CHAPTER TEN

Translational advancement of immunotherapeutics against pediatric central nervous system tumors

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Abstract

Pediatric central nervous system (CNS) tumors are the most common solid tumors in children and remain the leading cause of death amongst childhood cancer patients. Despite the intensity of standard cytotoxic regimens, many patients with high-grade tumors still experience relapse, at which time they have limited curative options. Even the survivors of childhood CNS tumors are often left with lifelong complications that negatively impact their quality of life. Immunotherapy holds the promise of tailored therapies that can improve outcomes and inflict fewer side effects. Early successes across against leukemia and some solid tumors have supported this promise, but this is only the infancy of targeted immunotherapies against pediatric CNS tumors. While this is a new, blossoming field, a robust and coordinated preclinical environment has spurred a spectrum of innovative clinical trials that serve as the ground floor for these new technologies. Here, we will review the current state of cellular therapy, immune checkpoint inhibition, cancer vaccines, and oncolytic viral therapy for children with CNS tumors.

1. Introduction

Pediatric central nervous system (CNS) tumors are the most common childhood solid tumors (Ostrom et al., 2021). Despite surgical advances and intensification of multimodal chemotherapeutic and radiation regimens, pediatric CNS tumors continue to be the leading cause of disease-related death among children and young adults (Pollack, Agnihotri, & Broniscer, 2019). A small portion of patients have seen improvements with the addition of novel molecularly-targeted agents (Fangusaro & Bandopadhayay, 2021). However, the prognosis for many children with high-grade tumors remains low, and some tumors such as H3K27M-mutated diffuse midline glioma (DMG) remain universally fatal even from diagnosis (Vitanza & Monje, 2019). For patients who achieve cure,

intensive conventional treatments can induce long-term toxicities including neurocognitive delays, endocrine disorders, ototoxicity, and secondary malignancies (Late Effects of Treatment for Childhood Cancer PDQR: Health Professional Version, 2002). Novel treatments that can provide a targeted approach for better clinical outcomes and less collateral damage are clearly needed.

Immunotherapeutics represent a broad class of agents ranging from immune effectors cells with direct cytolytic function to vaccines that induce endogenous immune activation against a tumor. While much of the recent experience has been in adults or for children with leukemia, dramatic clinical results have driven a surge of research in immune-based therapies for pediatric CNS tumors as well. Through rapid translational advancement from coordinated multi-national efforts, a range of innovative clinical trials have begun to explore the feasibility, tolerability, and preliminary clinical benefit of a range of immunotherapies. Much of the current experience is from early phase trials at single sites, but a willingness to exchange preliminary experiences and share ideas is fueling rapid translational success. Pediatric CNS tumors will have distinct obstacles compared to the story of immunotherapies against adult CNS tumors and against pediatric hematologic disease, but this story is now being written and quickly advancing to a new chapter of multi-site trials and a clearing understanding of efficacy. Here, we will assess components that make pediatric CNS tumors a distinct treatment challenge and take an inventory of the early clinical experience within the field of pediatric CNS immuno-oncology.

2. CAR T cells: introduction

Chimeric antigen receptors (CARs) are synthetic receptors that redirect T lymphocytes to recognize specific target antigens on cancer cells, bind them, and lyse them. The basic structure of a CAR consists of an extracellular binding domain, commonly a single-chain variable fragment (scFV), linked to a transmembrane region, spacer, and an intracellular CD3ζ domain. While they can be genetically engineered into a range of immune cells, the most common cell used is a T cell. First-generation CAR T cells were capable of recognizing target antigens independent of major histocompatibility complex (MHC) presentation. However, they were found to have limited efficacy due to inadequate signaling and limited

persistence (Brocker & Karjalainen, 1995). Second-generation CAR T cells engineered to have a co-stimulatory domain demonstrated enhanced cytotoxicity and proliferation and have since become the basis of a substantial portion of CAR T cell research (Krause et al., 1998). While third-generation CAR T cells also have been developed, their translational benefit over second gneration CAR T cells is unclear.

While CAR T cells have been deployed against a range of cancers, the most notable clinical success has been CD19-targeting CAR T cells for children with leukemia (Gardner et al., 2017; Maude et al., 2014). Considering the frequent ability to bridge children with leukemia to clinical trial, the clonal population of cells, and the fact that much of the cancer resides in the systemic circulation that CAR T cells can easily access, there were always destined to be challenges in translating this therapy into the relatively inhospitable and difficult-to-reach CNS. However, while often limited by the use of immune incompetent models, extensive preclinical investigations have demonstrated the ability to eliminate CNS tumors models both in vitro and in vivo (Ahmed et al., 2017; Ahmed et al., 2007; Donovan et al., 2020; Foster et al., 2022; Haydar et al., 2021; Majzner et al., 2022; Mount et al., 2018; Ravanpay et al., 2019; Vitanza et al., 2021; Vitanza et al., 2023). This robust preclinical experience, along with preliminary feasibility proven through both locoregional and systemic delivery of CAR T cells to adults has led to a flood of pediatric CNS CAR T cell trials.

3. CAR T cells: B7-H3 targeting

The immune checkpoint B7-H3 (CD276) is overexpressed in many pediatric tumors, including CNS tumors (Haydar et al., 2021; Majzner et al. 2019; Zhou et al., 2013). This potential therapeutic window led to the generation of B7-H3-targeting CAR T cells (B7-H3 CAR T cells) which consistently have been effective across a range of preclinical tumor models (Haydar et al., 2021; Majzner et al. 2019; Vitanza et al., 2023). The first clinical translation of B7-H3 CAR T cells against pediatric CNS tumors was in the first-in-human phase 1 clinical trial BrainChild-03 (NCT04185038), delivering repeatedly dosed intracranial B7-H3 CAR T cells at Seattle Children's (Vitanza et al., 2023). While the study has multiple arms, the published Arm C dedicated to children with diffuse intrinsic pontine glioma (DIPG) showed

that CAR T cells delivered introcerebroventricularly (ICV) were tolerable up to 10×10^7 cells per dose, with the most common side effects being fever, headache, nausea, and vomiting (Vitanza et al., 2025). The sole DLT was in a patient with progressive DIPG who experienced intratumoral hemorrhage following treatment at dose level 1 and was not seen again across 253 ICV doses. Of the 18 evaluable patients, 1 achieved a radiographic partial response while 15 had stable disease. The median survival was 19.8 months with three patients still alive more than 44 months from diagnosis – with two still receiving protocol therapy, including one that has received 81 total doses. Similar to other trials, CAR T cells were detectable in CSF in the majority of patients with cerebrospinal fluid (CSF) evidence of local immune activation (e.g. elevations in CXCL10, GM-CSF, and IFN-gamma post-infusion).

Another ongoing phase 1 trial delivering intracranial B7-H3 CAR T cells to children and young adults with DMG and other recurrent CNS tumors is currently enrolling at St. Jude's (Loc3CAR; NCT05835687). Preliminary reports described that the first 8 treated patients showed manageable tumor inflammation-associated neurotoxicity (TIAN) (Bertrand et al., 2024; Mahdi et al., 2023) with one case of severe neurotoxicity. Along with tolerability and survival, innovative correlative studies from this trial, including monitoring circulating tumor DNA levels in CSF and analyzing immune cells isolated from the CSF post-infusion, will hopefully shed further light on the activity of B7-H3 CAR T cells and their potential efficacy (Bertrand et al., 2024). Ultimately, the expansion of B7-H3 CAR T cell trials at multiple sites will provide important information regarding the manufacturing feasibility and clinical tolerability across programs as evidence for the advancement of these CAR T cells to planned phase 2 studies.

4. CAR T cells: EGFR targeting

Epidermal growth factor receptor (EGFR), another receptor for members of the EGF family, is commonly overexpressed in CNS tumors including EPN, GBM, and MB. An initial study of EGFRvIII-specific CAR T cells in adults with recurrent GBM demonstrated the feasibility and safety, but responses were hindered by subsequent tumor antigen loss (O'Rourke et al., 2017). Seattle Children's subsequently developed a distinct CAR utilizing the mAb806 scFv, providing the ability to bind both

the EGFRvIII variant as well as the overexpressed full-length EGFR that is not present on normal cells (Luwor et al., 2001; Ravanpay et al., 2019). A recently completed phase 1 study (BrainChild-02; NCT03638167) demonstrated safety and efficacy of locoregional delivery of multiple doses of EGFR CAR T cells in 4 children with recurrent or refractory EGFR-positive CNS tumors. Three of the 4 patients developed progressive disease, whereas, one patient with a spinal cord DMG had stable disease and subsequently achieved a complete response to conventional chemotherapy following CAR T cells (Gust et al., 2025). One challenge of this study was that many enrolled patients experienced rapid tumor progression while awaiting manufacturing of CAR T cells, precluding their ability to receive treatment. This obstacle is one common to the experience of other CAR T cell clinical trials for patients with aggressive tumor types due to the lag in treatment time between enrollment and subsequent treatment and highlights the need for development of more rapid manufacturing strategies and "off-the-shelf" CAR T cell products.

5. CAR T cells: GD2 targeting

GD2 is a diganglioside highly expressed on some tumors of neuroectodermal origin. GD2-targeting CAR T cells (GD2 CAR T cells) derived from the 14G2a scFv were first tested and showed therapeutic promise in children with neuroblastoma before their use was expanded to children with CNS tumors (Heczey et al., 2017). As DMG also highly express GD2, GD2 CAR T cells were deployed against preclinical patient-derived models and found to be highly effective (Mount et al., 2018). Notably, in vivo studies using patient-derived orthotopic xenograft tumors mice found fatal peritumoral inflammation so some initial clinical trials tailored inclusion and exclusion criteria to avoid this potential toxicity. The first clinical report of GD2 CAR T cells in children with CNS tumors was an interim analysis from Stanford University of a phase 1 trial testing escalating doses of second-generation 41BBz GD2-CAR T (NCT04196413) (Majzner et al., 2022). The initial report described 4 patients with DIPG or spinal DMG treated at the lowest dose level intravenously (IV) with GD2 CAR T (1×10^6 CAR T/kg); those with clinical benefit subsequently received additional ICV CAR T (30×10^6 CAR T). The group subsequently published a follow-up paper describing all patients treated in this initial study arm, including an

additional 8 patients treated at dose level 2 (2×10^6 CAR T/kg IV) (Monje et al., 2024). TIAN occurred in all treated patients but was manageable with supportive critical care interventions including CSF drainage, corticosteroids, and anti-cytokine medications. Though dose level 1 was tolerable, escalation of the IV dose resulted in 3 DLTs at dose level 2. Subsequently, some CAR T cell dosing was transitioned to ICV administration, which caused lower systemic toxicity and no DLTs. Of the 12 patients treated, 8 showed radiographic improvement, including 3 with substantial tumor reduction plus one ongoing complete response. Critically, neurologic improvements occurred in 9 of 11 patients. While a primary route of resistance to CAR T cell therapy for CNS patients has been proposed to be antigen-escape, GD2 antigen expression remained high even in progressing patients, suggesting antigen loss may not be the primary mechanism of resistance. Ongoing arms of the trial are continuing to evaluate ICV GD2 CAR T with or without preceding lymphodepletion, illuminating the potential role of augmenting the tumor immune microenvironment (TIME) for children with DIPG prior to CAR T cell therapy.

In a phase 1 trial conducted at Baylor College of Medicine, children with progressive high-grade GD2-positive CNS tumors were treated by systemic administration of autologous second-generation 41BBz GD2-specific CAR T or autologous GD2-CAR T co-expressing a constitutively active IL-7 receptor (C7R-GD2 CAR T) at doses of 1×10^7–3×10^7 cells/m^2 (GAIL-B; NCT04099797) following fludarabine (Flu) and cyclophosphamide (Cy) lymphodepletion (Lin et al., 2024). The C7R was designed to provide ongoing augmentation of CAR T function after activation by target antigen but without stimulation of bystander immune cells (Shum et al., 2017). Of the eleven treated patients, none experienced a DLT. Nearly all children treated with C7R-GD2 CAR T experienced grade 1 TIAN which was managed primarily with anakinra, an interleukin-1 receptor antagonist. The majority also developed cytokine release syndrome (CRS), with one patient experiencing grade 4 CRS. Treatment with C7R.GD2 CAR T cells resulted in transient improvement in neurologic deficits lasting 2–12 months, and 2 of 7 patients with DMG had a partial response. C7R.GD2 CAR T cell treated patients had increased circulating IL-6, IL-10, granzyme B, and IFN-gamma, suggesting improved immune activation of these cells. Post-mortem tumor analysis of one patient showed retained GD2 expression, again supporting that antigen escape may not be a primary resistance mechanism.

6. CAR T cells: HER2 targeting

HER2, a receptor for the epidermal growth factor (EGF) family, promotes proliferation and metastasis of cancer cells when activated. It is expressed in various solid tumors and is associated with poorer prognosis in some. Pediatric CNS tumors including ependymoma (EPN), GBM, and medulloblastoma (MB) may demonstrate HER2 surface overexpression, though at lower density than tumors with *HER2* gene amplification. Preclinical studies demonstrated that HER2-targeting CAR T cells (HER2 CAR T cells) could overcome low antigen density, showing antigen-specific killing of MB cell lines and efficacy in MB xenograft models (Ahmed et al., 2017).

In the first clinical trial to test HER2 CAR T against CNS tumors in the pediatric patients, Baylor College of Medicine used an FRP5 scFv-based second-generation CD28z HER2 CAR expressed on a virus specific T cell (VST) specific for CMV (HERT-GBM; NCT01109095) (Ahmed et al., 2017). Seventeen patients (10 adult, 7 pediatric) with progressive GBM were treated systemically with one or more doses of autologous HER2-CAR VSTs (1×10^6 to 1×10^8 cells/m^2) without lymphodepletion. There were no DLTs. One pediatric patient had a partial response lasting over 9 months, seven patients had stable disease lasting 8 weeks to 29 months, and eight patients had progressive disease. Three patients remained alive without progression at 24 months. While no significant expansion of the infused T cells was observed, they were detectable in peripheral blood up to 12 months post-infusion. A subsequent phase 1 study (iCAR; NCT02442297) tested locoregional delivery of escalating doses of HER2 CAR T, although results are not yet published. The group is now conducting an ongoing multi-site phase 1 trial (PBTC-059; NCT04903080) testing HER2 CAR T cells following lymphodepletion in children with progressive EPN.

The first pediatric intracranial CAR T cell trial in the United States was also investigating HER2 CAR T cells, specifically a second-generation 41BBz CAR T based on a trastuzumab-derived scFv (BrainChild-01; NCT03500991) (Vitanza et al., 2021). Published preliminary results of three heavily pre-treated patients with HGG or EPN receiving up to 9 doses of CAR T (1×10^7 to 2.5×10^7 cells/dose) revealed no DLTs and side effects including headache, localized spinal tumor site pain, and temporary worsening of pre-existing neurologic deficits consistent with the later described TIAN. Correlative studies revealed that CXCL10 and CCL2 cytokines were elevated in cerebrospinal fluid (CSF), along with an increased level of non-CAR T immune cells.

7. CAR T cells: IL-13Ra2 targeting

IL-13Ra2 is a membrane-bound protein overexpressed in multiple pediatric and adult CNS tumors. City of Hope has developed a CAR targeting this high affinity receptor by using an IL-13 mutein binding domain rather than a traditional scFv. These second-generation 41BBz CAR T cells have shown promise when administered locoregionally in adult clinical trials for progressive high-grade glioma (Brown et al., 2016). The group is now testing these cells in an ongoing pediatric trial using weekly intraventricular doses of autologous CAR T cells (Wang et al., 2023). The first 3 patients received treatment with CAR T cells alone, and the subsequent 3 received preceding Flu/Cy lymphodepletion. Preliminary reporting supports tolerability, with 3 of the 5 response-evaluable patients having transient disease stabilization or clinical improvement. Interestingly, single-cell analysis of CSF from these patients showed clonal expansion of CAR-negative T cells over time, suggesting activation and recruitment of endogenous T cells.

8. CAR T cells: multi-antigen targeting

To address antigen heterogeneity found within CNS tumors, multi-antigen targeting CAR T cells may be a focus of cellular therapy clinical trials in the future. Preclinical studies have shown that the proportion of cells targeted within a tumor by targeting multiple antigens is improved and that multivalent CAR T are able to effectively attack tumors expressing any or all targeted antigens (Bielamowicz et al., 2018; Hegde et al., 2016; Schmidts et al., 2023). At this time, BrainChild-04 (NCT05768880) is the only pediatric CNS trial delivering multi-antigen targeting CAR T cells – specifically, a quad-targeting product from pooled vectors that provides for the targeting of B7-H3, EGFR, HER2, and IL-13Ra2. There are no published results yet and treatment remains ongoing on two dose-escalating arms for DIPG and for children with recurrent/refractory CNS tumors.

9. CAR T cells: summary and future directions

Ultimately, early clinical studies of CAR T cells to treat pediatric patients with CNS tumors have shown feasibility and tolerability for both systemic and locoregional delivery, though patterns of variable toxicity

amongst target antigens or manufacturing strategies are emerging. Although radiographic responses have been somewhat limited, improvements in neurological symptoms and survival in some patients are encouraging. Enhancing tumor targeting through multivalent CAR T cells, co-targeting the TIME, or using of other synergistic combination therapies may improve outcomes further. Key questions remain about the optimal delivery methods and the role of lymphodepletion chemotherapy, which will likely require collaborative laboratory investigations with immunocompetent models to better inform future trials. While many questions remain and there are innumerable avenues to optimize these therapies, systemic and intracranial CAR T cells have been largely tolerable across a range of target antigens and in a spectrum of patients, supporting the advancement of these therapies.

10. Checkpoint inhibition: introduction

While many solid tumors have a complex TIME, the size of the CNS, its immune privilege, and the range of distinct CNS tumor pathologies that arise during childhood contribute to it being a unique and challenging anatomical compartment for immunotherapies. The immune landscape of pediatric CNS tumors is often broadly described as immunosuppressive or inert, prompting even greater challenges for effective immune-based therapies (Lieberman et al., 2019; Lin et al., 2018). These "immune cold" tumors exhibit limited infiltration of effector T cells while often harboring immunosuppressive cells, particularly myeloid-derived suppressor cells (MDSCs), including tumor-associated macrophages (TAMs) and microglia (Levine et al., 2024). MDSCs secrete inhibitory cytokines such as TGF-β and IL-10 that dampen T cell responses, fostering an environment that in many cases actively suppresses antitumor immunity (Sielska et al., 2013).

Pediatric CNS tumors also exhibit key immunological differences compared to their adult counterparts. Whereas adult GBM frequently shows higher PD-L1 expression and greater infiltration of exhausted T cells, pediatric tumors tend to have fewer adaptive immune components and are more reliant on innate immune suppression, such as from MDSCs and TAMs (Yao et al., 2023). Microglia, the resident immune cells of the CNS, play a dual role in this microenvironment, capable of both promoting and suppressing tumor growth depending on their activation state.

However, in many pediatric glioma, microglia adopt an immunosuppressive phenotype, releasing growth factors that support tumor progression while also preventing effective T cell infiltration (du Chatinier et al., 2023).

In addition to cellular immune suppression, the TIME undergoes profound metabolic and structural changes that further dampen immune responses. Hypoxia, a hallmark of aggressive brain tumors, fosters immune evasion by upregulating hypoxia-inducible factor 1-alpha (HIF-1α), which promotes the expansion of regulatory T cells and suppresses the function of cytotoxic lymphocytes (Feldman, 2024). Metabolic alterations, including increased lactate accumulation and glutamine metabolism, create an environment that is hostile to immune cell activity, leading to T cell exhaustion and dysfunction. Furthermore, these metabolic shifts can reinforce immunosuppressive signaling, suppressing effector T cell activation while promoting TAM polarization toward a pro-tumorigenic state (Wang et al., 2024).

Unlike many adult tumors, pediatric CNS tumors also tend toward lower tumor mutational burdens (TMB) (Patel et al., 2020), resulting in fewer neoantigens capable of stimulating a robust immune response. This lack of immunogenicity is further compounded by the presence of an intact blood-brain barrier (BBB), which restricts the trafficking of immune cells and systemically administered therapies into the tumor microenvironment. Additionally, the BBB can affect antigen presentation (Ramkissoon et al., 2017), potentially dampening the immune recognition of tumor cells. These barriers underscore the urgency of developing targeted immunotherapeutic strategies capable of overcoming tumor-induced immune suppression while effectively penetrating the CNS.

11. Checkpoint inhibition: established targets (PD-1, PD-L1, CTLA-4)

The immune system relies on a delicate balance between activation and inhibition to prevent autoimmunity while still effectively targeting malignant cells. Immune checkpoints such as programmed cell death protein 1 (PD-1), its ligand PD-L1 and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) serve as critical regulators of T cell activation and exhaustion. While CTLA-4 primarily functions in the early stages of T cell activation by competing with CD28 for binding to B7 molecules on antigen-presenting cells, PD-1 is more involved in later stages, suppressing

T cell responses in the tumor microenvironment (Wei, Duffy, & Allison, 2018). These inhibitory pathways can work in concert, with CTLA-4 preventing excessive T cell priming in lymphoid tissues and PD-1 limiting immune responses at the tumor site. Many tumors, including pediatric brain tumors, exploit these pathways by upregulating PD-L1, which may contribute to evasion of immune surveillance and induce T cell exhaustion.

Immune checkpoint blockade has been explored in pediatric CNS tumors, with PD-1, PD-L1, and CTLA-4 inhibitors being the most extensively studied. However, these therapies have shown limited efficacy in high-grade CNS malignancies, in part due to the highly immunosuppressive tumor microenvironment (Dunkel et al., 2023). Many pediatric glioma and MB express PD-L1 at variable levels, though often insufficiently to predict responsiveness to PD-1 inhibition alone (Gibney, Weiner, & Atkins, 2016). Furthermore, resistance mechanisms such as upregulation of additional inhibitory molecules or metabolic constraints on T cell function may further dampen checkpoint blockade efficacy. Alternative dosing schedules, such as neo-adjuvant approaches and novel immunotherapy combinations, continue to be investigated in ongoing pediatric clinical trials. Mismatch repair deficiency (MMRD) has emerged as the most promising biomarker for immune checkpoint blockade efficacy, particularly in HGG. While primary MMRD has historically been associated with poor outcomes in children with glioma, hypermutated glioma with MMRD exhibit significantly improved responses to immune checkpoint blockade compared with conventional chemoradiotherapy regimens (Das et al., 2023). In a recent large population-based study of children with primary MMRD and glioma, immune checkpoint blockade with the PD-1 inhibitor nivolumab and/or the CTLA-4 inhibitor ipilimumab was associated with a 60% increase in overall survival, (Negm et al., 2025).

12. Checkpoint inhibition: emerging targets (LAG-3, TIM-3, IDO)

Recent focus has shifted to lesser-studied immune checkpoints, such as LAG-3 and TIM-3, which are implicated in T cell exhaustion (Joller, Anderson, & Kuchroo, 2024). These molecules serve as additional layers of immunosuppression, limiting the effectiveness of PD-1/PD-L1 blockade (Tripathi et al., 2024). Studies in adult GBM suggest that dual inhibition

strategies may enhance antitumor responses, particularly in patients with pre-existing T cell infiltration (Huang et al., 2023).

One of these non-classical immune checkpoints, TIM-3 (*HAVCR1*), is widely expressed in different pediatric CNS tumors, including DMG (Ausejo-Mauleon et al., 2023; Tripathi et al., 2024), mainly within the TIME, primarily microglia and macrophages as these tumors are mostly devoid of T cells, and on the tumor cells. Targeting TIM-3 in preclinical DMG models led to promising survival, triggering a robust antitumor immune response and the induction of a long-lasting immune response mediated by the secretion of chemokines/cytokines that create a proinflammatory tumor microenvironment favoring a potent antitumor immune response (Ausejo-Mauleon et al., 2023). Further, clinical development has been hampered by the generalized poor results that TIM-3 monoclonal antibodies have yielded in early clinical trials for hematological diseases such as myelodysplasia and, thus, lack of interest in pharmaceutical companies to pursue further development of this molecule. Nevertheless, as more preclinical evidence emerges of the potential therapeutic benefit of targeting TIM-3 for pediatric/adult CNS tumors, a renewed spark for this target will open new opportunities.

Additionally, metabolic immune checkpoints such as indoleamine 2,3-dioxygenase (IDO) have demonstrated potential as modulators of antitumor immunity (Fujiwara et al., 2022). Indoximod, an IDO pathway inhibitor that, unlike traditional checkpoint inhibitors does not directly inhibit IDO enzymatic activity, disrupts tolerogenic signaling in antigen-presenting cells, has the potential to enhance T cell activation (Tang et al., 2021). Encouraging preliminary results a clinical trial testing indoximod showed prolonged survival in a subset of patients with recurrent pediatric CNS tumors, leading to the initiation of phase 2/3 trials (Johnson et al., 2024). Ultimately, given the complexity of immune evasion mechanisms within the CNS and the likely tailored approach for different tumor biologies, targeting both traditional and metabolic checkpoints may provide a more comprehensive approach to overcoming tumor immunosuppression.

13. Checkpoint inhibition: reversing the inhibition of immune clearance

Another method in which tumor cells evade immune clearance is by expressing "do not eat me" signals that inhibit phagocytosis by macrophages and other phagocytic cells. These signals can interact with

inhibitory receptors on phagocytic cells, preventing the engulfment and destruction of tumor cells (Feng et al., 2019). The most well-characterized "do not eat me" signal is CD47, which plays a critical role in immune evasion (Takimoto et al., 2019). It's interaction with signal regulatory protein alpha (SIRPα) on macrophages is responsible for initiating the inhibitory signaling pathway that prevents phagocytosis. Many CNS tumors, including HGG and DMG, exhibit high levels of CD47 expression, contributing to immune evasion and tumor progression (Huang et al., 2022; Jiang et al., 2024).

Therapeutic strategies targeting CD47 have demonstrated enhanced macrophage-mediated tumor clearance in preclinical glioma models. However, systemic CD47 blockade has been associated with significant off-target effects, including anemia and immune dysregulation, due to CD47's widespread expression on normal cells. To mitigate these toxicities, localized or tumor-specific delivery methods - such as nanoparticle-based CD47 inhibitors or bispecific antibodies that target both CD47 and tumor-associated antigens - are being developed to reduce systemic toxicity while maintaining therapeutic efficacy (Chao et al., 2019). Moreover, CD47 inhibition may inadvertently impair adoptive immune therapies, as macrophages can rapidly deplete engineered T cells, limiting the potential efficacy of combination approaches (Yamada-Hunter et al., 2024). Beyond CD47 blockade, multiple phagocytosis-inhibitory mechanisms serve as potentially impactful immunotherapy targets. For example, CD24 interacts with Siglec-10 on macrophages, leading to suppression of phagocytosis. Emerging evidence suggests that CD24 blockade can restore macrophage function and promote tumor cell clearance in preclinical models (Barkal et al., 2019). Additionally, MHC-I expression has been implicated in suppressing innate immune recognition of tumor cells by macrophages and natural killer (NK) cells, suggesting that tumors co-opt multiple pathways to evade destruction (Hu et al., 2024). PD-L1 and GD2 have also recently been demonstrated to function as "do not eat me" signals, supporting the idea that known immunotherapy targets may have additional previously unappreciated roles in immune evasion (Afzal et al., 2024; Theruvath et al., 2022). Combination therapies targeting multiple "do not eat me" signals may be required for effective macrophage-mediated tumor clearance in the face of complex interrelated TIME-related immunosuppressive mechanisms. While targeting any individual path of immune evasion may only provide limited clinical benefit in such aggressive and complex tumors,

targeting multiple mechanisms of immune evasion simultaneously may provide enhanced clinical benefit or synergistic immunotherapeutic effects.

Beyond immune checkpoint blockade and the inhibition of "do not eat me" signals, additional strategies targeting the TIME are being explored to enhance antitumor immunity in pediatric brain tumors. Given the distinct immunosuppressive landscape of these tumors - characterized by a lower TMB and unique immune evasion mechanisms – modulation of the TIME and metabolic microenvironment offers an alternative avenue for therapeutic intervention. One such approach involves targeting immunosuppressive cytokines, such as TGF-β, which play a pivotal role in maintaining an immune-excluded tumor state by suppressing cytotoxic T cell activity and promoting regulatory T cell (Treg) expansion. Given its broad immunosuppressive effects, TGF-β inhibition has been explored as an adjunct to immune checkpoint blockade, with early studies suggesting that dual blockade of TGF-β and PD-1 may enhance T cell infiltration and antitumor activity (Mariathasan et al., 2018). Additionally, TAMs contribute significantly to immune evasion by secreting pro-tumorigenic cytokines, such as CCL2 and IL-10, and by shaping metabolic conditions that inhibit adaptive immune responses. Strategies aimed at reprogramming TAMs from an immunosuppressive (M2-like) phenotype to a pro-inflammatory (M1-like) phenotype have shown promise in preclinical models. CSF1R inhibitors, which block macrophage colony-stimulating factor 1 receptor (CSF1R), have been investigated as a method to deplete pro-tumorigenic macrophages, though their efficacy as monotherapies has been limited. Combination approaches using CSF1R blockade with immune checkpoint inhibitors or metabolic modulators are currently under investigation to determine whether they can overcome resistance mechanisms and enhance immune-mediated tumor clearance (Zhang et al., 2024).

Furthermore, emerging evidence suggests that modulating metabolic pathways within the TIME, such as adenosine signaling and glutamate metabolism, may enhance immune responsiveness and improve the efficacy of immunotherapies. Adenosine accumulation in the TIME suppresses effector T cell activity while promoting Treg and MDSC expansion, leading to an immune-suppressive niche (Budhiraja et al., 2023). Adenosine receptor antagonists, particularly A2A receptor inhibitors, have demonstrated potential in reversing adenosine-mediated immunosuppression, particularly when used in combination with immune checkpoint inhibitors (Kutryb-Zajac et al., 2023). Similarly, metabolic dependencies

within the tumor microenvironment, such as increased glutamine metabolism, contribute to immune suppression by depriving T cells and dendritic cells of essential nutrients while fueling tumor growth. Glutaminase inhibitors, which block tumor-driven glutamine metabolism, have been investigated as a method to reshape the tumor microenvironment, enhancing the effectiveness of existing immunotherapies.

14. Checkpoint inhibition: summary and future directions

As the field advances, novel multimodal strategies combining immunotherapy, metabolic modulation, and microenvironment-targeting therapies will likely be required to overcome immune resistance and improve outcomes in pediatric brain tumors. More preclinical work is also required to understand the true dependency and translational impact of each of these immunogenic pathways, which likely vary on tumor biology and potentially between primary and metastatic disease. Finally, with the limited availability of systemic small molecule inhibitors and antibodies in the CNS, locoregional delivery of these therapeutics may need to expand either with intracerebroventricular or intratumoral delivery. The continued development of precision medicine approaches that account for the unique metabolic and immune characteristics of these tumors will be critical for enhancing treatment efficacy while minimizing toxicities.

15. Oncolytic viruses: introduction

Oncolytic virotherapy offers yet another promising field of immunotherapy. The concept behind this "not-so-new" approach is the utilization of either engineered or naturally occurring viruses to target and kill cancer cells while minimizing harm to healthy tissue. Oncolytic virotherapy dates back to the early 20th century when researchers observed that spontaneous viral infections sometimes led to the regression of certain cancers (Pelner, Fowler, & Nauts, 1958). In the 1950s, early trials demonstrated that injecting live viruses into patients with cancer could lead to tumor regression (Bierman et al., 1953). However, these experiments were met with a series of challenges, including immune system response, lack of viral specificity for cancer cells, and toxic adverse events (Kelly & Russell, 2007). It was not until the 1980's that the

development of virotherapy for CNS tumors gained traction when advances in molecular biology enabled scientists to engineer viruses that selectively target cancer cells, improving safety and efficacy. Oncolytic viruses (OVs) combine their tumor cell-killing ability with the capacity to trigger an anti-tumor immune response, thus harboring the potential to be synergistic with other therapeutic modalities already in use (Garcia-Moure et al., 2024). Multiple preclinical studies have highlighted the potential of using OVs as a treatment, demonstrating substantial tumor burden reduction and improved survival in animal models of glioma, MB, and other pediatric CNS cancers. Here, we will focus on advances in two of the most common approaches: herpes simplex virus (HSV) - the first virus approved by the FDA to treat cancer (Garcia-Moure et al., 2024) - and adenovirus.

16. Oncolytic viruses: preclinical efficacy

Herpes is a DNA virus with a robust cytotoxic and replication capacity and, importantly, is not integrative (Cripe et al., 2015). One study evaluated the HSV type I G207, which contains deletions in both copies of the neurovirulence gene γ134.5 and a disabling lacZ insertion within the ICP6 gene (Mineta et al., 1995). HSV-1 G207 proved to be safe when injected into the healthy cerebellum as well as in the developing brain (Bernstock et al., 2020; Radbill et al., 2007). In a separate study, HSV-1 G207 and M002 (encoding human interleukin-12) demonstrated potential efficacy in pediatric HGG (Friedman et al., 2018) and MB (Friedman et al., 2016). Similarly, HSV1716 (Seprehvir), demonstrated efficacy in preclinical studies of HGG and DMG via changes in cytoskeletal dynamics and molecular pathways related to cell polarity, migration, and movement (Cockle et al., 2017). HSV-1 rRp450, which expresses the rat CYP2B1 enzyme and activates the chemotherapeutic prodrug cyclophosphamide, prolonged overall survival in both ATRT and MB. The efficacy of HSV-1 rRp450 was further enhanced when cyclophosphamide was included in the treatment schedule (Studebaker et al., 2017). Altogether, these preclinical studies supported the translation of several HSV-1 variants to the clinical setting.

Adenovirus, another DNA (double-stranded) virus, has also been extensively researched as a potential therapy for CNS tumors. Specifically, the human adenovirus species C, serotype 5, has been a focus mainly due to its safety and easy manipulation in the lab (Garcia-Moure et al., 2024; Harrington et al., 2019). Delta-24-RGD was designed to specifically

replicate in and kill cancer cells due to the deletion of the Rb binding site (24 bp) in the adenoviral E1A protein, restricting the replication of this virus to cells with an aberrant Rb pathway (Fueyo et al., 2003). The native tropism of this virus was modified to infect preferentially through integrins (Fueyo et al., 2003). Delta-24-RGD has been evaluated in preclinical models of HGG and DIPG (Martinez-Velez et al. 2019; Martinez-Velez et al. 2019). Alonso and colleagues showed that Delta-24-RGD could infect, replicate, and improve the survival of immunodeficient and immunocompetent HGG and DIPG orthotopic models. This preclinical data served as the basis for a phase 1 trial for patients with newly diagnosed DIPG (NTC03178032), which will be discussed below.

VCN-01 is another adenovirus that also harbors a mutant Delta_E1A with a promoter that further restricts its replication to cancer cells. Additionally, VCN01 incorporates the hyaluronidase enzyme into its genome, allowing better distribution within the tumors (Rodriguez-Garcia et al., 2015). This virus extended the overall survival of mice bearing orthotopic PNET (Garcia-Moure et al. 2021) and had a good therapeutic effect in retinoblastoma (RB) models (Pascual-Pasto et al. 2019). Importantly, all the above viruses were delivered using direct injection or via a locoregional approach in RB models. Systemic delivery is a recognized challenge for OVs due to the risk of the virus being recognized and cleared by the innate and adaptive branches of the immune system. To address the hurdle, cellular carriers, such as mesenchymal stem cells (MSCs), have been evaluated. One example of this method is Celyvir, which incorporates ICOVIR-5, a previous version of the VCN-01 virus (Cascallo et al., 2007) loaded into MSCs. This method has also transitioned to early phase clinical trials (Gross et al., 2023). Similarly, the OV CRAd.S.pK7 was also loaded into MSC to facilitate its systemic delivery to the brainstem and to avoid its clearance by the immune system. However, the virus given alone failed to provide a therapeutic advantage in DMG preclinical models (Chastkofsky et al., 2021).

17. Oncolytic viruses: early clinical experience

Based on the promising preclinical studies, clinical trials were developed to evaluate the safety and efficacy of oncolytic virotherapy in pediatric patients. Several OVs, including HSV, adenovirus, poliovirus, and vesicular stomatitis virus (VSV), have already been tested in early-phase trials for pediatric CNS tumors.

One notable study involved using the engineered oncolytic HSVG207 in children with recurrent GBM. In this trial, the virus was administered directly into the tumor tissue. Results showed the treatment was well-tolerated, leading to possible efficacy in some patients although the overall survival did not appear to be substantially different from historical controls (Friedman et al., 2021). In the context of DIPG, Delta-24-RGD was evaluated in a phase 1 trial where the virus was administrated intratumorally to newly diagnosed patients and followed by radiotherapy. The data was encouraging, with the virus demonstrating a very safe profile with few adverse effects. The study had encouraging results with a median OS of 17.8 months and two long-term survivors (2/12) alive more than 5 years after viral injection (Gallego Perez-Larraya et al., 2022). The poliovirus PVSRIPO, an RNA virus, was evaluated in a phase Ib clinical trial for pediatric recurrent high-grade gliomas (NTC03043391). Interestingly, in this trial, the virus was delivered using convection-enhanced delivery (5×10^7) to 8 patients. The trial demonstrated that this method was overall safe, however some patients required bevacizumab to help manage post therapy inflammation (Thompson et al., 2023). Another RNA virus, the reovirus Reolysin (pelareorep) was evaluated in a phase 1 clinical trial for recurrent or refractory high-grade brain tumors (NTC02444546) in combination with GM-CSF (Schuelke et al., 2022). Unfortunately, this trial was closed due to both adverse effects and a lack of improvement in outcome.

18. Oncolytic viruses: early clinical experience

Ongoing research is needed to continue to optimize the delivery, efficacy, and safety of oncolytic virotherapy. Preexisting neutralizing antibodies pose a challenge for systemic delivery of OVs, and thus, cell carriers such as neural stem cells or MSCs are being assessed. Celyvir was the first in-child trial of autologous MSCs carrying the OV ICOVIR-5 in patients with advanced tumors, including MB and DMG (Ruano et al., 2020). Another strategy involves the use of stealth viruses, which are coated with different formulas and have the capacity to hide while they transition to the tumors. Additional research is focusing on overcoming immune resistance through the combination of oncolytic viruses with immune checkpoint activators or immune checkpoint inhibitors, such as anti-PD-1/PD-L1 therapies. Similarly, a Delta-24-RGD virus armed with the

positive immune modulator CD137L was shown to be superior to the parental virus in DMG models (Laspidea et al. 2022) This combination may help overcome immune suppression within the tumor microenvironment, allowing the body's own immune system to better recognize and attack cancer cells. Again, a better understanding of the TIME will allow us to develop improved viruses that can express molecules that support a proinflammatory ecosystem. Other important factors that should be considered and that were not be discussed here include viral immunodominance, innate immunity, etc.

Over the past decade, oncolytic virotherapy has finally been translated from the lab to the clinic for pediatric CNS tumors. OVs have proven safe in the clinic and have demonstrated both a positive antitumor effect and lack of toxicity. However, several hurdles remain in optimizing this therapeutic modality, including optimal viral delivery and overcoming immune responses, to become a meaningful curative therapy. Both preclinical and clinical studies suggest that oncolytic virotherapy holds great potential as an adjunctive or primary treatment for these devastating cancers. With continued research, collaboration, and innovation, OVs could soon become an integral part of the therapeutic arsenal against pediatric CNS tumors, offering new hope to children and their families facing these life-threatening conditions.

19. Vaccines: introduction

In situ vaccination with Coley's toxins elicited objective responses and evidence of systemic immunotherapeutic response nearly 150 years ago (McCarthy, 2006). Yet today, clinical advancement of anti-cancer vaccines could be characterized as lagging behind adoptive cellular therapies and immune checkpoint inhibitors. While prophylactic vaccines confer extraordinarily successful protection against viral mediated cancers such as hepatitis B, and human papilloma virus, therapeutic cancer vaccines have not shown ability to demonstrate rapid polyclonal T cell responses necessary to overcome intratumoral heterogeneity and immunosuppression (Alizadeh et al., 2015). Development of novel mRNA-based vaccines promise to overcome these hurdles given their unique ability to be customized for maximal expression and immunogenicity. These features coupled with their amenability to translation and iteration can be leveraged to overcome grand challenges in the treatment of refractory brain tumors.

RNA is customizable for payload packaging into nanoparticle, usually lipid based or can be leveraged to deliver antigen presenting signals for dendritic cells and CAR T cells (Mendez-Gomez et al., 2024; Sayour et al., 2017). The ability to deliver a myriad of personalized targets in a single payload that is simultaneously amenable to commercialization are significant advantages of mRNA-based therapy (Falcone & Andrews, 1991; Hornung et al., 2006; Rodgers, Wang, & Kiledjian, 2002; Sayour et al., 2018; Schmidt et al., 2009). RNA can be customized for modulation of the innate immune system through engineering the 5' and 3' ends, through unique cap designs, internal ribosomal entry sequences, untranslated regions to promote translation or immunogenicity and polyAAA length or type (e.g. polyUUU) (Bradrick, Walters, & Gromeier, 2006; Bung et al., 2010; Conry et al., 1995; Holcik & Liebhaber, 1997; Holtkamp et al., 2006; Kore & Shanmugasundaram, 2008; Rodgers, Wang, & Kiledjian, 2002; Yu & Russell, 2001). Coupled with ability to modify coding regions for silenced/ unsilenced antigens and tandem payload delivery of mRNAs delivering immunomodulatory signals (i.e. mRNAs encoding for cytokines, chemokines, co-stimulatory molecules, and antibodies) or siRNA/ shRNA targeting immunoregulatory axes, mRNA vaccines can be customized in a near infinite, and personalized, manner (Broos et al., 2016; Kariko et al., 2005).

20. Vaccines: CNS tumor targeting

In CNS tumors, initial work has largely involved dendritic cell therapies presenting mRNA antigens. In a landmark study, Mitchell and colleagues demonstrated that RNA loaded DC vaccines encoding for CMV matrix protein pp65 (tumor associated antigen expressed in GB) could substantially improve median and long-term survival outcome in patients with primary GBM (Mitchell et al., 2015). Notably, this study utilized tetanus toxoid during intradermal DC vaccinations, which was shown to elicit a CD4 memory recall response in draining lymph nodes that facilitated mRNA loaded DC migration in a CCL3 dependent manner (Mitchell et al., 2015). Follow up studies confirmed promising findings, and several additional trials have been conducted and planned for other refractory brain tumors including MB and DIPG. In these studies, instead of single antigen targeted, mRNA is manufactured from whole tumor derived transcriptome from patient derived biopsies (Nair et al., 2015). From these biopsies, total RNA is extracted from

patient tumors, which contains ribosomal, transfer and messenger RNA, although the latter only makes up a tiny fraction. Total RNA then undergoes reverse transcription (RT)-PCR to create a cDNA library which can be in vitro transcribed and amplified into a purified mRNA product representing the entire brain tumor transcriptome. This approach has been shown to elicit hyperexpanded T cell receptor (TCR) clonotypes in patients with MB undergoing treatment with mRNA loaded DC vaccines and ex vivo activated T cells (activated ex vivo following culture with mRNA loaded DCs) (Flores et al., 2019).

Recently, one of the first reports on direct mRNA vaccine therapy in brain tumors was published (Mendez-Gomez et al., 2024). In a small cohort of 4 patients with GBM, mRNA wrapped into multilamellar lipid particle aggregates (LPAs) elicited rapid modulation of the brain tumor microenvironment in tandem with systemic activation of DC and T cells. These RNA-LPAs were administered intravenously to artificially mimic systemic viral infection against mRNA payloads encoding for pp65 and personalized total tumor mRNA. In all patients, there was evidence of T cell responses against tumor associated antigens expressed in GBM. In one patient, there was significant enhancement thought to be fulminant tumor recurrence, but upon biopsy there were no tumor cells, but rather reactive gliosis and immune cells (Mendez-Gomez et al., 2024).

21. Vaccines: summary and future directions

While findings are early, they represent promise that the tumor induced immunosuppression can be rapidly overcome with therapeutic mRNA cancer vaccines while engendering polyclonal T cell responses against TAAs.

22. Conclusions

Novel and effective therapies are urgently needed to help improve outcomes and the quality of life of children with CNS tumors. Immunotherapy has transformed the way many adults with various cancers are treated; however, the signal for its use in pediatric CNS malignancies is still emerging. While early research studies have shown that several strategies are feasible, safe, and tolerable, their efficacy in comparison to the

current standards of care must still be evaluated in the majority of cases. These therapies also still require substantial optimization to overcome key barriers including the unique heterogeneity of pediatric CNS tumors across different biological subgroups as well as within individual tumors. Delivery also continues to be a critical obstacle as many promising therapeutics simply do not reach their targets in the required concentrations or augment the TIME for the required length of time. It is almost certain that different therapeutics will have variable optimal delivery routes, ranging from systemic, to intratumoral, to intracerebroventricular. Furthermore, key questions remain including the utility of lymphodepleting chemotherapy prior to immunotherapy, how the unique tumor immune microenvironment affects efficacy, and which combination of therapies may improve outcomes. Improved preclinical models that have evolved from the admittingly lacking orthotopic xenograft approach will be important, as will the invaluable correlative biospecimens obtained from the courageous patients enrolling on these often first-in-human clinical trials. Deliberate evaluation of immunocorrelatives and refining of neuroimaging guidelines for evaluation of immune based therapies for CNS tumors will be critical to maximizing what we learn from each clinical trial and how we benefit immediate, as well as future, patients. Importantly, close collaborations between scientists and clinical researchers across the globe, are essential for the development of pediatric CNS tumor immunotherapies to advance at the pace our patients demand and deserve.

23. Competing interests statement

E.J.S. is an inventor on issued and pending patents related to mRNA vaccines and cancer immunotherapy, some of which are licensed to iOncologi, Inc. E.J.S serves on the advisory boards of NTX (Nature's Toolbox) and Siren Biotechnology. M.M.A is an inventor on issued and pending patents related to oncolytic viruses. N.A.V. holds equity in and serves as the Scientific Advisory Board Chair for BrainChild Bio, Inc. N.A.V. is also an inventor on issued and pending patents related to CAR T cell therapies. T.B.D. is a consultant for Regeneron.

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