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Review

CAR T cell therapies for diffuse midline glioma

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Diffuse midline glioma (DMG) is a fatal pediatric cancer of the central nervous system (CNS). The location and infiltrative nature of DMG prevents surgical resection and the benefits of palliative radiotherapy are temporary; median overall survival (OS) is 9–11 months. The tumor immune microenvironment (TIME) is 'cold', and has a dominant immunosuppressive myeloid compartment with low levels of infiltrating lymphocytes and proinflammatory molecules. Because survival statistics have been stagnant for many decades, and therapies targeting the unique biology of DMG are urgently needed, this has prompted the clinical assessment of chimeric antigen receptor (CAR) T cell therapies in this setting. We highlight the current landscape of CAR T cell therapy for DMG, the role the TIME may play in the response, and strategies to overcome treatment obstacles.

Highlights

The lack of effective therapies for DMG stems from tumor location, complex molecular features, and immunosuppressive tumor immune microenvironment.

CAR therapy can effectively elicit an immune response in immunologically cold diffuse midline glioma tumors.

Locoregional infusions of CAR T cells minimize therapy related toxicities, promote proinflammatory immune engagement, and may reduce immunosuppressive responses.

Combinatorial targeting approaches are crucial for addressing tumor heterogeneity.

Further understanding the tumor microenvironment and complex biology of DMG will be necessary to improve CAR T cell efficacy to promote long-term responses.

Diffuse midline glioma

DMG is a universally lethal pediatric and adolescent cancer of the CNS that is derived from mutant oligodendroglial precursor-like cells (OPC-like) found in the pons (diffuse intrinsic pontine glioma, DIPG), thalamus, midbrain, and spinal cord [1–10]. DMG accounts for 50% of all pediatric high-grade gliomas (HGGs) and presents the highest mortality rate of any cancer (20–25% of all pediatric cancer-related deaths), with a median overall survival (OS) of less than 1 year [1,11].

The molecular complexities of DMG are major contributors to poor outcomes, typically driving resistance to conventional therapies [12–16]. Failure of conventional approaches is in part due to the restricted drug delivery to the tumor site incurred by the blood–brain barrier (BBB) [1] and because the critical location of the tumor restricts surgical intervention (Figure 1) [17]. To date, the standard of care management of DMG patients is limited to palliative radiotherapy (RT) with immunosuppressive corticosteroids to manage tumor-associated edema [1].

In 2021, the 5th edition of the World Health Organization *Classification of Tumors of the Central Nervous System* aligned tumor classification with the hallmark epigenetic alterations seen across patients – 'DMG, H3K27-altered' [18]. Global hypomethylation of histone H3 at lysine 27 (H3K27) is the characteristic feature of DMG, and stems from recurring somatic mutations in the H3 genes *HIST1H3B/C* (H3.1K27M) and *H3F3A* (H3.3K27M) [3,7,8,13]. In wild-type H3 (wt-H3) DMGs, global hypomethylation is promoted by the overexpression of the Enhancer of zeste homologs inhibitory protein (*EZHIP*) which represses the H3K27 methyltransferase activity of Polycomb repressor complex 2 (PRC2) [3,7,13,19]. Additional recurring mutations are found in patient tumors depending on the age of diagnosis and tumor location [10]. Together, these molecular lesions cooperate to drive tumor heterogeneity and promote adaptation to the therapeutic pressures of RT, chemotherapies, or precision therapies [14–16]. Although the age- and location-dependent mutational profiles of DMG provide us with the beginnings of a unique therapeutic targeting toolkit (Figure 1) [13], DMG tumors harbor a relatively low mutational burden [20]. Nevertheless, co-occurring alterations in *TP53*, *AVCR1*, *MYC*, *PDGFRA*, and elements of the *PI3K* pathway are common [2,12,13,15], and may play a role in the immunosuppressive (cold) tumor immune

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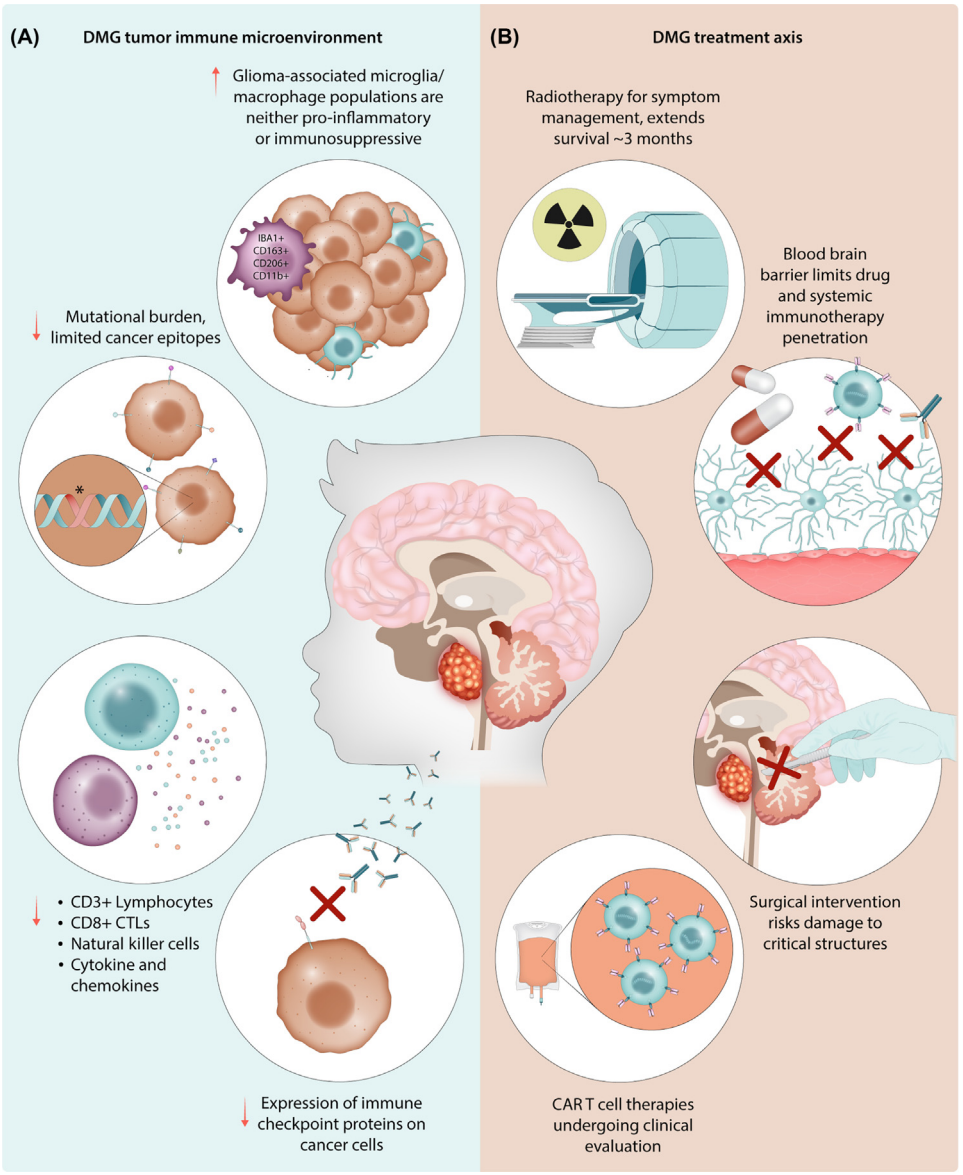

Figure 1. Diffuse midline glioma (DMG) microenvironment and treatment axis. (A) Glioma-associated macrophages and microglia (GAMs) represent the largest proportion of immune cells in the tumor immune microenvironment (TIME) and are characterized by the expression of $CD11b^+$, $IBA1^+$, $CD163^+$, and $CD206^+$. However, they do not fit the classical macrophage M1 or M2 phenotypes. DMG tumors have a low mutational burden, and consequently a low amount of cancer neoepitopes. The cold TIME is characterized by limited infiltration of various lymphocyte populations including cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, as well as low levels of proinflammatory cytokines. In addition, immune checkpoints are downregulated or absent on the surface of cancer cells, limiting the application of immune checkpoint inhibitor (ICI) therapy. (B) Palliative radiotherapy is the only standard treatment and is largely used to manage cerebral edema and neurological symptoms without significant improvement of overall survival. The blood–brain barrier protects the brain from pathogens, although it can also prevent various drugs from reaching the tumor site. Surgical resection of tumors is not possible owing to their critical location within the pons. Chimeric antigen receptor (CAR) T cell therapies are an emerging therapeutic option for DMG.

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microenvironment (TIME) [15,21–23]. We explore here the cold TIME that is shaped by the unique topography, genetics, and epigenetics of DMG, and the potential of CAR T cell therapies to provide a piece of the complex puzzle of treatments necessary to improve patient outcomes. Specifically, we discuss antigens, clinical efficacy, and the safety of CAR T therapies currently being assessed in DMG clinical trials, as well as their limitations and potential strategies to overcome treatment obstacles.

The tumor immune microenvironment

Advances in stereotactic biopsy have helped to elucidate the complex TIME of DMG, revealing that DMGs are immunologically 'cold' (Figure 1) [17,24,25]. Poor immune cell infiltration is conserved across patients [10], with sparse $CD3^+$ lymphocytes and $CD8^+$ cytotoxic subsets, few natural killer (NK) cells, and a lack of cytokines and chemokines (namely IL-6, IL-1A, CCL3, IL1B, and CCL4) [25,26]. Glioma-associated macrophages and microglia (GAMs) expressing $CD11b^+$, $IBA1^+$, $CD163^+$, and $CD206^+$ represent the largest proportion of total immune infiltrates in tumors located in the pons (i.e., DIPG) that is known to be less proinflammatory than most other cancers [17,25]. Interestingly, despite similar phenotypic expression profiles, DMG GAMs demonstrate divergent transcriptional signatures from glioblastoma GAMs, which show increased expression of genes associated with chemotaxis, cellular activation, and inflammatory activation markers, and are therefore likely influenced by tumor-derived factors [25]. DMG GAMs, however, show an enrichment for transcripts of genes associated with cell adhesion, angiogenesis, and extracellular matrix (ECM) remodeling that can be seen in an immunosuppressive microenvironment [25,27]. Although macrophages cocultured with HGG can increase their expression of immunosuppressive markers, this may be less uniform in DIPG, and could be related to genomic subgroup [17]. DIPGs of the H3.1 genotype express a more immunosuppressive phenotype than the H3.3 genotype, which are typically more inert; interestingly, this does not dictate survival outcomes [17,26]. DMG GAMs also demonstrate increased expression of MHC antigen-presenting genes; however, they lack the supportive cytokines and chemokines to effectively engage the immune network [17,25]. Ultimately, DMG GAMs may not fit neatly into the classical M1 versus M2 macrophage tumorigenic paradigm, particularly when compared to other HGGs.

The cold DMG TIME can be further exacerbated by the therapeutic use of corticosteroids such as dexamethasone because they increase the expression of cytotoxic T lymphocyte-associated antigen 4 (CTLA4), potentially disengaging the limited T cell infiltrate from initiating an immune response [1,2]. RT may act as a double-edged sword in the local TIME, and the evidence suggests that it drives immunosuppression by negatively impacting on local lymphocyte populations [28]. By contrast, it has also been demonstrated that RT promotes increased antigen presentation and antitumor immune recruitment through proinflammatory signaling [28]. Indeed, the limited accessibility of biopsy tissues from DMG patients, particularly following RT, has played a significant role in our lack of understanding of how RT modulates the TIME, with important ramifications for the selection of precision therapies thereafter.

CAR T cell therapy as a promising immuno-oncology (IO) approach for DMG

The use of IO approaches such as immune checkpoint inhibitors (ICIs) has been successful across several types of solid cancers [21,29]. Disappointingly, efficacy has not been translated to DMG patients, possibly owing to the dependency of ICIs on local immune networks for target engagement [15,23]. Furthermore, the low mutational burden of DMGs negatively impacts on the expression of immune checkpoint proteins and cancer neoepitopes, thus rendering these approaches ineffective (Figure 1) [15,17,23]. Adoptive cell therapies such as CAR T cells offer an attractive approach for immune cell engagement and proinflammatory responses (Box 1), and studies indicate that the unique DMG TIME may support their use. We provide below an overview of the current landscape of CAR T cell therapy in DMG, its challenges, and future directions.

Box 1. CAR T cell design and mechanism of action

CAR T therapies directed to tumor-associated antigens (TAAs) provide hope in overcoming the cold TIME of DMG and promote increased response and survival. A CAR is a fusion protein incorporating four main components: an antigen-binding domain, typically composed of an antibody-derived single-chain variable fragment (scFv), tethered cytokine or ligand, a flexible hinge region, a transmembrane domain, and an intracellular signaling domain derived from the CD3ζ subunit of the T cell receptor (TCR) complex (Figure 3) [62]. The inclusion of an additional costimulatory domain such as 4-1BB (CD137) or CD28 is commonplace in CAR design and has demonstrated increased persistence and more durable treatment responses [62]. Variations in these components can improve CAR T effectiveness and reduce treatment-associated toxicities by influencing antigen recognition capabilities and CAR signaling efficacy [43,62,71]. To this end, novel CAR T designs can include additional auxiliary genes, such as cytokine and chemokine receptors, to enhance CAR T cell adhesion, tracking, and infiltration into tumors and to induce a more favorable TIME [62]. In autologous CAR T cell development, patient T cells are isolated from the peripheral blood by leukapheresis and transduced with CAR transgenes before expansion *ex vivo* and reinfusion into the patient. Effective T cell engagement and tumor destruction occurs through recognition of the TAA that is highly expressed on the surface of cancer cells in a MHC-independent manner. This results in the activation of effector T cell signaling pathways, thereby prompting the release of cytotoxic granules perforin and granzyme B, as well as proinflammatory cytokines IL-2, IL-6, IFN-γ, and TNF-α [62]. Selection of an appropriate TAA is crucial to ensure that on-tumor/off-target (OT/OT) toxicities are minimized, and an ideal TAA is therefore expressed homogeneously across tumor cells but is absent or poorly expressed in healthy tissues [92]. In addition, several factors beyond CAR T design influence therapeutic efficacy. These include T cell subset phenotypes, macrophage populations, and circulating cytokines and chemokines [62,75]. Early results of CAR T cell therapies for DMG patients herald new promise of longer-term survival; however, inter- and intratumoral heterogeneity may play a role in reduced response, and the TIME of DMG also influences the therapeutic efficacy of these sophisticated therapies [39,43,58].

The use of CAR T cells has been a breakthrough for the treatment of hematological malignancies. The first therapy, tisangenlecleucel (commercially known as Kymriah), was approved by the FDA in 2017 for the treatment of children and young adults with refractory/relapsed (R/R) B lineage acute lymphocytic leukemia (B-ALL) [30]. Less than a decade later, six commercial products are available for the treatment of various B lineage malignancies, including multiple myeloma (Table 1) [31]. This has paved the way for extending the use of CAR T cells in the clinical assessment of solid tumors, although few have delivered robust survival benefits [32]. Given the immunological features of DMG, CAR T cell therapy presents a promising therapeutic approach, and eight clinical trials in DMG are active at the time of writing (Table 2).

Table 1. FDA-approved CAR T cell products

Generic name	Brand name	Year approved	Target antigen	Clinical trial	Disease	Patient population
Tisangenlelcleucel	Kymriah	2017	CD19	NCT02435849	B cell acute lymphoblastic leukemia (ALL)	Children and young adults with relapsed or refractory B cell ALL
		2018		NCT02445248	B cell non-Hodgkin lymphoma (NHL)	Adults with relapsed or refractory B cell NHL
Axicabtagene ciloleucel	Yescarta	2017	CD19	NCT02348216	B cell ALL	Adults with relapsed or refractory B cell NHL
				NCT03105336	Follicular lymphoma (FL)	Adults with relapsed or refractory FL
Brexucabtagene autoleucel	Tarartus	2020	CD19	NCT02601313	Mantle cell lymphoma (MCL)	Adults with relapsed or refractory MCL
				NCT02614066	B cell ALL	Adults with relapsed or refractory B cell ALL
Lisocabtagene maraleucel	Breyanzi	2021	CD19	NCT03575351	B cell NHL	Adults with relapsed or refractory B cell NHL
Ideacabtagene vicleucel	Abecma	2021	BCMA	NCT03361748	Multiple myeloma	Adults with relapsed or refractory multiple myeloma
Ciltacabtagene autoleucel	Carvykti	2022	BCMA	NCT03548207	Multiple myeloma	Adults with relapsed or refractory multiple myeloma

Table 2. CAR T cell clinical trials for DMG

Study title	Target	Delivery	Phase	Status	Year	Clinical trial number
HER2-specific CAR T cell locoregional immunotherapy for HER2-positive recurrent/refractory pediatric CNS tumors (DIPG excluded)	HER2	ICV	1	Recruiting	2018	NCT03500991
EGFR806-specific CAR T cell locoregional immunotherapy for EGFR-positive recurrent or refractory pediatric CNS tumors (DIPG excluded)	EGFR806	ICV	1	Active, not recruiting	2018	NCT03638167
Study of B7-H3-specific CAR T cell locoregional immunotherapy for diffuse intrinsic pontine glioma/diffuse midline glioma and recurrent or refractory pediatric central nervous system tumors	B7-H3	ICV	1	Recruiting	2019	NCT04185038
GD2 CAR T cells in diffuse intrinsic pontine gliomas (DIPG) and spinal diffuse midline glioma (DMG)	GD2	IV/ICV	1	Recruiting	2019	NCT04196413
C7R-GD2.CAR T cells for patients with GD2-expressing brain tumors (GAIL-B)	GD2	IV	1	Recruiting	2019	NCT04099797
CAR T cells after lymphodepletion for the treatment of IL13Rα2-positive recurrent or refractory brain tumors in children	IL13Ralpha2	ICV	1	Recruiting	2020	NCT04510051
Study of B7-H3, EGFR806, HER2, and IL13-zetakine (quad) CAR T cell locoregional immunotherapy for pediatric diffuse intrinsic pontine glioma, diffuse midline glioma, and recurrent or refractory central nervous system tumors	B7-H3, EGFR806, HER2, and IL-13-zetakine	ICV	1	Not yet recruiting	2023	NCT05768880
Leveraging chimeric antigen receptor-expressing T cells for children with diffuse midline glioma	GD2	IV	1	Recruiting	2023	ACTRN12622000675729

Disialoganglioside GD2

Disialoganglioside GD2 is an attractive CAR T cell target expressed on several solid and CNS tumors [33–36] while remaining largely absent or poorly expressed in healthy tissues [33,34,37]. Screening the cell surface of patient-derived DMG cells revealed high and uniform expression of GD2 in H3K27M-mutant cells, and dramatic efficacy of GD2-targeting CAR T cell therapy in preclinical models [38]. Thus, a Phase 1 clinical trial (NCT04196413) began evaluating intravenous (IV) infusion of autologous GD2 CAR T cell therapy, followed by optional repeated intracerebroventricular (ICV) dosing, for young adult and pediatric H3K27M-mutant DMG patients [39]. Preliminary data demonstrated radiographical and clinical benefits in three of four patients, including marked tumor reductions and neurological improvement; the one non-responsive patient in this initial report exhibited elevated immunosuppressive cytokines in cerebral spinal fluid (CSF), such as transforming growth factor β (TGF-β) [39]. The addition of ICV dosing improved or stabilized responses and correlated with significantly lower levels of cytokine release syndrome (CRS), promotion of proinflammatory cytokine/chemokines in the CNS, and decreased immunosuppressive myeloid cells detected in the CSF compared to IV infusions. Intratumoral inflammation can cause transient worsening of neurological symptoms attributable to the neuroanatomical location of the tumor, and intratumoral edema in structures such as the brainstem can transiently obstruct CSF flow and cause hydrocephalus. Such tumor inflammation-associated neurotoxicity (TIAN) [40] was seen in each of these four subjects to a variable degree, which was anticipated and managed with neurointensive measures [39]. These preliminary results hold promise for continued targeting of GD2 in DMG and other cancers [39,41,42], and multiple active clinical trials for DMG patients are currently under assessment (Table 2). Two additional Phase 1 clinical trials (NCT04099797, ACTRN12622000675729) are also currently recruiting DMG (and DIPG) patients as well as patients with other CNS tumors.

B7-homolog 3 protein (B7-H3)

B7-H3, also known as CD276, is also an attractive therapeutic target because of its overexpression across multiple cancers, including DMG [43–48]. In an open, single-site, Phase 1 clinical trial

(BrainChild-03; NCT04185038) evaluating B7-H3 CAR T cells in DMG (among other CNS tumors), CAR T cells were administered ICV on a repeated, outpatient, every-other-week dosing regimen, and the first three treated patients received a total of 40 doses [43]. Evaluation of cytokines in serum and CSF demonstrated increased immune engagement following localized infusions and may represent local on-target immune activation. Furthermore, circulating CAR T cells were detectable in the CSF of patients post-treatment, suggesting there is at least brief persistence in the CNS compartment. Serial proteomic evaluations in two patients were feasible and showed elevations in multiple immunomodulatory proteins, including CD14, CD163, CSF-1, and PD-L2, that may lead to suppression of CAR T cell activity [43]. Preliminary tolerability of intracranially delivered B7-H3 CAR T cells is consistent with two other brief reports [45,46] and holds promise for patients with DMG.

Quad CAR targeting: B7-H3, EGFR806, HER2, and IL-13Rα2

A world first Phase 1 quad-targeting CAR trial (BrainChild-04; NCT05768880) targeting B7-H3, HER2, EGFR806, and IL-13-zetakine (IL-13Rα2) recently opened for children and young adults (age 1–26 years) with DMG/DIPG patients as well as other R/R CNS tumors. Instead of a conventional mono-antigen targeting CAR, patients enrolled in this study will receive a CAR T cell product generated from pooled vector for a heterogeneous CAR T cell population expressing as many as four CARs by different subsets as a means to address tumor heterogeneity.

Epidermal growth factor receptor (EGFR)

The EGFR is a well-documented therapeutic target implicated in tumor progression [49,50] and is overexpressed in some DMGs [51]. Phase 1 clinical trials evaluating EGFR CAR T therapy against various tumors including HGG, lung, and pancreatic cancer have been shown to be safe and feasible with some clinical efficacy [52–54]. Non-pontine DMG patients have been enrolled in a Phase 1 trial (BrainChild-02; NCT03638167) targeting the cancer specific epitope EGFR806 [55]; however, clinical data have not yet been published. In a Phase 2 trial in adults with HGG (NCT01454596), patients demonstrated tolerable responses to EGFRvIII CAR T cells that were given ICV [53,56]. In this study, CAR T cells were detectable via qPCR and flow cytometry in all 10 patients; however, CAR T cells could not be detected in peripheral blood 1 month post-treatment. Encouragingly, there was no evidence of on-tumor/off-target (OT/OT) toxicities of IV infused EGFR CAR products that have documented CAR^+ trafficking to tumor sites [53]. Patients also presented with varying degrees of CRS and immune effector cell-associated neurotoxicity syndrome (ICANS), but did not display any evidence of OT/OT, which is consistent with other EGFR CAR therapies [56]. Post-treatment decreases in antigen density, infiltration of immunosuppressive regulatory T cells, and upregulation of inhibitory pathways played a significant role in stalling antitumor responses [53], which is also consistent with other EGFR-based therapeutic approaches [57].

ERBB2 receptor tyrosine kinase (HER2)

HER2 has been investigated as a therapeutic target for non-pontine CNS tumors in both pediatric and adult patients at progression (NCT03500991, NCT02442297), and demonstrated tolerability and safety [58,59]. Both studies utilized either ICV or tumoral cavity infusions using a fractionated/multi-infusion approach. The first study demonstrated an increase of proinflammatory molecules CXCL10, CCL2, G-CSF, GM-CSF, IFN-α2, IL-10, IL12-p70, IL-15, IL-1α, IL-6, IL-7, and TNF-α following treatment; however, CAR T cells were less frequently detected than in patients receiving B7-H3 CAR T cells [58]. There were notable increases in non-CAR T cell populations following infusions, which may suggest the ability of CAR T cells to influence immune engagement (directly or indirectly), but how tumor location, CAR T cell engineering, or prior therapy may affect intracranial CAR T cell persistence is still poorly understood. In the second study, the treatment was effective

in promoting tumor reduction, localized inflammation, and cytokine and chemokine signaling in responding patients (6 of 17 evaluable patients) and few to no OT/OT related toxicities. The median overall survival post-treatment was 11.1 months (95% CI, 4.1–27.2 months), and 24.5 months (95% CI, 17.2 and 34.6 months) post-diagnosis; however, no conclusions could be drawn on the overall survival benefit [59].

Interleukin 13 receptor subunit α2 (IL-13Rα2)

IL-13Rα2 is highly expressed in several CNS tumors including DMG [60,61] and is targetable through membrane-bound IL-13 fused to the intracellular CAR signaling domains [62]. Minimal expression of IL-13Rα2 is observed in healthy tissues, except for the testis, prompting the potential of therapeutically targeting IL-13Rα2 in DMG. A Phase 1 clinical trial (NCT04510051) for pediatric patients with IL-13Rα2-positive brain tumors is currently recruiting, with clinical data yet to be published. IL-13Rα2 directed CAR T cell therapy is also under investigation in adult patients with glioblastoma (NCT02208362) where the treatment regimen consists of multiple doses of IL-13Rα2 CAR, either ICV or intratumorally based on progression eligibility. Preliminary data for a single patient demonstrated a transient complete response including elimination of metastatic glioblastoma spinal tumors, clinical improvement, and discontinuation of adjuvant therapies [61,63]. This correlated with detectable levels of CAR T cells and proinflammatory cytokines in the CSF, although disease recurrence due to reduced IL-13Rα2 expression ultimately resulted in poor responses to subsequent infusions. Preliminary data has so far supported the feasibility and tolerability of IL-13Rα2 in glioblastoma with potential to be extended to DMG as a combinatorial target [61,63]. A second Phase 1 clinical trial (NCT04003649) using IL-13Rα2 CAR T in combination with monoclonal antibody (mAb) therapies nivolumab (PD1) and ipilimumab (CTLA-4) is currently underway; however, low expression of checkpoint proteins could present a limiting factor in DMG [15,17].

Therapy-related toxicities

Achieving a localized inflammatory response is an important component of the therapeutic efficacy of CAR T cell therapies, and is often an indicator of CAR T cell activation [64]. However, inflammatory responses and acute therapy-related toxicities can be harmful and life-threatening, especially in the context of CNS tumors where expansion of the compartment is not possible (Figure 2). Therefore, minimizing the incidence of toxicity or expertly managing it are crucial for the advancement of such cellular therapies.

On-target/off-tumor toxicities

OT/OT toxicities are an important consideration in the development of CAR T therapies (Figure 2A). B cell aplasia is a common OT/OT toxicity observed in CD19-directed CAR T cell therapies; however, it is effectively managed with IV immunoglobulin infusions. OT/OT toxicities have also been observed in some EGFR-directed CAR therapies in other cancer types [52,54], although there are only limited reports for DMG and other CNS tumors [39,53,58]. OT/OT toxicities can be mediated through single-chain fragment variable (scFv) antibody modifications that target cancer-specific epitopes or high tumor-associated antigen (TAA)-expressing cells [39,43,55,58]; however, it remains an important consideration when addressing tumor heterogeneity and identifying new TAAs.

Cytokine release syndrome

The onset of a potentially lethal CRS has been seen following various CAR T cell treatments, promoting a systemic inflammatory episode resulting in fever, rigors, and in severe cases, organ failure [62,64,65]. Importantly, the onset of CRS is heavily mediated through the production of the proinflammatory cytokines IL-6, IFN-γ, TNF-α, and IL-1, as well as inducible nitric oxide

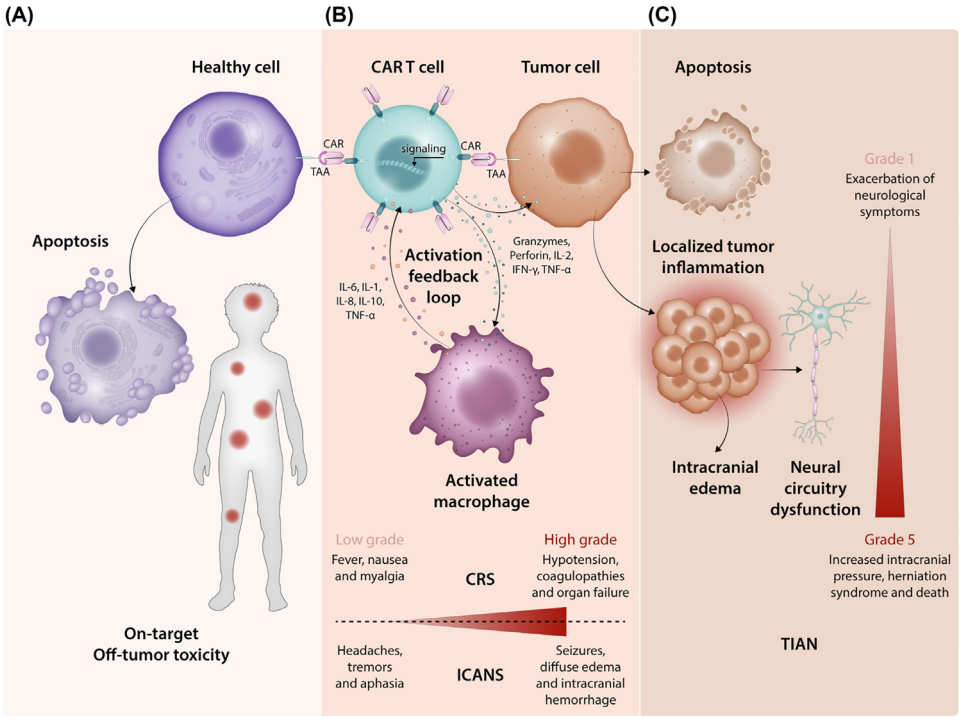

Figure 2. Chimeric antigen receptor (CAR) T cell therapy-associated toxicities. (A) On-target/off-tumor toxicity occurs when healthy cells express a tumor-associated antigen (TAA) on their surface and undergo apoptosis initiated by CAR T cells. (B) Following engagement with TAA, CAR T cells produce inflammatory signaling molecules including granzymes, perforin, IL-2, TNF-α, and IFN-γ that initiate cell recruitment and cytolysis of the cancer cells. Circulating macrophages become activated and produce their own inflammatory cytokines such as IL-6, IL-1, IL-10, and TNF-α, which creates an activation feedback loop. Increased production of IL-6 and IL-1 is implicated in the onset of systemic inflammatory episodes cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which is potentially lethal in severe cases. (C) Tumor inflammation-associated neurotoxicity (TIAN) occurs in patients with central nervous system (CNS) disease and results from localized inflammation of the tumor site, leading to symptoms secondary to increased edema, or from neural circuitry dysfunction. TIAN is graded according to changes in baseline deficits.

(Figure 2B) [65]. These are predominantly secreted by circulating myeloid cells, mainly macrophages, in response to CAR T cell activation, and are thought to induce an activation feedback loop (Figure 2B) [65–69]. As many as 90% of patients receiving IV CAR T cells experience some degree of low-grade CRS, whereas 27–47% may develop more severe symptoms [65]. It is therefore crucial to ensure that therapeutic efficacy and therapy-related toxicities are balanced and considered, particularly within the CNS compartment [64].

Immune effector cell-associated neurotoxicity syndrome

ICANS is another therapy-related toxicity seen in several IO approaches that can be associated with CRS or can develop independently [65]. Patients can exhibit an array of neurological symptoms including headaches, tremors, aphasia, and can also result in intracranial pressure (ICP), hemorrhage, and coma [65,70]. The pathophysiology of ICANS is not yet fully understood; however, it is suggested to involve a similar mechanism to CRS, resulting in damage to the BBB and, subsequently, stimulation of the systemic immune system and CNS microglia populations that drive a neurotoxic inflammatory response (Figure 2B) [65]. Notably, ICANS has not been reported as a frequent occurrence in DMG CAR T cell trials.

Tumor inflammation-associated neurotoxicity

TIAN is a potential new syndrome that can be experienced by patients with CNS malignancy and is distinct from CRS and ICANS. TIAN involves localized inflammation at the tumor site, resulting in critical local edema that poses risks of neuronal dysfunction, post-treatment pseudo-progression, increased ICP, herniation of tissue structures, and obstruction of CSF flow (Figure 2C) [40]. Grading of TIAN has been proposed but has not yet been prospectively described in pediatric CNS CAR T cell trials. Incorporation of individual neurologic changes, such as headache, cranial nerve palsies, or weakness, into the context of a broader syndrome – as has been done with CRS and other immunotoxic disorders – will hopefully better describe this distinct biologic indication and enhance toxicity comparisons across trials. In turn, this will aid in treatment paradigms that can be crafted to improve safety as more pediatric cellular therapy trials become available.

Although management protocols have been developed for CRS and ICANS, there may be variability in their effectiveness depending on the tumor location and across potentially distinct biologic syndromes such as TIAN [30,66]. As the field continues its rapid advance, multi-institutional collaborations will surely begin to describe the roles of immunosuppressive treatments that have been well described in patients with hematologic malignancies.

Future directions

Locoregional infusions and the TIME

Traditionally, CAR T cells have been administered as a single bolus IV infusion [31]. Repeated, fractionated, locoregional infusions have recently demonstrated tolerability and preliminary responsiveness across patients, while also abrogating severe systemic toxicity [39,43,58,71]. This is supported by lower levels of IL-6 and IL-1 following locoregional infusions compared to IV, lower serum cytokine levels compared to CSF, and a lower incidence of acute toxicities [39,58]. Furthermore, increases in immunosuppressive myeloid cells and TGF-β cytokines were greater in patients following IV infusions, suggesting that the infusion approach may impact on myeloid populations [39]. Therefore, it could be suggested that the immunologically cold TIME of DMG could positively (or not negatively) contribute to toxicity and tolerability, and this requires further investigation. DMG GAM populations may also play a role in CAR T cell efficacy that extends beyond therapy-related toxicities, and studies suggest that tumor-specific subsets can play distinct roles eliciting pro- and antitumor responses [72]. For instance, divergencies in the immunosuppressive TIME of pediatric DMG patients compared to adolescents and young adults also requires consideration [10]. Although microglia are the dominant brain-derived immune cells in pediatric DMG, macrophages predominate in adults [10], which may therefore influence CAR T treatment selection, antigen density, T cell fitness, and inflammatory responses [25,73,74]. Furthermore, patients who present lower absolute macrophage populations have shown greater durable responses than those who do not [75].

Effective T cell trafficking is reliant on an adequate native signaling network (CCL3, CCL3, IL-1A, IL-6) to recruit $CD8^+$ cytotoxic T cells to the tumor site [76], which is potentially problematic in the cold TIME of DMG. Locoregional infusions can effectively deliver CAR T cells directly to the tumor site, and in turn engage a localized inflammatory response capable of promoting immune cell infiltration [77–79]. The possible recruitment of immunosuppressive myeloid populations which may in turn inhibit CAR T efficacy is a factor requiring consideration as these pathways are investigated further.

Engineering CAR T strategies

Novel engineering strategies to improve the biological activity of CAR T cells may also benefit CAR T cell persistence and efficacy in DMG. Variations in CAR T cell persistence can stem from several

factors including CAR T cell design (Box 1), manufacturing procedures, T cell subsets, and the influence of the TIME [79–82]. Therapeutic efficacy is highly dependent on T cell fitness, which is characterized by the presence of exhaustion markers such as PD-1, TIM-3, CTLA-4, TIGIT, and LAG-3 [82]. The expression of these markers can result from constant activation of CAR signaling domains, mainly due to repeated exposure to its target antigen [79,83]. However, it can also be influenced by CAR T cell designs that promote tonic signaling [5]. For instance, CD28 costimulatory domains are typically more potent in terms of cytotoxic activity, but are more prone to exhaustion, whereas 4-1BB domains are associated with more long-term, sustained responses, with lower rates of T cell expansion and a reduced incidence of toxicities [2,4]. In the context of DMG, tumor burden, antigen density, and scFv affinity are important factors to consider in the design of CAR T cells because they will influence CAR T cell activation thresholds, and therefore the expression of exhaustion markers [2,4]. In addition, with the increased use of multi-fractionated dosing strategies, an interesting question is presented – what level of persistence is required in these settings? (see Outstanding questions).

Armored CAR designs aim to boost the function of CAR T cells by incorporating coexpressed immunomodulatory domains to improve cell persistence, cytotoxic capabilities, and/or the ability to positively influence the TIME. These can include additional secreted cytokines to create a more favorable TIME, chemokine receptors for greater tumor homing, and coexpression of additional ligands such as 4-1BBL and CD40L for engagement of other immune populations, as well as knockdown, or inhibition of exhaustion markers [2]. For instance, a second-generation GD2 CAR T cell coexpressing IL-15 demonstrated superior engraftment and antitumor control compared to a third-generation CAR T cell in mice with glioblastoma and DMG [7]. In addition, coexpression of IL-15 in CAR T cells used in the treatment of glioblastoma demonstrated greater antitumor responses and increased engagement of other immune entities, including $CD8^+$, NK, and B cells [84]. Furthermore, myeloid cells expressing IL-15Rα were simultaneously killed, thus negating their immunosuppressive signaling [84]. As such, an increased understanding of DMG GAM populations may elucidate similar engineering strategies that could be exploited for DMG and other CNS tumors.

Target selection and tumor heterogeneity

Although CAR T cell therapies have been successful in other cancers, tumor heterogeneity, and consequently the identification of pan-cancer antigens, remains one of the most crucial challenges. Studies evaluating CAR T cells across almost all cancer types have demonstrated loss of effectiveness owing to antigen escape or treatment-resistant populations arising from intratumor heterogeneity (Figure 3A). To address this, several novel strategies are being developed (Figure 3B). Coadministration of CD19- and CD22-directed CAR T cells has shown promising clinical efficacy in pediatric B-ALL patients with R/R disease [85]. Encouragingly, this combinatorial strategy has improved complete response rates and lowered the incidence of relapse compared to single-target CD19 CAR T cells [85]. Topically, the clinical introduction of quad-targeting CAR T cell therapy aims to extend the engineering limits of multi-antigen targeting.

Adaptor CAR technology is an emerging therapeutic strategy with potential in DMG and many other cancers (Figure 3C). In principle, it improves flexibility of CAR targeting through multiple adaptor molecules that bind to different TAAs and engage CAR T cells through a CAR-specific binding tag [86–89]. These adaptor molecules allow targeting of heterogeneous populations, while also serving as activation/inhibitory modulators to mediate acute toxic events [86–89]. Adaptor molecules may circumvent some barriers to the development of CAR approaches for DMG and other CNS tumors because they are typically no larger than an IgG molecule, and are thus BBB permeable [86]. Further preclinical assessment will be necessary to understand whether this approach can address DMG heterogeneity and the management of toxicities.

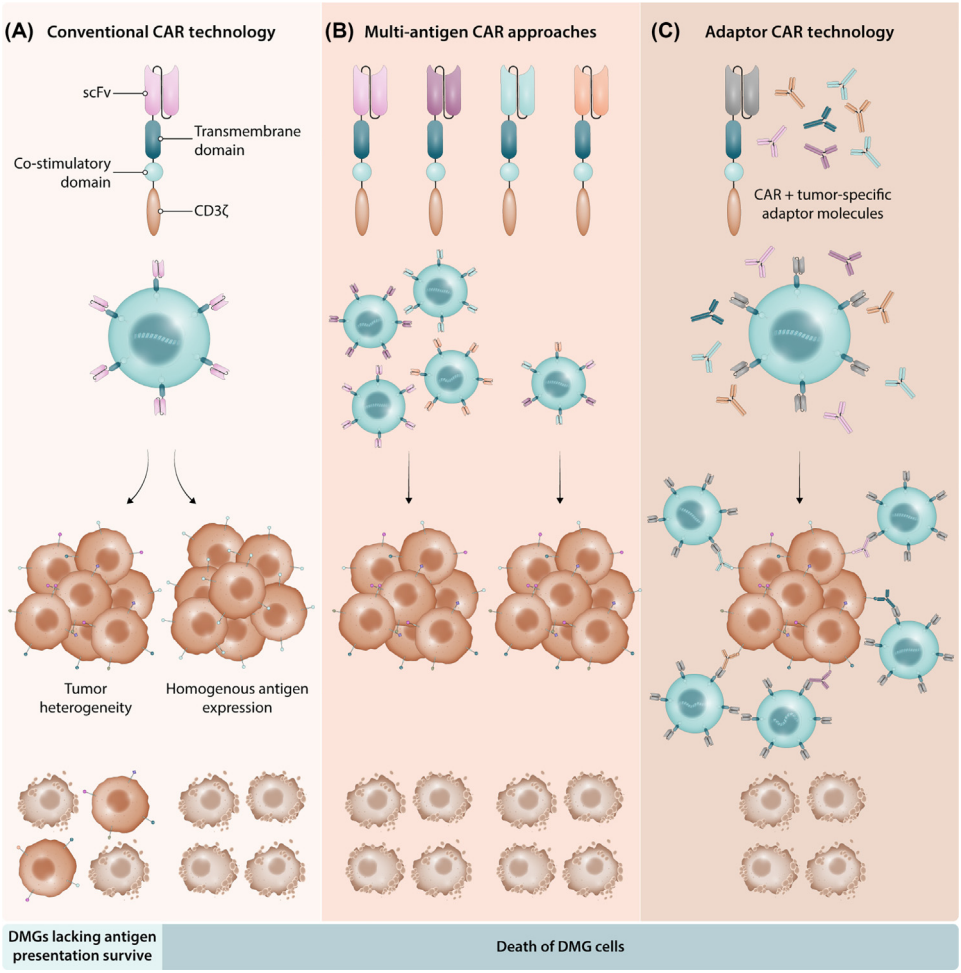

Figure 3. Chimeric antigen receptor (CAR) T cell design and treatment strategies to address tumor heterogeneity. (A) A conventional CAR comprises an antibody single-chain variable fragment (scFv) for antigen recognition, a transmembrane domain, a costimulatory domain, and a CD3ζ signaling domain. Activation of the CAR is initiated via recognition of a tumor-associated antigen (TAA) expressed on cancer cells and results in cell death. Heterogeneous expression of TAA can result in selection of antigen-negative populations and relapse. (B) Multi-antigen CAR approaches aim to address tumor heterogeneity by targeting two or more antigens simultaneously. This involves the production of multiple, mono-target conventional CAR products, or a single product designed to express multiple scFvs. (C) Adaptor CAR technology employs the use of antibody-like molecules that bind to TAA and engage CAR T cells through an adaptor molecule-specific tag. Adaptor CAR T cells can be used to control CAR T cell activity. Abbreviation: DMG, diffuse midline glioma.

Concluding remarks

Although these approaches hold promise for DMG, the limited accessibility of living tumor samples for TAA phenotyping and treatment stratification remains a significant challenge [17,24]. In addition, the production of multiple CAR constructs per patient introduces economic challenges given the financially demanding production costs associated with therapy development [90,91]. Importantly, several questions around the impact of the TIME and unique DMG GAM populations on CAR T cell efficacy remain unanswered, and the best CAR T strategies to overcome tumor heterogeneity and enhance CAR T cell persistence remain to be established (see Outstanding questions). Nonetheless, the novel developments in CAR T technology hold promise for the exploration of this strategy in cold TIMEs, particularly in DMG.

Outstanding questions

Can intracranially delivered CAR T cells induce sustained responses in children and young adults with CNS tumors, including DMG?

What degree of CAR T cell persistence is required in the CNS, and can repeated fractionated dosing overcome this barrier?

Are multi-antigen targeting engineering strategies sufficient to overcome tumor heterogeneity?

How can we more efficiently identify novel TAAs for CAR T cell development in DMG?

What other elements of the local immune TIME can influence antitumor effects, treatment resistance, and CAR T cell engagement?

Can CAR T cells be combined with other emerging therapeutics?

How can we most safely deliver intracranial CAR T cells to preserve quality of life while we aim for a cure?

How does divergent TIME in children versus adults with DMG affect CAR T cell selection and responses?

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Declaration of interests

M.D.D. is a parent to a child lost to diffuse intrinsic pontine glioma (DIPG) and is the founder and a Director of the not-for-profit charity RUN DIPG Pty Ltd. The other authors declare no conflicts of interest.

References

1. Vitanza, N.A. and Monje, M. (2019) Diffuse intrinsic pontine glioma: from diagnosis to next-generation clinical trials. *Curr. Treat. Options Neurol.* 21, 37
2. Aziz-Bose, R. and Monje, M. (2019) Diffuse intrinsic pontine glioma: molecular landscape and emerging therapeutic targets. *Curr. Opin. Oncol.* 31, 522
3. Theeler, B.J. *et al.* (2020) NCI-CONNECT: comprehensive oncology network evaluating rare CNS tumors – histone mutated midline glioma workshop proceedings. *Neurooncol. Adv.* 2, vdaa007
4. Gillespie, S. and Monje, M. (2018) An active role for neurons in glioma progression: making sense of Scherer's structures. *Neuro Oncol.* 20, 1292–1299
5. Monje, M. *et al.* (2011) Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. *Proc. Natl. Acad. Sci. U. S.A.* 108, 4453–4458
6. Nagaraja, S. *et al.* (2017) Transcriptional dependencies in diffuse intrinsic pontine glioma. *Cancer Cell* 31, 635–652
7. Nagaraja, S. *et al.* (2019) Histone variant and cell context determine H3K27M reprogramming of the enhancer landscape and oncogenic state. *Mol. Cell* 76, 965–980
8. Haag, D. *et al.* (2021) H3.3-K27M drives neural stem cell-specific gliomagenesis in a human iPSC-derived model. *Cancer Cell* 39, 407–422
9. Jessa, S. *et al.* (2022) K27M in canonical and noncanonical H3 variants occurs in distinct oligodendroglial cell lineages in brain midline gliomas. *Nat. Genet.* 54, 1865–1880
10. Liu, I. *et al.* (2022) The landscape of tumor cell states and spatial organization in H3-K27M mutant diffuse midline glioma across age and location. *Nat. Genet.* 54, 1881–1894
11. Hoffman, L.M. *et al.* (2018) Clinical, radiologic, pathologic, and molecular characteristics of long-term survivors of diffuse intrinsic pontine glioma (DIPG): a collaborative report from the International and European Society for Pediatric Oncology DIPG Registries. *J. Clin. Oncol.* 36, 1963–1972
12. Werbrouck, C. *et al.* (2019) TP53 pathway alterations drive radioresistance in diffuse intrinsic pontine gliomas (DIPG). *Clin. Cancer Res.* 25, 6788–6800
13. Findlay, I.J. *et al.* (2022) Pharmaco-proteogenomic profiling of pediatric diffuse midline glioma to inform future treatment strategies. *Oncogene* 41, 461–475
14. Jardim, D.L. *et al.* (2021) The challenges of tumor mutational burden as an immunotherapy biomarker. *Cancer Cell* 39, 154–173
15. Persson, M.L. *et al.* (2022) The intrinsic and microenvironmental features of diffuse midline glioma: Implications for the development of effective immunotherapeutic treatment strategies. *Neuro-Oncology* 24, 1408
16. Jackson, E.R. *et al.* (2023) ONC201 in combination with paxalisib for the treatment of H3K27-altered diffuse midline glioma. *Cancer Res.* Published online May 17, 2023. https://doi.org/10.1158/0008-5472.CAN-23-0186
17. Lieberman, N.A.P. *et al.* (2019) Characterization of the immune microenvironment of diffuse intrinsic pontine glioma: implications for development of immunotherapy. *Neuro-Oncology* 21, 83–94
18. Louis, D.N. *et al.* (2021) The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro-Oncology* 23, 1231–1251
19. Khuong-Quang, D.A. *et al.* (2012) K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol.* 124, 439–447
20. Sun, C.X. *et al.* (2023) Generation and multi-dimensional profiling of a childhood cancer cell line atlas defines new therapeutic opportunities. *Cancer Cell* 41, 660–677
21. Sun, H. *et al.* (2020) Specific TP53 subtype as biomarker for immune checkpoint inhibitors in lung adenocarcinoma. *EBioMedicine* 60, 102990
22. Cao, H. *et al.* (2020) Differential kinase activity of ACVR1 G328V and R206H mutations with implications to possible TβRI cross-talk in diffuse intrinsic pontine glioma. *Sci. Rep.* 10, 6140
23. Foster, J.B. *et al.* (2023) Translational considerations for immunotherapy clinical trials in pediatric neuro-oncology. *Neoplasia* 42, 100909
24. Williams, J.R. *et al.* (2020) Progress in diffuse intrinsic pontine glioma: advocating for stereotactic biopsy in the standard of care. *Neurosurg. Focus.* 48, E4
25. Lin, G.L. *et al.* (2018) Non-inflammatory tumor microenvironment of diffuse intrinsic pontine glioma. *Acta Neuropathol. Commun.* 6, 51
26. Plant, A.S. *et al.* (2018) Immunophenotyping of pediatric brain tumors: correlating immune infiltrate with histology, mutational

load, and survival and assessing clonal T cell response. *J. Neurooncol.* 137, 269–278
27. Gordon-Weeks, A. and Yuzhalin, A.E. (2020) Cancer extracellular matrix proteins regulate tumour immunity. *Cancers (Basel)* 12, 3331
28. Ashrafizadeh, M. *et al.* (2020) Damage-associated molecular patterns in tumor radiotherapy. *Int. Immunopharmacol.* 86, 106761
29. Wagner, L.M. and Adams, V.R. (2017) Targeting the PD-1 pathway in pediatric solid tumors and brain tumors. *OncoTargets Ther.* 10, 2097
30. Maude, S.L. *et al.* (2018) Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Engl. J. Med.* 378, 439–448
31. Cappell, K.M. and Kochenderfer, J.N. (2023) Long-term outcomes following CAR T cell therapy: what we know so far. *Nat. Rev. Clin. Oncol.* 20, 359–371
32. Marofi, F. *et al.* (2021) CAR T cells in solid tumors: challenges and opportunities. *Stem Cell Res Ther* 12, 81
33. Navid, F. *et al.* (2010) Anti-GD2 antibody therapy for GD2-expressing tumors. *Curr. Cancer Drug Targets* 10, 200–209
34. Nazha, B. *et al.* (2020) Disialoganglioside GD2 expression in solid tumors and role as a target for cancer therapy. *Front. Oncol.* 10, 1000
35. Golinelli, G. *et al.* (2020) Targeting GD2-positive glioblastoma by chimeric antigen receptor empowered mesenchymal progenitors. *Cancer Gene Ther.* 27, 558–570
36. Yu, J. *et al.* (2018) Anti-GD2/4-1BB chimeric antigen receptor T cell therapy for the treatment of Chinese melanoma patients. *J. Hematol. Oncol.* 11, 1
37. Gargett, T. *et al.* (2022) GD2-targeting CAR-T cells enhanced by transgenic IL-15 expression are an effective and clinically feasible therapy for glioblastoma. *J. Immuno Ther. Cancer* 10, e005187
38. Mount, C.W. *et al.* (2018) Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M+ diffuse midline gliomas. *Nat. Med.* 24, 572–579
39. Majzner, R.G. *et al.* (2022) GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. *Nature* 603, 934–941
40. Mahdi, J. *et al.* (2023) Tumor inflammation-associated neurotoxicity. *Nat. Med.* 29, 803–810
41. Straathof, K. *et al.* (2020) Antitumor activity without on-target off-tumor toxicity of GD2–chimeric antigen receptor T cells in patients with neuroblastoma. *Sci. Transl. Med.* 12, eabd6169
42. Del Bufalo, F. *et al.* (2023) GD2-CART01 for relapsed or refractory high-risk neuroblastoma. *N. Engl. J. Med.* 388, 1284–1295
43. Vitanza, N.A. *et al.* (2022) Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: preliminary first-in-2 human bioactivity and safety. *Cancer Discov.* 13, 114–131
44. Du, H. *et al.* (2019) Antitumor responses in the absence of toxicity in solid tumors by targeting B7-H3 via chimeric antigen receptor T Cells. *Cancer Cell* 35, 221–237
45. Tang, X. *et al.* (2020) Bioactivity and safety of B7-H3-targeted chimeric antigen receptor T cells against anaplastic meningioma. *Clin. Transl. Immunol.* 9, e1137
46. Tang, X. *et al.* (2021) Administration of B7-H3 targeted chimeric antigen receptor-T cells induce regression of glioblastoma. *Signal Transduct. Target. Ther.* 6, 125
47. Majzner, R.G. *et al.* (2019) CAR T cells targeting B7-H3, a pan-cancer antigen, demonstrate potent preclinical activity against pediatric solid tumors and brain tumors. *Clin. Cancer Res.* 25, 2560–2574
48. Haydar, D. *et al.* (2021) Cell-surface antigen profiling of pediatric brain tumors: B7-H3 is consistently expressed and can be targeted via local or systemic CAR T-cell delivery. *Neuro Oncol.* 23, 999–1011
49. Tomas, A. *et al.* (2014) EGF receptor trafficking: consequences for signaling and cancer. *Trends Cell Biol.* 24, 26–34
50. Uribe, M.L. *et al.* (2021) EGFR in cancer: signaling mechanisms, drugs, and acquired resistance. *Cancers (Basel)* 13, 2748
51. Li, G. *et al.* (2012) Expression of epidermal growth factor variant III (EGFRvIII) in pediatric diffuse intrinsic pontine gliomas. *J. Neurooncol.* 108, 395–402
52. Liu, Y. *et al.* (2020) Anti-EGFR chimeric antigen receptor-modified T cells in metastatic pancreatic carcinoma: a phase I clinical trial. *Cytotherapy* 22, 573–580
53. O'Rourke, D.M. *et al.* (2017) 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.* 9, eaaa0984
54. Zhang, Y. *et al.* (2021) Phase I clinical trial of EGFR-specific CAR-T cells generated by the piggyBac transposon system in advanced relapsed/refractory non-small cell lung cancer patients. *J. Cancer Res. Clin. Oncol.* 147, 3725–3734
55. Ravanpay, A.C. *et al.* (2019) EGFR806-CAR T cells selectively target a tumor-restricted EGFR epitope in glioblastoma. *Oncotarget* 10, 7080–7095
56. Goff, S.L. *et al.* (2019) Pilot trial of adoptive transfer of chimeric antigen receptor-transduced T cells targeting EGFRvIII in patients with glioblastoma. *J. Immunother.* 42, 126–135
57. Sampson, J.H. *et al.* (2010) Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *J. Clin. Oncol.* 28, 4722–4729
58. Vitanza, N.A. *et al.* (2021) Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis. *Nat. Med.* 27, 1544–1552
59. Ahmed, N. *et al.* (2017) HER2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma: a phase 1 dose-escalation trial. *JAMA Oncol.* 3, 1094–1101
60. Maggs, L. *et al.* (2021) CAR T cell-based immunotherapy for the treatment of glioblastoma. *Front. Neurosci.* 15, 535
61. Brown, C.E. *et al.* (2018) Optimization of IL13Rα2-targeted chimeric antigen receptor T cells for improved anti-tumor efficacy against glioblastoma. *Mol. Ther.* 26, 31–44
62. Rafiq, S. *et al.* (2020) Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat. Rev. Clin. Oncol.* 17, 147–167
63. Brown, C.E. *et al.* (2016) Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N. Engl. J. Med.* 375, 2561–2569
64. Chohan, K.L. *et al.* (2023) CAR-T cell therapy: the efficacy and toxicity balance. *Curr. Hematol. Malig. Rep.* 18, 9–18
65. Morris, E.C. *et al.* (2022) Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. *Nat. Rev. Immunol.* 22, 85–96
66. Giavridis, T. *et al.* (2018) CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat. Med.* 24, 731–738
67. Guo, H. *et al.* (2021) Focused evaluation of the roles of macrophages in chimeric antigen receptor (CAR) T cell therapy associated cytokine release syndrome. *Cancer Biol. Med.* 19, 333–342
68. Fischer, J.W. and Bhattarai, N. (2021) CAR-T cell therapy: mechanism, management, and mitigation of inflammatory toxicities. *Front. Immunol.* 12, 693016
69. Shimabukuro-Vornhagen, A. *et al.* (2018) Cytokine release syndrome. *J. Immunother. Cancer* 6, 56
70. Siegler, E.L. and Kenderian, S.S. (2020) Neurotoxicity and cytokine release syndrome after chimeric antigen receptor T cell therapy: insights into mechanisms and novel therapies. *Front. Immunol.* 11, 1973
71. Vitanza, N.A. *et al.* (2023) Locoregional CAR T cells for children with CNS tumors: clinical procedure and catheter safety. *Neoplasia* 36, 100870
72. Van Ginderachter, J.A. *et al.* (2019) Ontogeny of tumor-associated macrophages. *Front. Immunol.* 10, 1799
73. Glass, R. and Synowitz, M. (2014) CNS macrophages and peripheral myeloid cells in brain tumours. *Acta Neuropathol.* 128, 347–362
74. Hambardzumyan, D. *et al.* (2016) The role of microglia and macrophages in glioma maintenance and progression. *Nat. Neurosci.* 19, 20–27
75. Jain, M.D. *et al.* (2021) Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. *Blood* 137, 2621
76. Galeano Niño, J.L. *et al.* (2020) Cytotoxic T cells swarm by homotypic chemokine signalling. *eLife* 9, e56554
77. Sagnella, S.M. *et al.* (2022) Locoregional delivery of CAR-T cells in the clinic. *Pharmacol. Res.* 182, 106329
78. Nguyen, D.T. *et al.* (2022) CAR T cell locomotion in solid tumor microenvironment. *Cells* 11, 1974
79. Sterner, R.C. and Sterner, R.M. (2021) CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* 11, 69

80. Guedan, S. *et al.* (2019) Engineering and design of chimeric antigen receptors. *Mol. Ther. Methods Clin. Dev.* 12, 145
81. Shah, N.N. and Fry, T.J. (2019) Mechanisms of resistance to CAR T cell therapy. *Nat. Rev. Clin. Oncol.* 16, 372–385
82. Daniela, G.M.T. *et al.* (2021) Understanding T cell phenotype for the design of effective chimeric antigen receptor T cell therapies. *J. Immuno Ther. Cancer* 9, e002555
83. Liu, C. *et al.* (2021) Speed and location both matter: antigen stimulus dynamics controls CAR-T cell response. *Front. Immunol.* 12, 748768
84. Markella, Z. *et al.* (2023) IL15 modification enables CAR T cells to act as a dual targeting agent against tumor cells and myeloid-derived suppressor cells in GBM. *J. Immuno Ther. Cancer* 11, e006239
85. Romero, D. (2023) Promise with co-administration of CAR T cells. *Nat. Rev. Clin. Onco.* 20, 1
86. Arndt, C. *et al.* (2020) Adaptor CAR platforms – next generation of T cell-based cancer immunotherapy. *Cancers* 12, 1302
87. Ma, J.S.Y. *et al.* (2016) Versatile strategy for controlling the specificity and activity of engineered T cells. *Proc. Natl. Acad. Sci. U. S. A.* 113, E450
88. Sutherland, A.R. *et al.* (2020) Modular chimeric antigen receptor systems for universal CAR T cell retargeting. *Int. J. Mol. Sci.* 21, 7222
89. Nixdorf, D. *et al.* (2023) Adapter CAR T cells to counteract T-cell exhaustion and enable flexible targeting in AML. *Leukemia* 37, 1298–1310
90. Bishop, D.C. *et al.* (2018) PiggyBac-engineered T cells expressing CD19-specific CARs that lack IgG1 Fc spacers have potent activity against B-ALL xenografts. *Mol. Ther.* 26, 1883–1895
91. Choi, G. *et al.* (2022) Price and prejudice? The value of chimeric antigen receptor (CAR) T-cell therapy. *Int. J. Environ. Res. Public Health* 19, 12366
92. Wei, J. *et al.* (2019) Target selection for CAR-T therapy. *J. Hematol. Oncol.* 12, 62