigen receptor-engineered T cells. Hum Gene Ther 2022; 33:223–236.

50. Zhang G, Zhao Y, Liu Z, *et al.* GD2 CAR-T cells in combination with Nivolumab exhibit enhanced antitumor efficacy. Transl Oncol 2023; 32:101663.

51. Engelhardt B. Regulation of immune cell entry into the central nervous system. Results Probl Cell Differ 2006; 43:259–280.

52. Wilson EH, Weninger W, Hunter CA. Trafficking of immune cells in the central nervous system. J Clin Invest 2010; 120:1368–1379.

53. Patterson JD, Henson JC, Breese RO, *et al.* CAR-T cell therapy for pediatric brain tumors. Front Oncol 2020; 10:1582.

54. Galea I, Bernardes-Silva M, Forse PA, *et al.* An antigen-specific pathway for CD8 T cells across the blood-brain barrier. J Exp Med 2007; 204: 2023–2030.

55. Brown CE, Aguilar B, Starr R, *et al.* Optimization of IL13Rα2-targeted chimeric antigen receptor T cells for improved antitumor efficacy against glioblastoma. Mol Ther 2018; 26:31–44.

56. Akhavan D, Alizadeh D, Wang D, *et al.* CAR-T cells for brain tumors: lessons learned and road ahead. Immunol Rev 2019; 290:60–84.

57. Mahdi J, Dietrich J, Straathof K, *et al.* Tumor inflammation-associated neuro- ■■ toxicity. Nat Med 2023; 29:803–810.

This study is very important because it details the tumor inflammation-associated neurotoxicity (TIAN) and proposes a grading scale and discusses the potential management with the goal of standardizing both reporting and management.

58. Kienzler JC, Zakelis R, Marbacher S, *et al.* Changing the paradigm of intracranial hypertension in brain tumor patients: a study based on noninvasive ICP measurements. BMC Neurol 2020; 20:268.

59. Del Bufalo F, De Angelis B, Caruana I, *et al.* GD2-CART01 for relapsed or ■■ refractory high-risk neuroblastoma. N Engl J Med 2023; 388:1284–1295.

In this study, the authors reported data on 27 patients affected by relapsed or refractory neuroblastoma treated in a phase 1/2 trial with GD2-CART cells demonstrating feasibility and safety. They demonstrated also that activation of the suicide gene controlled side effects and which can be a very important mechanism of safety in case of serious side events. As secondary outcome, they reported good response data: seventeen children had a response to the treatment (overall response, 63%); nine patients had a complete response, and eight had a partial response. Among the patients who received the recommended dose, the 3-year overall survival and event-free survival were 60 and 36%, respectively.