

Translational considerations for immunotherapy clinical trials in pediatric neuro-oncology



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ABSTRACT

While immunotherapy for pediatric cancer has made great strides in recent decades, including the FDA approval of agents such as dinutuximab and tisagenlecleucel, these successes have rarely impacted children with pediatric central nervous system (CNS) tumors. As our understanding of the biological underpinnings of these tumors evolves, new immunotherapeutics are undergoing rapid clinical translation specifically designed for children with CNS tumors. Most recently, there have been notable clinical successes with oncolytic viruses, vaccines, adoptive cellular therapy, and immune checkpoint inhibition. In this article, the immunotherapy working group of the Pacific Pediatric Neuro-Oncology Consortium (PNOC) reviews the current and future state of immunotherapeutic CNS clinical trials with a focus on clinical trial development. Based on recent therapeutic trials, we discuss unique immunotherapy clinical trial challenges, including toxicity considerations, disease assessment, and correlative studies. Combinatorial strategies and future directions will be addressed. Through internationally collaborative efforts and consortia, we aim to direct this promising field of immuno-oncology to the next frontier of successful application against pediatric CNS tumors.

Introduction

The immune system has long garnered attention as a potentially powerful ally in the fight against cancer. Recent decades have seen significant advances in the clinical implementation of immunotherapy for a

wide range of tumors, including pediatric cancers. However, these advances are only beginning to reach children with central nervous system (CNS) tumors, the deadliest pediatric cancers. The initial clinical implementation of immunotherapeutics against CNS tumors has been hampered by unique considerations forced by the clinical, biologic, and

Abbreviations: CNS, central nervous system; PNOC, Pacific pediatric neuro-oncology consortium; OV, oncolytic virus; FDA, Food and Drug Administration; HSV-1, herpes simplex virus 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; TME, tumor microenvironment; HGG, high-grade glioma; DIPG, diffuse intrinsic pontine glioma; DMG, diffuse midline glioma; ATRT, atypical teratoid rhabdoid tumor; GBM, glioblastoma; IL, interleukin; ACT, Adoptive cellular therapy; ICI, immune checkpoint inhibitor; ctDNA, circulating tumor-DNA; miRNAs, microRNA; exoDNA, exosomal-DNA; CTC, circulating tumor cell; CSF, cerebral spinal fluid; TMB, tumor mutational burden; ddPCR, digital droplet PCR; sPD-L1, soluble PD-L1; sB7-H3, soluble CD276; SCFA, small chain fatty acids; TIL, tumor infiltrating lymphocytes; MDSC, myeloid derived suppressor cell.

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Table 1
Immune based clinical trials through the Pacific Pediatric Neuro-Oncology Consortium (PNOC).

Trial #	Study Name	NCT
PNOC005	A phase 1 study of modified measles virus for the treatment of children and young adults with recurrent medulloblastoma or recurrent atypical teratoid rhabdoid tumors (ATRT)	NCT02962167
PNOC007	H3.3K27M Specific Peptide Vaccine Combined with poly-ICLC and Nivolumab for the Treatment of newly diagnosed HLA-A2 (02:01)+ H3.3K27M Positive Diffuse Intrinsic Pontine Glioma (DIPG) and newly diagnosed HLA-A2 (02:01)+ H3.3K27M Positive Midline Gliomas	NCT02960230[14]
PNOC013	A Safety and Pharmacokinetic Study of Single Agent REGN2810 in Pediatric Patients with Relapsed or Refractory Solid or Central Nervous System (CNS) Tumors and a Safety and Efficacy Trial of REGN2810 in Combination with Radiotherapy in Pediatric Patients	NCT03690869
PNOC018	Genetically Modified Cells (KIND T Cells) for the Treatment of HLA-A*0201-Positive Patients With H3.3K27M-Mutated Glioma	NCT05478837
PNOC019	A Randomized, Double-Blinded, Pilot Trial of Neoadjuvant Checkpoint Inhibition followed by Combination Adjuvant Checkpoint Inhibition in Children and Young Adults with Recurrent or Progressive High Grade Glioma (HGG)	NCT04323046
PNOC020	A Study of RNA-lipid Particle (RNA-LP) Vaccines for Newly Diagnosed Pediatric High-Grade Gliomas (pHGG) and Adult Glioblastoma (GBM)	NCT04573140
PNOC025	A Phase 1 Study of Magrolimab in Children and Adults with Recurrent or Progressive Malignant Brain Tumors	NCT05169944
PNOC028	A Phase 1 Study of Intra-Tumoral Injections of Ex Vivo Expanded Natural Killer Cells in Children and Young Adults with Recurrent or Progressive Supratentorial Malignant Brain Tumors	Not available
PNOC029	Nivolumab and DAY101 for the treatment of newly diagnosed or recurrent craniopharyngioma in children and young adults	NCT05465174

immunosuppressive features of solid tumors in the brain and spine. This following review from the immunotherapy working group within the Pacific Pediatric Neuro-Oncology Consortium (PNOC) and Children's Brain Tumor Network (CBTN) focuses on highlighting themes on translation of immunotherapies to clinical trials for pediatric CNS tumors. We review recent trials and advances in oncolytic viruses, vaccines, adoptive cell therapy, and checkpoint inhibition, followed by a discussion of important aspects in trial development, including toxicity, correlative studies, and combinatorial opportunities.

The immunotherapy landscape in pediatric CNS tumors

Oncolytic viruses

Oncolytic viruses (OVs) represent a unique type of anticancer agents at the interface of biological- and immunotherapies. The field achieved a milestone in 2015 with the approval by the US Food and Drug Administration (FDA) of the herpes simplex virus 1 (HSV-1) expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) named T-VEC (talimogene laherparepvec) for the treatment of advanced melanoma [1]. More recently Teserpaturev, a triple-mutated HSV-1 OV, has been approved in Japan for the treatment of malignant glioma in adults [2]. OVs employ either naturally occurring viruses that take advantage of the molecular makeup of the cancer cells or viruses modified in the laboratory to achieve cancer specificity. OVs have the ability to convert tumors from immunologically 'cold' to 'hot' through induction of proinflammatory pathways within the tumor microenvironment (TME) [3]. This characteristic makes them very attractive for treating pediatric CNS tumors considering their low tumor mutational burden (TMB) and paucity of infiltrating immune cells. In preclinical studies, adenoviruses and herpes viruses are the most extensively studied, demonstrating tolerability and efficacy in different models including atypical teratoid rhabdoid tumor (ATRT), diffuse intrinsic pontine glioma (DIPG), high-grade glioma (HGG), and medulloblastoma [4–7]. Most of these approaches utilize intratumoral administration rather than systemic, with viral carriers such as neural or mesenchymal cells also evaluated [8]. Recent trials using either the HSV-1 G207 for patients with recurrent or progressive HGG (NCT02457845) [9] or DNX-2401 for newly diagnosed DIPG (NCT03178032) [10] reported safety and a degree of efficacy across both studies. Additional viruses are also being used in ongoing and recent trials including modified measles virus (PNOC005, NCT02962167; Table 1), as well as modified polio and rhinovirus viruses (PVSRIPO, NCT03043391). Refinements aimed at enhanced efficacy may be achieved through combinatorial therapeutic partners, repetitive intratumoral dosing regimens, or improved potency

of the virus (e.g. armed with ligands that further drive the immune system) [11].

Vaccine therapies

Cancer vaccines have had a long history since Coley's toxins in the late 1800's but have yet to be fully realized against CNS tumors. Peptide vaccines have been traditionally employed as a source of antigenic material to induce response in malignant CNS tumors, but tumor specific antigen targeting remains challenging. EGFRVIII, a tumor specific antigen, has been utilized as a peptide target (conjugated to keyhole limpet hemocyanin) in adult glioblastoma (GBM) but did not meet efficacy goals in a phase III study [12]. Multiple overexpressed antigens (e.g. survivin, EphA2, interleukin (IL)-13 receptor- α 2, YKL-40, and gp100) are also being leveraged as therapeutic targets with promising activity [13]. PNOC utilized an H3.3K27M specific peptide vaccine coupled with polyinosinic:polycytidylic acid (poly(I:C)) for HLA-A2 restricted patients with DMG (PNOC007, NCT02960230; Table 1). While overall survival was similar to historical controls, a subset of patients with expansion of vaccine-induced specific cytotoxic T cells had a superior median survival (>16 months) [14]. This report included characterization of peripheral PD-1+ CD8+ T cells that were exhausted following the vaccine, suggesting potential efficacy from a combination with anti-PD-1 therapy and spurring the addition of nivolumab to the clinical trial (PNOC007, NCT02960230; Table 1). Alternatively, some studies have used autologous cellular vaccines targeting total tumor material using irradiated cells [15], cell lysate [16], and whole tumor mRNA [17]. Vaccine trials using mRNA loaded dendritic cells are underway in medulloblastoma (Re-MATCH trial; NCT01326104), HGG (ACTION trial; NCT03334305), and DIPG (NCT03396575). While promising, HLA-restricted epitopes make development of peptide vaccines for the general population challenging, which can be especially limiting for relatively rare diseases such as pediatric CNS tumors. However, mRNA vaccines in lipid-nanoparticles could be available to all patient haplotypes, tailored for flexibility in commercialization, and available for rapid deployment. These systems have been piloted against adult GBM, but are now underway for children with HGG (PNOC020, NCT04573140; Table 1).

Adoptive cellular therapy

Adoptive cellular therapy (ACT) has become one of the fastest-growing fields under investigation against pediatric CNS tumors. ACT involves ex vivo manipulation of immune cells that are then administered to a patient for anti-tumor effect. The majority of cellular therapy has primarily focused on T cell manipulation for anti-cancer activity,

though similar technology can be applied to other effector cells, such as NK cells [18] and macrophages [19]. Manipulation can include simple expansion of the immune cells, stimulation with a particular tumor-associated target, or modification to introduce a T cell receptor (TCR) or chimeric antigen receptor (CAR) on the cell surface. Infusion of expanded T and NK cells for a cohort of relapsed medulloblastoma patients was initially published over thirty years ago [20], with newer expansion techniques being utilized in recent [21] and upcoming clinical trials (PNOC028, registration pending; Table 1). T cells expanded and selected for the targets WT1, PRAME, and survivin are also currently being tested (REMIND trial, NCT03652545) [22]. Building on the momentum of six FDA-approved CAR T cell therapies for hematologic malignancies, CAR T cells are now being tested against pediatric CNS tumors with exciting initial reports. CAR T cells targeting HER2 were the first to be intracranially delivered and reported for pediatric CNS tumor patients (BrainChild-01, NCT03500991) [23], with additional ongoing trials targeting EGFR806 (BrainChild-02, NCT03638167), B7-H3 (BrainChild-03, NCT04185038) [24], GD2 (NCT04196413, GAIL-B; NCT04099797) [25], IL-13RA2 (NCT04510051), and the first multi-antigen targeting trial against pediatric CNS tumors (BrainChild-04, NCT05768880). Preliminary reports from the first patients treated with B7-H3-, GD2-, and HER2-specific CAR T cells have shown that it is feasible to manufacture and deliver fractionated doses that allow for repeated intracranial infusions and can induce locoregional immune activation [23–25]. B7-H3- and GD2-specific CAR T cells may also be demonstrating early evidence of efficacy against both pontine and spinal DMG, though, if there has been clinical benefit, it has not been universal [24,25]. Ultimately, these initial studies are still ongoing and as more patients are treated at higher dose levels there may be a clearer signal of clinical efficacy. Regardless, the preliminary feasibility of these trials has invigorated international efforts to build the next generation of these therapies that will be directed against novel targets, incorporate multi-antigen targeting, and feature secondary genetic modifications to boost efficacy and chemotaxis. For example, a novel targeting approach will be featured in an upcoming trial using TCR targeting H3K27M, the pathognomonic mutation in DMG (PNOC018, NCT05478837; Table 1). Future trials will also look to optimize delivery. While initial studies are using systemic and intracranial dosing, the optimal route of delivery remains an ongoing question in the field. When given intravenously, CAR T cells certainly can traffic to the brain; however, this approach likely requires larger effector cell doses and increases the risk of systemic toxicities [25,26]. Even if intracranial delivery is determined to be superior to systemic dosing, there is no consensus on where in the CNS ACT should be delivered. It is possible delivery will depend on location and extent of disease, as well as potentially tumor biology-driven microenvironmental considerations.

Checkpoint blockade and immunoregulatory antibodies

Immune checkpoint inhibitors (ICIs) are a class of humanized monoclonal antibodies that target inhibitory receptors and ligands (i.e., PD-1, PD-L1 and CTLA-4) expressed on tumor cells as well as T lymphocytes and antigen presenting cells to restore anti-tumor immune responses and allow for persistent activation of T cells. ICIs have been investigated in pediatric CNS tumors due to the successful results seen in adult patients with metastatic melanoma, non-small cell lung cancer and renal cell carcinoma, but have demonstrated limited efficacy except in patients with congenital mismatch repair deficiency (PBT0045, NCT02359565) [27–30]. Indeed, replication repair deficient pediatric HGG tumors treated with ICIs had objective responses and a promising median survival of 2 years when compared to historical controls with median post-relapse survival of only 2.6 months [31]. Alternative dosing schedules, such as neo-adjuvant approaches (PNOC019, NCT04323046; Table 1) and immunotherapy combinations (PNOC013, NCT03690869 and PNOC029, NCT05465174; Table 1), continue to be investigated. In addition, immune modulatory antibodies targeting cells beyond T cells

are actively being explored, such as magrolimab, an anti-CD47 antibody that disrupts the “don’t eat me” signal to macrophages (PNOC025, NCT05169944; Table 1), and CD40-agonists that stimulate dendritic cells, lymphocytes and macrophages (NCT03389802). Ultimately, the efficacy of antibody-based therapy may be hindered by the blood-brain barrier and may require additional disruption, such as focused ultrasound or intratumoral administration.

Toxicity considerations

Systemic inflammation

Immune-based therapies have the potential to induce systemic inflammation from activation of different facets of the immune system. This is well described in the trials of CAR T cell therapy and bi-specific T cell engagers for hematologic malignancies, in which patients can develop symptoms ranging from isolated fever to those mirroring macrophage activating syndrome/hemophagocytic lymphohistiocytosis [32–34]. These adverse events have been termed cytokine release syndrome (CRS) and are driven by elevated levels of circulating cytokines, such as IL-6. Similarly, immune-effector cell associated neurotoxicity syndrome (ICANS) is an acute constellation of neurologic symptoms that most often includes confusion, headaches, and language disturbances, but can involve encephalopathy, seizures, and fatal cerebral edema [35]. In a prospective study aiming to assess the occurrence of CRS and ICANS in CD19-directed CAR T cell therapy, 44% (19/43) of patients experienced neurotoxicity, and ICANS correlated with severity of CRS, prior abnormal CNS neuroimaging findings, and higher systemic (but not CSF) CAR T cell numbers [36]. The study suggests that ICANS is the result of systemic inflammation affecting the brain. CRS and ICANS do not seem to occur nearly as frequently in intracranially delivered CAR T cells for CNS tumors [23–25,37], which may have been anticipated due to the lack of systemic inflammation from locoregional CNS delivery.

ICIs have been well tolerated in pediatric CNS tumor clinical trials thus far, but ICIs are also associated with systemic immune-related adverse events (irAEs). Inhibition of immune checkpoints, necessary for balanced immune homeostasis, can lead to activation of autoreactive T cells and resultant autoimmune related activity [38–40]. The exact mechanism that leads to irAEs is unknown but is thought to be secondary to ICIs reduction of T cell self-tolerance and an increase in autoantibody production as well as inflammatory cytokines. irAEs can occur in any organ system, but most commonly present in the gastrointestinal tract, skin, lungs, endocrine glands, and liver [41]. Rare fatal irAEs include autoimmune cardiac and neurologic toxicities. Severe irAEs occur more commonly when anti-PD1 monoclonal antibody is given in combination with anti-CTLA4 monoclonal antibody rather than as monotherapies [40,42]. While several studies in melanoma and small cell lung cancer have postulated a positive association with the development of irAEs and anti-tumor responses using ICIs, this remains controversial and not universally observed across all tumor types [43].

Local inflammation

Pseudoprogression is a term frequently used in immunotherapy to describe transient local inflammatory changes at the site of CNS tumors following therapy. CAR T cells targeting tumors in the brain and spinal cord have the potential to cause local inflammation at the site of the tumor as T cells are activated, with subsequent neurologic sequelae. Pre-clinically, this raised concerns with GD2-directed CAR T cells in murine models of DMG where a small portion of mice with pontine tumors and all mice with thalamic tumors died secondary to localized swelling, hydrocephalus, and herniation [44]. Early clinical data suggests there may be a risk of worsening existing neurologic deficits, which is often self-limiting or responds to anti-inflammatory interventions [23–25]. Localized inflammation in the tumor in the setting of CAR T cells for CNS

tumors has recently been termed tumor inflammation-associated neurotoxicity (TIAN) [45]. There are relevant questions about how to distinguish these symptoms from clinical progression or if truly localized neurologic toxicity composes a distinct constellation that deserves unique consideration, as with CRS and ICANS. On-target/off-tumor effects from CAR T cells may also produce local inflammation in the brain, and must be carefully assessed, although additional biopsy or post-mortem analysis may be necessary for full characterization.

The use of ICIs have also been associated with symptoms of local tumor inflammation or pseudoprogression due to infiltration of activated immune cells in a number of cancers, such as metastatic melanoma and non-small cell lung cancer, but the incidence in primary CNS tumors is unknown [46]. Patients with DIPG treated with the PD-1 inhibitor pembrolizumab on the PBT-045 trial experienced new/increased neurological symptoms and decreased progression-free and overall survival compared to historical controls [47], causing their subsequent exclusion from the trial. The incidence in other CNS tumors is less well described.

Similarly, data on local inflammation after treatment with oncolytic viruses is still scarce. Pseudoprogression was observed in several patients treated with the G207 virus (NCT02457845), suggesting local inflammation that was confirmed on paired samples pre- and post-treatment with increased tumor infiltration [9]. In DIPG patients treated with DNX-2401 (NCT03178032), local inflammation after treatment was not specifically noted, but two patients developed either hemiparesis or tetraparesis following biopsy and therapy [10]. Since gadolinium was not used in the imaging protocol, pseudoprogression could not be analyzed in this trial, although access to a paired pre-post biopsy also showed increased immune infiltration in the tumor.

Approach to toxicity management

As the initial wave of phase 1 adoptive cellular therapy trials for children with CNS tumors is still ongoing, our understanding of irAEs is evolving. The treatment for severe irAEs includes suspending treatment such as ICIs and beginning corticosteroid treatment. Additional immunomodulators may be necessary if clinical symptoms do not improve with steroids. Specific immunomodulators will be recommended based on the irAEs experienced, e.g. immune-related-colitis can be treated with infliximab and immune-related-hepatitis with agents like mycophenolate mofetil or budesonide.

Bevacizumab can also be used as an alternative to corticosteroids to treat pseudoprogression, thereby limiting side effects and direct inhibition of lymphocytes. The relationship to recent surgery, potential for upcoming urgent surgery, and risk of hemorrhage may be relevant to a discussion about the role and timing of bevacizumab. Elevated intracranial pressure may need to be relieved through pharmacologic or neurosurgical interventions and these discussions may be altered based on the presence and type of CNS catheter that a patient may have in place.

As noted above, preliminary evidence of children receiving CAR T cells for CNS tumors, in particular for those receiving intracranial dosing, suggests that severe CRS may not be a risk. Notably, different research teams may capture self-resolving metronomic fever immediately following CAR T cell infusions individually as fever or as grade 1 CRS. However, as individual cellular therapies may have a distinct risk of trafficking into the serum and inducing systemic toxicity, either due to the target or the CAR construction, vigilance in monitoring for CRS will continue to be important. Treatment of CRS may include anti-pyretics, inhibition of IL-6 receptor, steroids, and supportive care as reviewed in the EBMT/EHA CAR-T Cell Handbook [48]. Lymphodepleting chemotherapy such as cyclophosphamide and fludarabine used in preparation for adoptive cellular therapy has been linked to increased incidence of CRS and neurotoxicity in the past, however further investigation has shown this may be more related to the degree of systemic CAR T cell proliferation of specific products rather than conditioning regimens [49–51]. Nevertheless, there is inconsistency in the use of lymphodepleting chemotherapy

preparative regimens in CAR T cell trials for CNS tumors, with many trials avoiding their use.

Clinical trials of CAR T cells for pediatric CNS tumors may also have different approaches to preemptive management, including required anti-epileptic medications or mandated inpatient admissions to the oncology team, transplant team, or intensive care unit (ICU). Therefore, acute management will be impacted by the level of baseline care. In general, detailed neurologic exams are critical and early neurology team involvement for toxicities is recommended. Cooperative groups are now working on consensus guidelines for management and a better understanding of the pathophysiology of these neurological findings that are most likely distinct from ICANS.

Disease assessment and correlative studies

A major obstacle to clinical trials using immune-based therapeutics is accurately identifying disease response. Imaging in this context can be challenging to interpret. As first described in the setting of radiotherapy [52], pseudoprogression describes increased contrast-enhancement and lesion size following therapy, but ultimately reflects only transient changes that remain stable or improve over time without a change in therapy. Tumor directed immunotherapy can lead to pseudoprogression and other atypical radiographic patterns which can be confused with true tumor progression or treatment failure. In addition, clinical symptoms from tumor pseudoprogression mimic true progression, further muddying the picture in real time. Additionally, anti-inflammatory agents given for symptom management following immunotherapy may affect intra- or peri-tumoral inflammation which decreases tumor bulk on neuroimaging rather than classic disease response.

Traditional response criteria based on conventional imaging such as magnetic resonance imaging (MRI) is not reliably predictive of immunotherapeutic outcomes [46,53–55]. This can lead to premature discontinuation of immunotherapy as well as inaccurate conclusions from new immune mediated strategies. Therefore, novel imaging techniques and serially evaluable biomarkers are needed to better assess immunotherapeutic efficacy and safety, as well as tumor evolution and immune response.

Specimens for tumor and immune monitoring

To get a better understanding of the immunological changes and select possible predictive markers for response to immunotherapy, multiple techniques can be applied on several specimens to enrich the clinical trial setting (Fig. 1). Molecular profiling of the tumor at diagnosis will offer unique insight into the immune composition at diagnosis, and we propose additional subsequent monitoring of immune changes through serial sampling of other compartments including peripheral blood, CSF, bone marrow, and stool (Fig. 1). These modalities are aimed at harm minimization whilst also providing clinically relevant information in real-time throughout a patient's disease journey.

Flow cytometry

Unlike hematological malignancies, flow cytometry is not frequently used for CNS malignancies. However, this technique has been explored in fresh tissue to characterize immune cell infiltration across multiple pediatric CNS malignancies [56,57]. To fully appreciate the effect of a therapy on the tumor and its TME, repetitive sampling of the tumor could be performed. However, repetitive sampling is not standard practice and is invasive for the patient. Studying more accessible immune compartments, such as blood, CSF, or bone marrow, could provide valuable information on changes in the immune landscape and functional dynamics in a patient with a CNS tumor. Ideally, this correlates with the TME and/or disease response or can discriminate between real progression and pseudoprogression. In a study for adult brain tumor patients, flow cytometry and CyTOF on blood samples showed an increase

Specimens for immune monitoring of pediatric CNS tumors

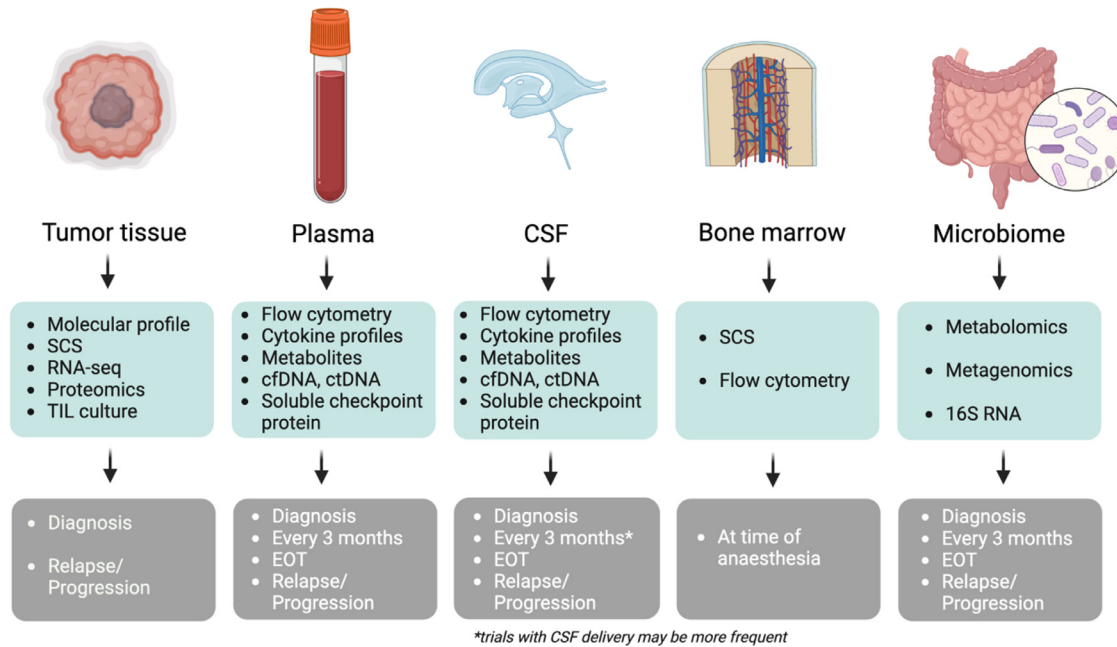


Fig. 1. Specimens for immune monitoring of pediatric CNS tumors. Characterization of the immune landscape from multiple compartments for application in clinical trials for pediatric CNS malignancies. Longitudinal liquid biopsies (blood, tumor tissue, CSF), microbiome and bone marrow can be utilized for a comprehensive evaluation with the suggested techniques. CSF: Cerebral Spinal Fluid; SCS: Single Cell Sequencing (different techniques available); ctDNA: circulating tumor DNA; cfDNA: circulating free DNA; RNA-seq: RNA-sequencing (bulk); TIL: tumor infiltrating lymphocytes; EOT: End Of Treatment. Created with BioRender.com.

of myeloid derived suppressor cells (MDSCs), but not regulatory T cells, for glioblastoma patients compared to other CNS tumor entities [58]. Higher intratumoral MDSCs correlated with poorer survival, underpinning the MDSC as an important target for immunotherapy [58]. Furthermore, they demonstrated that the immune signature in the blood changed during treatment [58]. More recently CNS CAR T cell trials have utilized flow cytometry on patient blood and CSF to identify circulating CAR T cells [23–25].

The role of the bone marrow compartment and its interplay with CNS malignancies needs to be further clarified. An interesting finding by Chongsathidkiet and colleagues was the accumulation of T cells in the bone marrow of patients with glioblastoma, in combination with low CD4 levels systemically [59]. This study raises the question if sequestration of T cells in bone marrow can be qualified as a T cell dysfunction, and therefore a possible target for therapy. Longitudinal flow cytometry analysis of blood/bone marrow and CSF will not only provide information on disease progression but could also be used as a tool in predicting immune treatment responsiveness. Multiple platforms can be used to design brain tumor specific flow cytometry panels, evaluating subsets and functionality of T/B/NK and myeloid cells.

Single cell sequencing

The unique advantage of single cell RNA sequencing (scRNA-seq) over bulk RNA-seq, is the ability to profile the transcriptome of thousands of individual cells. This technique has developed tremendously over recent years, resulting in single cell sequencing (SCS) “atlases” for several pediatric CNS tumors, and to a lesser extent, its microenvironment. Detailed scRNA-seq studies have been useful for appreciating the heterogeneity and functional differences in brain residing macrophages. A recent paper of Liu et al where they dissected a cohort of H3-K27M DMG patients by SCS, including the TME, revealed age dependent differences in the immune infiltrate of DMGs [60]. They found higher expression of the TAM-secreted ligand *OSM* in adult TAMs, with higher corresponding receptor *OSMR* in adult tumor cells, indicating immune-

mediated engagement of a previously validated pathway [60]. The recent report on GD2-directed CAR T cell therapy for DMG performed scRNA-seq on blood and CSF of treated patients, where they identified distinct myeloid populations in both compartments, as well as during peak inflammation and late in course at the time of progression [25]. Moreover, scRNA-seq profiling has also been performed on CSF and blood to monitor the dynamic changes of leukocytes and myeloid cells during treatment for other brain metastases and non-oncologic disorders such as multiple sclerosis [61,62]. Proteomics will be the next step to validate the findings of SCS on a protein level. Further characterization of the TME, such as expression profiles and T cell surface receptors for various pediatric brain tumors, will aid in understanding the interaction between immune cells and tumors, paving the way for the development of novel immunotherapy strategies.

Tumor free markers of immunotherapy selection and response

Liquid biopsy

Considerable recent efforts have focused on the discovery and development of liquid biopsy-based techniques to identify biomarkers used to guide treatment recommendation and response. As it stands, circulating tumor-DNA (ctDNA) [63,64], methylated-ctDNA [65], circulating tumor micro-RNA (circulating miRNAs) [66,67], circulating tumor exosomal-DNA (exoDNA) [68], circulating tumor cells (CTCs) [69], and soluble proteins have been identified in the blood, CSF and urine of patients. While these have been studied to identify tumor markers, they are yet to be considered standard clinical approaches and are generally conducted as research. Below we describe tumor-related biomarkers that may aid in the development and optimization of immunotherapies for the treatment of CNS malignancies.

Tumor free nucleic acid biomarkers of pediatric CNS tumors

Analysis of circulating genomic material is an exciting frontier for the non-invasive tumor genotyping of pediatric CNS cancer. Targeted approaches such as nanopore sequencing of ultra-short ctDNA fragments

and digital droplet PCR (ddPCR) are the most commonplace. These methods have been used to identify hallmark mutations in children with HGGs (such as H3K27M in DMG) [70–72], as predictive markers of disease response to investigational agents [73], and to track treatment response in medulloblastoma [74]. These properties make cfDNA biomarkers attractive for monitoring treatment response and potentially for the selection of the most appropriate immunoncology-based therapies. Next generation sequencing (FDA approved Memorial Sloan Kettering-Integrated Molecular Profiling of Actionable Cancer Targets – MSK-IMPACT) have also recently been used to detect somatic alterations in the CSF of 30/64 pediatric CNS cancer patients, and was found to be strongly positively associated with the presence of disseminated disease [75]. Although these studies did not inform treatment, they do highlight the potential of such approaches to be used to characterize the somatic alterations that give rise to these cancers, and, to stratify patients to relevant immunological treatments. Finally, low-coverage whole genome analysis from serially collected CSF of medulloblastoma patients was recently shown to identify measurable residual disease and predict patient outcome [64]. These techniques will continue to be evaluated across multiple tumor types and incorporated into upcoming clinical trials to evaluate applicability in discerning treatment response in the context of immune based therapies.

Protein inflammatory marker analysis in clinical trials

Plasma soluble tumor checkpoint proteins, including soluble PD-L1 (sPD-L1) [76], and soluble immune cell checkpoints such as soluble CTLA-4 from T_{regs} and soluble PD-1 from CD4+ T cells [77], have been identified in the blood and CSF of patients. This may act as a surrogate to genomics for the selection and/or monitoring of efficacy for pediatric brain tumor patients treated with immunotherapies. Serum and CSF levels of sPD-L1 in adults with HGG are significantly elevated compared to healthy controls, with CSF PD-L1 levels more indicative of aggressive tumor features [76].

As many ACT trials for children with CNS tumors incorporate locoregional delivery, enrolled patients on these studies have CNS catheters such as an Ommaya in place. Beyond allowing for a more localized delivery and the potential for less systemic toxicity, this neurosurgical technology also allows for frequent CSF sampling with minimal invasiveness. These precious biospecimens may be integral to future paradigms of disease assessment and tumor evolution, especially considering the known limitations of neuroimaging in the setting of possible pseudoprogression following immunotherapy [78]. Serial longitudinal chemokine/cytokine analysis has already been found to be feasible [23]. On Seattle Children's BrainChild-01 and -03 trials, delivering locoregional HER2 and B7-H3 CAR T cells, respectively, to children with recurrent/refractory CNS tumors and DIPG/DMG revealed elevations of critical analytes via multiplex assay [23,24]. For example, CXCL10, a type 1 CXC chemokine ligand that has been reported to improve effector cell migration into gliomas, was elevated post infusion in the CSF [23,24,79]. "IFN- γ " was also elevated, which is notable as IFN- γ can activate macrophages and promote cytotoxic T cell function [80–82]. However, there were only limited changes in serum chemokine/cytokine concentrations, correlating with the lack of systemic toxicities. Similar results were seen in Stanford's GD2-directed CAR initial report, where plasma cytokines such as IL-6 were elevated following intravenous delivery of CAR, but not after doses delivered into the CSF [25]. Similar to the Seattle trials, CSF levels of proinflammatory cytokines such as IFN- γ were elevated in the CSF following intracerebroventricular doses [25]. The understanding of the role of chemokines and cytokines of particular CAR T cell activity may fuel the next wave of cellular engineering, in which secretion of IL-12, IL-15, IL-18, and others may improve chemotaxis and cytotoxicity [83–85]. Beyond CAR T, other immune based clinical trials for pediatric CNS tumors have measured serum cytokines, with varied fluctuations depending on the type of therapy [86–88], and suggest continued monitoring in the serum will also be important for systemically delivered therapies.

Another emerging method of detection includes whole proteome analysis or targeted proteomics of patient biospecimens, such as CSF and serum. In BrainChild-03, serial targeted proteomics was piloted and showed feasibility in detecting the ACT target, B7-H3, in the CSF and serum of treated patients [24]. It was also possible to capture modulation of several other potentially important immunoregulatory peptides, such as BCL10, CXCL13, HAVCR2 (TIM-3), ICOSLG (ICOS ligand), PDCD1LG2 (PD-L2), TNF receptor super family member 14 (TNFRSF14), and VCAM-1 [24]. Using parallel platforms to validate findings of locoregionally treated patients, particularly in the CSF, may direct the field toward establishing early signs of treatment response and tumor evolution.

Future work will hopefully include both serial circulating tumor DNA and serial circulating proteomic assessments as they have already proven feasible in other clinical settings [64,89]. An ideal state may be that serial circulating chemokines/cytokines, genomics, and proteomics can be triangulated to ensure biospecimens are optimally used to craft the best potential treatment and allow iterations to optimize future therapies. Ultimately, immuno-neuro-oncology working groups should aim to align these assays to allow for analysis across trials and collaboratively build towards more effective immune based therapies.

Microbiome

The microbiome describes the totality of bacterial, fungal, and viral species in the body of which most reside in the gut [90]. The microbiome has a strong interaction with the immune system and can regulate T cell immunity in a variety of diseases, including malignancies. Although the composition of the microbiome varies greatly within individuals and among populations [91], its prognostic value and even therapeutic applications for patients with cancer has become evident over the last decade. In humans, profound correlations were found between immunotherapy responses and the microbiome composition, particularly for ICI treatment [92,93]. In addition, dysbiosis and treatment response was reported for a CAR T trial in adult B-cell malignancies, where the use of antibiotics four weeks before the start of treatment correlated negatively with outcome and positively with neurotoxicity [94]. As a next step, studies with feces transplantation for patients resistant to ICI are now well underway and have started to report positive results in a small cohort of patients [95].

Little is known about the relationship between the microbiome and tumor pathogenesis in pediatric brain tumors, or the predictive value of therapy response. However, it is thought that there is strong, two-way biochemical signaling between the microbiome and the CNS. In a group of meningioma and HGG patients, less diversity of the microbial ecosystem was found in brain tumor patients compared to healthy controls, but no significant differences between the meningioma and glioma groups [96]. Brain tumor groups lacked small chain fatty acids (SCFA)-producing probiotics. This metabolite is thought to play an important role in the so-called *gut-brain-axis*, reducing secretion of proinflammatory cytokines, and inducing FoxP3 regulation and IL-10 production [97]. Recently, the first data presented on DIPGs and the microbiome revealed a dysbiosis at diagnosis, where the *Firmicutes/Bacteroidetes* ratio identified certain bacterial family members were associated with reduced progression free survival [98].

Incorporating longitudinal feces collection, and ideally metabolites in plasma, into immunotherapy clinical trials for pediatric brain tumors can generate biomarkers to predict treatment response and provide possible targets for microbiome manipulation. One of the major challenges will be to build uniformity in terms of sample collection, sample processing, data analysis and data presentation [99]. We propose four time points for the collection of the microbiome: at diagnosis, during radiotherapy (if applicable), during chemotherapy (if applicable), and end of treatment/at time of progression. 16S RNA is the most common and least expensive mode of analysis, with a reliable reference library and therefore the most feasible. However, DNA metagenomics/metabolomics (shotgun) will generate more information and

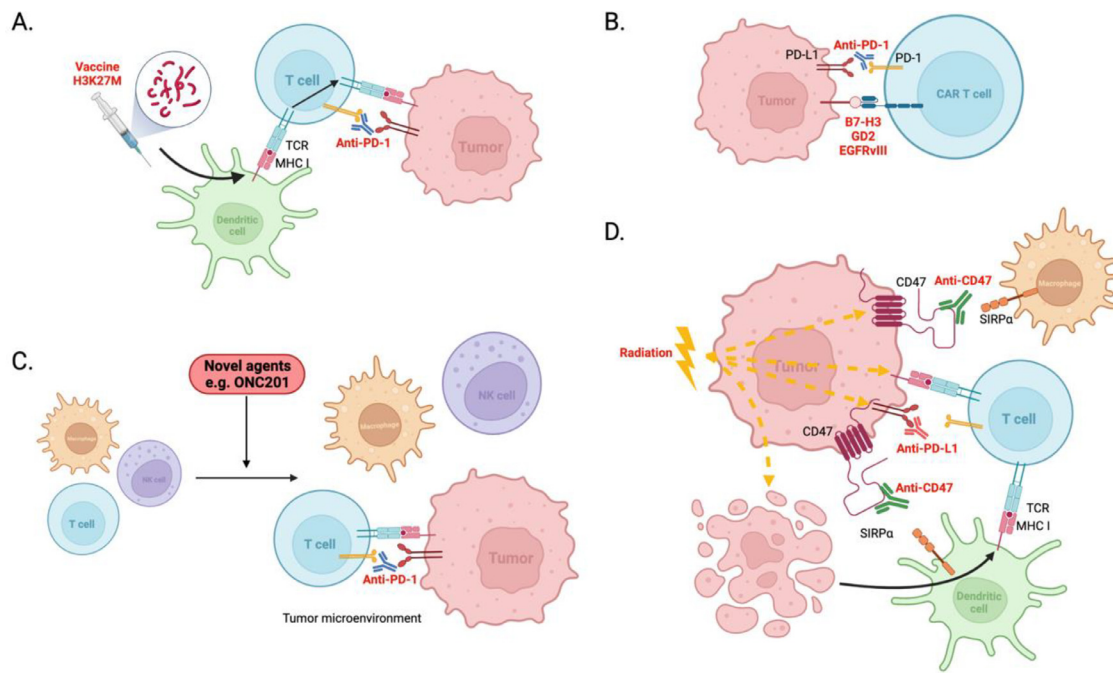


Fig. 2. Potential strategies to warm the cold TME in central nervous system tumors. **A.** Cancer vaccines, including H3K27M peptide vaccine, activates and guides immune cells to the tumor. Combination with ICIs prevent activated T cells from becoming exhausted and promotes their pro-tumor effects. **B.** CAR T cell therapy, such as B7-H3-, GD2- and EGFRvIII-directed CAR T cells in combination with ICI to reduce CAR T cell exhaustion and potentiate pro-tumor activity. **C.** Novel agents such as ONC201 recruit immune cells including T cells, NK cells and macrophages into the tumor microenvironment promoting immunogenic tumor cell death. Combination with ICIs may potentiate the immunological effects of ONC201. **D.** Radiation therapy upregulates surface expression of immune modulatory and checkpoint proteins including CD47 and PD-L1 on tumors, priming for combination with ICI and magrolimab. Radiation-induced cell death promotes the uptake of tumor-associated antigens by dendritic cells that are presented to T cells. Radiation therapy increases MHC I expression on tumor cells, further suggesting a benefit to combine with other immunotherapies. Created with BioRender.com.

possibly new biomarkers to correlate to treatment effect or toxicity outcome.

Combination therapy and future studies

Despite the major advances over the past several decades, several hurdles still remain for effective immune activation in the CNS. Ongoing preclinical studies and upcoming clinical trials are aimed at overcoming these roadblocks. Here we review several of these roadblocks and active approaches toward them including specifically the cold TME, T cell exhaustion, and tumor antigen heterogeneity.

Warming the cold tumor microenvironment of pediatric CNS tumors

Many patients fail to respond to therapies designed to harness their own immune system in the fight against their cancer, particularly so for pediatric patients diagnosed with CNS malignancies. This is in part because CNS tumors harbor an immunologically cold TME, characterized by few tumor infiltrating lymphocytes (TILs), and featuring abundant immunosuppressive myeloid cells (such as microglia and macrophages) and secrete immunosuppressive cytokines [14,100–102]. To circumvent the cold TME, combination therapies that target the tumor and the TME is a potentially beneficial approach. As discussed above, ICIs can help promote an immune response in T cells, and have been actively studied as combinatorial approaches with vaccines [103,104] (PNOC007; NCT02960230, Table 1), and CAR T cells [105], (Fig. 2A and B).

Another way to warm the TME (and promote the activity of ICIs) is to use anticancer drugs that modulate the tumor and its surrounding TME (Fig. 2C). Preliminary results for the brain-penetrant, small molecule, ‘ONC201’, used in the treatment of DMG, shows that it is not only an anti-DMG therapy [106], but also promotes recruitment of immune cells into the tumor in other cancer types [107,108]. Immune cells shown to

be recruited include CD3+ T cells, including CD8+ T cells in a patient with mantle cell lymphoma and murine colorectal cancers, and NK cells infiltrating in colorectal cancers [107,108], suggesting a combination with ICIs might be a promising approach. Indeed, combining ONC201 with an anti-PD-1 monoclonal antibody enhanced tumor regression in mice harboring colorectal carcinomas compared to anti-PD-1 monotherapy [108].

Radiotherapy is one of the primary treatments across different CNS tumors and has been shown to have both a proinflammatory effect and an immune suppressive effect, including release of immunosuppressive cytokines and direct toxicity to local lymphocytes [109]. Intriguingly, studies have shown damage caused by radiation treatment increases release of tumor-associated antigens that are then presented to T cells via antigen presenting cells (APCs) [110,111]. Thus radiotherapy unmasks the tumor to the immune system, providing a rationale for combining radiation with ICIs [110]. This has been investigated and proposed safe in the recently completed phase I trial in adults with newly diagnosed GBM [112]. Both PD-L1 and CD47 have been shown to be upregulated on cells post radiation [113]. Accordingly, radiation combined with dual PD-L1 and CD47 inhibition resulted in increased tumor regression in murine lung cancer models [113]. CD47 blockade (magrolimab) also augmented cross-priming between dendritic cells and T cells, promoting a more potent anti-tumor immune response [114]. Thus, radiation in combination with magrolimab will also be explored in children, adolescents, and adults with recurrent or progressive malignant brain tumors (Fig. 2D).

Combination strategies to reduce T cell exhaustion

Despite the success of ACT in hematologic malignancy, many solid tumor patients still fail to respond, even with the addition of ICIs. Thus, novel methods to enhance and sustain T cell activity are necessary. The

PI3K pathway is one of the most overactivated oncopathways in pediatric CNS tumors [115,116], but also plays an important role in driving T cells to exhaustion [117]. Inhibition of PI3K- δ (*PIK3CD*) and PI3K- γ (*PIK3CG*) in T cells promotes the durability of activated T cells to drive sustained anticancer effects [117]. This suggests that combining PI3K inhibition and CAR T cell therapy, or cancer vaccine, could be a promising approach. Expanding CAR T cells with PI3K inhibitors *ex vivo* has shown to enhance their function *in vivo* [118]; however, studies investigating PI3K inhibitors and CAR T therapy both given *in vivo* are warranted. Further, combining an HPV16 E7(49–57) peptide vaccine with the PI3K inhibitor wortmannin resulted in an increase of E7-reactive T cells compared to the vaccine alone, leading to a greater reduction in tumor growth in a TC-1 murine model [119]. It must be noted, that the PI3K pathway is also necessary to activate T cells, thus the timing of treatment is an important consideration. In addition, PI3K- δ is important for sustained T_{reg} function, which is known to suppress anticancer immunity. Inhibition of PI3K- δ (as well as PI3K- α/δ) led to a 50% reduction in T_{reg} infiltration in mice harboring colorectal cancers [120]. Immunosuppressive macrophages are also known to reduce the anti-cancer benefits of T cells. Excitingly, pan-inhibition of PI3K with copanlisib used in combination with anti-PD-1 antibody decreased both T_{regs} and immunosuppressive macrophages, as well as MDSCs promoting an anticancer response [121].

The tyrosine kinase inhibitor dasatinib (targeting PDGFRA) in combination with the mTOR inhibitor everolimus, have been shown to extend survival in a DMG murine model, and importantly, everolimus improved retention of dasatinib in the CNS [122]. In addition to dasatinib's anti-tumor effect, Mestermann et al. have shown that dasatinib can inhibit CAR T cell activity [123], likely through its SFK inhibitor function without affecting the viability of the T cells [124]. Recent work by Weber et al. showed that transient rest of CAR T cells through exposure to dasatinib can improve exhaustion and prevent tonic signaling from the CAR domain to improve effector function [125]. Thus a combinatorial approach for DMG utilizing intermittent dasatinib and everolimus paired with CAR T cells may provide both an anti-tumor effect and boost to T cell function.

A myriad of additional strategies are also being studied to improve T cell exhaustion, in particular in the context of CAR T cells, and may also be important in CNS tumor targeting. These include targeting transcription factors such as Tox [126], epigenetic regulators such as TET2 [127] and DNMT3A [128], cytokine signaling such as dominant negative TGF- β receptor [129], metabolic regulators [130,131], and adjustments in CAR engineering and manufacturing strategies [132].

Addressing tumor antigen heterogeneity

Pediatric CNS tumors represent a wide range of diagnoses, and specific histologies often harbor significant heterogeneity in their transcriptional and mutational profiles. As such, it may be difficult to target one single antigen without the risk of escape phenomenon from selective pressure or downregulation of the antigen. Prior immunotherapies targeting a single antigen such as EGFRvIII [133] and IL13RA2 [37] with CAR T cells in adult GBM have shown down regulation of the target antigen at the time of relapse. Thus, multiple strategies are being explored to overcome this roadblock.

As discussed above, mRNA vaccines offer the possibility of HLA independent multi-antigen targeting [134]. Due to their relatively nimble production, mRNA vaccines could be adjusted over time for an individual patient, offering the potential for real-time adjustments to evolving tumor landscapes.

Using T cells targeting multiple antigens is another potential mechanism to address heterogeneity, if multiple targets have been identified in the tumor population. As discussed above, multi-targeted CTLs are currently being studied in an ongoing clinical trial (REMIND trial, NCT03652545) [22]. Multi-antigen directed CAR T cells have also been studied pre-clinically in GBM models with advantages over single-

antigen targeting CAR T cells [135–137]. Upcoming CAR studies will combine targets against B7-H3, IL13RA2, HER2, and EGFR (Brainchild-04, NCT05768880). As CAR T cells targeting intracellular proteins have now become possible [138], many additional oncogenic/tumor-dependent antigens may now also be potential targets to help overcome tumor heterogeneity.

Conclusion

The field of immunotherapy is a promising and rapidly evolving scientific arena with multiple technologies applicable and attractive for targeting pediatric CNS tumors. The unique aspects of igniting the immune system in the CNS bring both therapeutic challenges and distinctive potential toxicities, making preclinical studies and clinical trial design critical. With advances in non-invasive tumor monitoring techniques, there is potential to more clearly delineate efficacy and toxicity, as well as tailoring sequential therapies to overcome tumor evolution. Much work remains in fully engaging an immunotherapeutic response in children with CNS tumors, but - with coordinated efforts across pediatric medical centers and CNS focused clinical consortia - the initial signals of efficacy can hopefully blossom into long-standing cures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] RH Andtbacka, HL Kaufman, F Collichio, T Amatrua, N Senzer, J Chesney, KA Delman, LE Spitler, I Puzanov, SS Agarwala, et al., Talimogene laherparepvec improves durable response rate in patients with advanced melanoma, *J. Clin. Oncol.* 33 (2015) 2780–2788.
- [2] JE Frampton, Teseraturev/G47Delta: first approval, *BioDrugs* 36 (2022) 667–672.
- [3] HL Kaufman, FJ Kohlhapp, A Zloza, Oncolytic viruses: a new class of immunotherapy drugs, *Nat. Rev. Drug Discov.* 14 (2015) 642–662.
- [4] M Garcia-Moure, M Gonzalez-Huarriz, S Labiano, E Guruceaga, E Bandres, M Zalacain, L Marrodan, C de Andrea, M Villalba, N Martinez-Velez, et al., Delta-24-RGD, an oncolytic adenovirus, increases survival and promotes proinflammatory immune landscape remodeling in models of AT/RT and CNS-PNET, *Clin. Cancer Res.* 27 (2021) 1807–1820.
- [5] AW Studebaker, BJ Hutzen, CR Pierson, KB Haworth, TP Cripe, EM Jackson, JR Leonard, Oncolytic herpes virus rRp450 shows efficacy in orthotopic xenograft group 3/4 medulloblastomas and atypical teratoid/rhabdoid tumors, *Mol. Ther. Oncol.* 6 (2017) 22–30.
- [6] N Martinez-Velez, M Garcia-Moure, M Marigil, M Gonzalez-Huarriz, M Puigdelloses, J Gallego Perez-Larraya, M Zalacain, L Marrodan, M Varela-Guruceaga, V Laspedea, et al., The oncolytic virus Delta-24-RGD elicits an antitumor effect in pediatric glioma and DIPG mouse models, *Nat. Commun.* 10 (2019) 2235.
- [7] G Pascual-Pasto, M Bazan-Peregrino, NG Olaciregui, CA Restrepo-Perdomo, A Mato-Berciano, D Ottaviani, K Weber, G Correa, S Paco, M Vila-Ubach, et al., Therapeutic targeting of the RB1 pathway in retinoblastoma with the oncolytic adenovirus VCN-01, *Sci. Transl. Med.* 11 (2019).
- [8] MI Chastkofsky, KC Pituch, H Katagi, M Zannikou, L Ilut, T Xiao, Y Han, AM Sonabend, DT Curiel, ER Bonner, et al., Mesenchymal stem cells successfully deliver oncolytic virotherapy to diffuse intrinsic pontine glioma, *Clin. Cancer Res.* 27 (2021) 1766–1777.
- [9] GK Friedman, JM Johnston, AK Bag, JD Bernstock, R Li, I Aban, K Kachurak, L Nan, KD Kang, S Totsch, et al., Oncolytic HSV-1 G207 immunovirotherapy for pediatric high-grade gliomas, *N. Engl. J. Med.* 384 (2021) 1613–1622.
- [10] J Gallego Perez-Larraya, M Garcia-Moure, S Labiano, A Patino-Garcia, J Dobbs, M Gonzalez-Huarriz, M Zalacain, L Marrodan, N Martinez-Velez, M Puigdelloses, et al., Oncolytic DNX-2401 virus for pediatric diffuse intrinsic pontine glioma, *N. Engl. J. Med.* 386 (2022) 2471–2481.
- [11] V Laspedea, M Puigdelloses, S Labiano, L Marrodan, M Garcia-Moure, M Zalacain, M Gonzalez-Huarriz, N Martinez-Velez, I Ausejo-Mauleon, D de la Nava, et al., Exploiting 4-1BB immune checkpoint to enhance the efficacy of oncolytic virotherapy for diffuse intrinsic pontine gliomas, *JCI Insight* 7 (2022).
- [12] M Weller, N Butowski, DD Tran, LD Recht, M Lim, H Hirte, L Ashby, L Mechtler, SA Goldlust, F Iwamoto, et al., Rindopimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial, *Lancet Oncol.* 18 (2017) 1373–1385.
- [13] H Okada, P Kalinski, R Ueda, A Hoji, G Kohanbash, TE Donegan, AH Mintz, JA Engh, DL Bartlett, CK Brown, et al., Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with α -type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma, *J. Clin. Oncol.* 29 (2011) 330–336.
- [14] S Mueller, JM Taitt, JE Villanueva-Meyer, ER Bonner, T Nejo, RR Lulla, S Goldman, A Banerjee, SN Chi, NS Whipple, et al., Mass cytometry detects H3.3K27M-specific vaccine responses in diffuse midline glioma, *J. Clin. Invest.* 130 (2020) 6325–6337.
- [15] H Okada, FS Lieberman, KA Walter, LD Lunsford, DS Kondziolka, GK Bejjani, RL Hamilton, A Torres-Trejo, P Kalinski, Q Cai, et al., Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of patients with malignant gliomas, *J. Transl. Med.* 5 (2007) 67.
- [16] RM Prins, H Soto, V Konkankit, SK Odesa, A Eskin, WH Yong, SF Nelson, LM Liao, Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy, *Clin. Cancer Res.* 17 (2011) 1603–1615.
- [17] SK Nair, T Driscoll, D Boczkowski, R Schmittling, R Reynolds, LA Johnson, G Grant, H Fuchs, DD Bigner, JH Sampson, et al., Ex vivo generation of dendritic cells from cryopreserved, post-induction chemotherapy, mobilized leukapheresis from pediatric patients with medulloblastoma, *J. Neurooncol.* 125 (2015) 65–74.
- [18] TJ Laskowski, A Biederstadt, K Rezvani, Natural killer cells in antitumor adoptive cell immunotherapy, *Nat. Rev. Cancer* (2022).
- [19] NR Anderson, NG Minutolo, S Gill, M Klichinsky, Macrophage-based approaches for cancer immunotherapy, *Cancer Res.* 81 (2021) 1201–1208.
- [20] Y Okamoto, K Shimizu, K Tamura, Y Miyao, M Yamada, Y Matsui, N Tsuda, H Takimoto, T Hayakawa, H Mogami, An adoptive immunotherapy of patients with medulloblastoma by lymphokine-activated killer cells (LAK), *Acta Neurochir. (Wien)* 94 (1988) 47–52.
- [21] S Khatua, LNJ Cooper, DI Sandberg, L Ketonen, JM Johnson, ME Rytting, DD Liu, H Meador, P Trikha, RJ Nakkula, et al., Phase I study of intraventricular infusions of autologous ex vivo expanded NK cells in children with recurrent medulloblastoma and ependymoma, *Neuro. Oncol.* 22 (2020) 1214–1225.
- [22] E Hwang, F Fortiz, R Cruz, A Geiger, M Grant, H Lang, A Shibli, S Burnett, C Lazarski, J Tanna, et al., IMM-19. Outcomes of Pediatric Patients with High-Risk CNS Tumors Treated with Multi-tumor associated antigen specific T cell (TAA-T) therapy: the ReMIND trial, *Neuro-Oncol.* 24 (2022) i85–i86.
- [23] NA Vitanza, AJ Johnson, AL Wilson, C Brown, JK Yokoyama, A Künkele, CA Chang, S Rawlings-Rhea, W Huang, K Seidel, et al., Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis, *Nat. Med.* (2021).
- [24] NA Vitanza, AL Wilson, W Huang, K Seidel, C Brown, JA Gustafson, JK Yokoyama, AJ Johnson, BA Baxter, RW Koning, et al., Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: preliminary first-in-human bioactivity and safety, *Cancer Discov.* (2022).
- [25] RG Majzner, S Ramakrishna, KW Yeom, S Patel, H Chinnasamy, LM Schultz, RM Richards, L Jiang, V Barsan, R Mancusi, et al., GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas, *Nature* 603 (2022) 934–941.
- [26] J Theruvath, E Sotillo, CW Mount, CM Graef, A Delaidelli, S Heitzeneder, L Labanieh, S Dhingra, A Leruste, RG Majzner, et al., Locoregionally administered B7-H3-targeted CAR T cells for treatment of atypical teratoid/rhabdoid tumors, *Nat. Med.* (2020).
- [27] JD Wolchok, V Chiarion-Sileni, R Gonzalez, P Rutkowski, JJ Grob, CL Cowey, CD Lao, J Wagstaff, D Schadendorf, PF Ferrucci, et al., Overall survival with combined nivolumab and ipilimumab in advanced melanoma, *N. Engl. J. Med.* 377 (2017) 1345–1356.
- [28] RJ Motzer, NM Tannir, DF McDermott, O Aren Frontera, B Melichar, TK Choueiri, ER Plimack, P Barthelemy, C Porta, S George, et al., Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma, *N. Engl. J. Med.* 378 (2018) 1277–1290.
- [29] J Brahmer, KL Reckamp, P Baas, L Crino, WE Eberhardt, E Poddubskaia, S Antonia, A Pluzanski, EE Vokes, E Holgado, et al., Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer, *N. Engl. J. Med.* 373 (2015) 123–135.
- [30] E Bouffet, V Larouche, BB Campbell, D Merico, R de Borja, M Aronson, C Durno, J Krueger, V Cabric, V Ramaswamy, et al., Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency, *J. Clin. Oncol.* 34 (2016) 2206–2211.
- [31] A Das, S Sudhama, D Morgenstern, A Coblenz, J Chung, SC Stone, N Alsafwani, ZA Liu, OAA Karsaneh, S Soleimani, et al., Genomic predictors of response to PD-1 inhibition in children with germline DNA replication repair deficiency, *Nat. Med.* 28 (2022) 125–135.
- [32] SL Maude, TW Laetsch, J Buechner, S Rives, M Boyer, H Bittencourt, P Bader, MR Verneris, HE Stefanski, GD Myers, et al., Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia, *N. Engl. J. Med.* 378 (2018) 439–448.
- [33] DA Lichtenstein, F Schischlik, L Shao, SM Steinberg, B Yates, HW Wang, Y Wang, J Inglefield, A Dulau-Florea, F Ceppi, et al., Characterization of HLH-like manifestations as a CRS variant in patients receiving CD22 CAR T cells, *Blood* 138 (2021) 2469–2484.
- [34] RA Gardner, O Finney, C Annesley, H Brakke, C Summers, K Leger, M Bleakley, C Brown, S Mgebroff, KS Kelly-Spratt, et al., Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults, *Blood* 129 (2017) 3322–3331.
- [35] BD Santomasso, LJ Nastoupil, S Adkins, C Lacchetti, BJ Schneider, M Anadkat, MB Atkins, KJ Brassil, JM Caterino, I Chau, et al., Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO guideline, *J. Clin. Oncol.* 39 (2021) 3978–3992.
- [36] J Gust, OC Finney, D Li, HM Brakke, RM Hicks, RB Futrell, DN Gamble, SD Rawlings-Rhea, HK Khalatbari, GE Ishak, et al., Glial injury in neurotoxicity after pediatric CD19-directed chimeric antigen receptor T cell therapy, *Ann. Neurol.* 86 (2019) 42–54.
- [37] CE Brown, D Alizadeh, R Starr, L Weng, JR Wagner, A Naranjo, JR Ostberg, MS Blanchard, J Kilpatrick, J Simpson, et al., Regression of glioblastoma after chimeric antigen receptor T-cell therapy, *N. Engl. J. Med.* 375 (2016) 2561–2569.
- [38] J Choi, SY Lee, Clinical characteristics and treatment of immune-related adverse events of immune checkpoint inhibitors, *Immune Netw.* 20 (2020) e9.
- [39] M Ellithi, R Elair, GV Chang, MA Abdallah, Toxicities of immune checkpoint inhibitors: Its-ending adverse reactions and more, *Cureus* 12 (2020) e6935.
- [40] Q Durrechou, C Domblides, B Sionneau, F Lefort, A Quivy, A Ravaud, M Gross-Goupil, A Daste, Management of immune checkpoint inhibitor toxicities, *Cancer Manag. Res.* 12 (2020) 9139–9158.
- [41] MA Postow, R Sidlow, MD Hellmann, Immune-related adverse events associated with immune checkpoint blockade, *N. Engl. J. Med.* 378 (2018) 158–168.
- [42] JA Marin-Acevedo, RM Chirila, RS Dronca, Immune checkpoint inhibitor toxicities, *Mayo Clin. Proc.* 94 (2019) 1321–1329.
- [43] M Conroy, J Naidoo, Immune-related adverse events and the balancing act of immunotherapy, *Nat. Commun.* 13 (2022) 392.
- [44] CW Mount, RG Majzner, S Sundares, EP Arnold, M Kadapakkam, S Haile, L Labanieh, E Hulleman, PJ Woo, SP Rietberg, et al., Potent antitumor efficacy of anti-GD2 CAR T cells in H3-K27M(+) diffuse midline gliomas, *Nat. Med.* 24 (2018) 572–579.
- [45] J Mahdi, J Dietrich, K Straathof, C Roddie, BJ Scott, TB Davidson, LM Prolo, TT Batchelor, CJ Campen, KL Davis, et al., Tumor inflammation-associated neurotoxicity, *Nat. Med.* 29 (2023) 803–810.
- [46] H Urban, E Steidl, E Hattingen, K Filipski, M Meissner, M Sebastian, A Koch, A Strzelczyk, MT Forster, P Baumgarten, et al., Immune checkpoint inhibitor-induced cerebral pseudoprogression: patterns and categorization, *Front. Immunol.* 12 (2021) 798811.
- [47] E Hwang, L Kilburn, A Onar, T Lin, T Young-Poussaint, D Mitchell, A Margol, I Dunkel, S Gilheeny, M Fouladi, IMM-09. Outcome of patients with recurrent diffuse intrinsic pontine glioma (DIPG) treated with pembrolizumab (anti-PD-1): a Pediatric Brain Tumor Consortium study (PBT045), *Neuro-Oncol.* 20 (2018) i100–i100.
- [48] Ayuketang FA, Jager U (2022). *Management of cytokine release syndrome (CRS) and HLH*. Kroger N, Gribben J, Chabannon C, Yakoub-Agha I and Einsele H (eds): Cham (CH), pp. 135–139.

- [49] KA Hay, LA Hanafi, D Li, J Gust, WC Liles, MM Wurfel, JA Lopez, J Chen, D Chung, S Harju-Baker, et al., Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy, *Blood* 130 (2017) 2295–2306.
- [50] J Gust, KA Hay, L-A Hanafi, D Li, D Myerson, LF Gonzalez-Cuyar, C Yeung, WC Liles, M Wurfel, JA Lopez, et al., Endothelial activation and blood–brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells, *Cancer Discov.* 7 (2017) 1404–1419.
- [51] KL Lowe, CL Mackall, E Norry, R Amado, BK Jakobsen, G Binder, Fludarabine and neurotoxicity in engineered T-cell therapy, *Gene Ther.* 25 (2018) 176–191.
- [52] LC Hygino da Cruz, I Rodriguez, RC Domingues, EL Gasparetto, AG Sorensen, Pseudoprogression and pseudoresponse: imaging challenges in the assessment of post-treatment glioma, *Am. J. Neuroradiol.* 32 (2011) 1978–1985.
- [53] C Dromain, C Beigelman, C Pozzessere, R Duran, A Digkila, Imaging of tumour response to immunotherapy, *Eur. Radiol. Exp.* 4 (2020) 2.
- [54] BB Kasten, N Udayakumar, JW Leavenworth, AM Wu, SE Lapi, JE McConathy, AG Sorace, AK Bag, JM Markert, JM Warram, Current and future imaging methods for evaluating response to immunotherapy in neuro-oncology, *Theranostics* 9 (2019) 5085–5104.
- [55] R Garcia-Figueiras, S Baleato-Gonzalez, A Luna, J Munoz-Iglesias, L Oleaga, JA Vallejo Casas, T Martin-Noguerol, J Broncano, MC Areses, JC Vilanova, Assessing immunotherapy with functional and molecular imaging and radiomics, *Radiographics* 40 (2020) 1987–2010.
- [56] AM Griesinger, DK Birks, AM Donson, V Amani, LM Hoffman, A Waziri, M Wang, MH Handler, NK Foreman, Characterization of distinct immunophenotypes across pediatric brain tumor types, *J. Immunol.* 191 (2013) 4880–4888.
- [57] AS Plant, S Koyama, C Sinai, IH Solomon, GK Griffin, KL Ligon, P Bandopadhyay, R Betensky, R Emerson, G Dranoff, et al., Immunophenotyping of pediatric brain tumors: correlating immune infiltrate with histology, mutational load, and survival and assessing clonal T cell response, *J. Neurooncol.* 137 (2018) 269–278.
- [58] TJ Alban, AG Alvarado, MD Sorensen, D Bayik, J Volovetz, E Serbinowski, EE Mulkearns-Hubert, M Sinyuk, JS Hale, GR Onzi, et al., Global immune fingerprinting in glioblastoma patient peripheral blood reveals immune-suppression signatures associated with prognosis, *JCI Insight* 3 (2018).
- [59] P Chongsathidkiet, C Jackson, S Koyama, F Loebel, X Cui, SH Farber, K Woroniecka, AA Elsamadicy, CA Dechant, HR Kemeny, et al., Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors, *Nat. Med.* 24 (2018) 1459–1468.
- [60] I Liu, L Jiang, ER Samuelsson, S Marco Salas, A Beck, OA Hack, D Jeong, ML Shaw, B Englinger, J LaBelle, et al., The landscape of tumor cell states and spatial organization in H3-K27M mutant diffuse midline glioma across age and location, *Nat. Genet.* 54 (2022) 1881–1894.
- [61] D Schafflick, CA Xu, M Hartlehnert, M Cole, A Schulte-Mecklenbeck, T Lautwein, J Wolbert, M Heming, SG Meuth, T Kuhlmann, et al., Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis, *Nat. Commun.* 11 (2020) 247.
- [62] C Rubio-Perez, E Planas-Rigol, JL Trincado, E Bonfill-Texidor, A Arias, D Marchese, C Moutinho, G Serna, L Pedrosa, R Iurlaro, et al., Immune cell profiling of the cerebrospinal fluid enables the characterization of the brain metastasis microenvironment, *Nat. Commun.* 12 (2021) 1503.
- [63] F Mouliere, CG Smith, K Heider, J Su, Y van der Pol, M Thompson, J Morris, JCM Wan, D Chandrananda, J Hadfield, et al., Fragmentation patterns and personalized sequencing of cell-free DNA in urine and plasma of glioma patients, *EMBO Mol. Med.* 13 (2021) e12881.
- [64] APY Liu, KS Smith, R Kumar, L Paul, L Bihannic, T Lin, KK Maass, KW Pajtl, M Chintagumpala, JM Su, et al., Serial assessment of measurable residual disease in medulloblastoma liquid biopsies, *Cancer Cell* 39 (2021) 1519–1530 e1514.
- [65] F Nassiri, A Chakravarthy, S Feng, SY Shen, R Nejad, JA Zuccato, MR Voisin, V Patil, C Horbinski, K Aldape, et al., Detection and discrimination of intracranial tumors using plasma cell-free DNA methylomes, *Nat. Med.* 26 (2020) 1044–1047.
- [66] J Wang, F Che, J Zhang, M Zhang, S Xiao, Y Liu, L Zhou, Q Su, C You, Y Lu, et al., Diagnostic and prognostic potential of serum cell-free microRNA-214 in glioma, *World Neurosurg.* 125 (2019) e1217–e1225.
- [67] MA Bustos, N Rahimzadeh, S Ryu, R Gross, LT Tran, VM Renteria-Lopez, RI Ramos, A Eisenberg, P Hothi, S Kesari, et al., Cell-free plasma microRNAs that identify patients with glioblastoma, *Lab. Invest.* 102 (2022) 711–721.
- [68] A Piazza, P Rosa, L Ricciardi, A Mangraviti, L Pacini, A Calogero, A Raco, M Miscusi, Circulating exosomal-DNA in glioma patients: a quantitative study and histopathological correlations—a preliminary study, *Brain Sci.* 12 (2022).
- [69] C Muller, J Holtschmidt, M Auer, E Heitzer, K Lamszus, A Schulte, J Matschke, S Langer-Freitag, C Gasch, M Stoupiec, et al., Hematogenous dissemination of glioblastoma multiforme, *Sci. Transl. Med.* 6 (2014) 247ra101.
- [70] S Mueller, P Jain, WS Liang, L Kilburn, C Kline, N Gupta, E Panditharatna, SN Magge, B Zhang, Y Zhu, et al., A pilot precision medicine trial for children with diffuse intrinsic pontine glioma-PNOC003: a report from the pediatric neuro-oncology consortium, *Int. J. Cancer* 145 (2019) 1889–1901.
- [71] E Izquierdo, P Proszek, G Pericoli, S Temelso, M Clarke, DM Carvalho, A Mackay, LV Marshall, F Carceller, D Hargrave, et al., Droplet digital PCR-based detection of circulating tumor DNA from pediatric high grade and diffuse midline glioma patients, *Neurooncol. Adv.* 3 (2021) vda013.
- [72] D Li, ER Bonner, K Wierzbicki, E Panditharatna, T Huang, R Lulla, S Mueller, C Koschmann, J Nazarian, AM Saratsis, Standardization of the liquid biopsy for pediatric diffuse midline glioma using ddPCR, *Sci. Rep.* 11 (2021) 5098.
- [73] E Cantor, K Wierzbicki, RS Tarapore, K Ravi, C Thomas, R Cartaxo, V Nand Yadav, R Ravindran, AK Bruzek, J Wadden, et al., Serial H3K27M cell-free tumor DNA (cf-tDNA) tracking predicts ONC201 treatment response and progression in diffuse midline glioma, *Neuro. Oncol.* 24 (2022) 1366–1374.
- [74] L Escudero, A Llor, A Arias, A Diaz-Navarro, F Martinez-Ricarte, C Rubio-Perez, R Mayor, G Caratu, E Martinez-Saez, E Vazquez-Mendez, et al., Circulating tumour DNA from the cerebrospinal fluid allows the characterisation and monitoring of medulloblastoma, *Nat. Commun.* 11 (2020) 5376.
- [75] AM Miller, L Szalontay, N Bouvier, K Hill, H Ahmad, J Rafailov, AJ Lee, MI Rodriguez-Sanchez, O Yildirim, A Patel, et al., Next-generation sequencing of cerebrospinal fluid for clinical molecular diagnostics in pediatric, adolescent and young adult (AYA) brain tumor patients, *Neuro Oncol.* (2022).
- [76] S Liu, Y Zhu, C Zhang, X Meng, B Sun, G Zhang, Y Fan, X Kang, The clinical significance of soluble programmed cell death-ligand 1 (SPD-L1) in patients with gliomas, *Front. Oncol.* 10 (2020) 9.
- [77] D Gu, X Ao, Y Yang, Z Chen, X Xu, Soluble immune checkpoints in cancer: production, function and biological significance, *J. Immunother. Cancer* 6 (2018) 132.
- [78] H Okada, M Weller, R Huang, G Finocchiaro, MR Gilbert, W Wick, BM Ellingson, N Hashimoto, IF Pollack, AA Brandes, et al., Immunotherapy response assessment in neuro-oncology: a report of the RANO working group, *Lancet Oncol.* 16 (2015) e534–e542.
- [79] F Nishimura, JE Dusa, J Eguchi, X Zhu, A Gambotto, WJ Storkus, H Okada, Adoptive transfer of type 1 CTL mediates effective anti-central nervous system tumor response: critical roles of IFN-inducible protein-10, *Cancer Res.* 66 (2006) 4478–4487.
- [80] D Duluc, M Corvaisier, S Blanchard, L Catala, P Descamps, E Gamelin, S Ponsoda, Y Delneste, M Hebban, P Jeannin, Interferon-gamma reverses the immunosuppressive and protumoral properties and prevents the generation of human tumor-associated macrophages, *Int. J. Cancer* 125 (2009) 367–373.
- [81] P Bhat, G Leggett, N Waterhouse, IH Frazer, Interferon-gamma derived from cytotoxic lymphocytes directly enhances their motility and cytotoxicity, *Cell Death. Dis.* 8 (2017) e2836.
- [82] GP Dunn, CM Koebel, RD Schreiber, Interferons, immunity and cancer immunoediting, *Nat. Rev. Immunol.* 6 (2006) 836–848.
- [83] B Hu, J Ren, Y Luo, B Keith, RM Young, J Scholler, Y Zhao, CH June, Augmentation of antitumor immunity by human and mouse CAR T cells secreting IL-18, *Cell Rep.* 20 (2017) 3025–3033.
- [84] M Chmielewski, C Kopecky, AA Hombach, H Abken, IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression, *Cancer research* 71 (2011) 5697–5706.
- [85] G Krenciute, BL Prinzing, Z Yi, MF Wu, H Liu, G Dotti, IV Balyasnikova, S Gottschalk, Transgenic expression of IL15 improves antitumor activity of IL13Ralpha2-CAR T cells but results in antigen loss variants, *Cancer Immunol. Res.* 5 (2017) 571–581.
- [86] JL Lasky, EH Panosyan, A Plant, T DAVIDSON, WH YONG, RM PRINS, LM LIAU, TB MOORE, Autologous tumor lysate-pulsed dendritic cell immunotherapy for pediatric patients with newly diagnosed or recurrent high-grade gliomas, *Anticancer Research* 33 (2013) 2047–2056.
- [87] J Fangusaro, DA Mitchell, M Kocak, GW Robinson, PA Baxter, EI Hwang, J Huang, A Onar-Thomas, IJ Dunkel, M Fouladi, et al., Phase 1 study of pomalidomide in children with recurrent, refractory, and progressive central nervous system tumors: a pediatric brain tumor consortium trial, *Pediatr. Blood. Cancer* 68 (2021) e28756.
- [88] MA Shevtsov, AV Kim, KA Samochernych, IV Romanova, BA Margulis, IV Guzhova, IV Yakovenko, AM Ischenko, WA Khachatryan, Pilot study of intratumoral injection of recombinant heat shock protein 70 in the treatment of malignant brain tumors in children, *Onco. Targets Ther.* 7 (2014) 1071–1081.
- [89] JR Whiteaker, K Sharma, MA Hoffman, E Kuhn, L Zhao, AR Cocco, RM Schoenherr, JJ Kennedy, U Voytovich, C Lin, et al., Targeted mass spectrometry-based assays enable multiplex quantification of receptor tyrosine kinase, MAP Kinase, and AKT signaling, *Cell Rep. Method.* 1 (2021).
- [90] A Dzutsev, JH Badger, E Perez-Chanona, S Roy, R Salcedo, CK Smith, G Trinchieri, Microbes and cancer, *Annu. Rev. Immunol.* 35 (2017) 199–228.
- [91] Human Microbiome Project C, Structure, function and diversity of the healthy human microbiome, *Nature* 486 (2012) 207–214.
- [92] M Vezizou, JM Pitt, R Daillere, P Lepage, N Waldschmidt, C Flament, S Rusakiewicz, B Routy, MP Roberti, CP Duong, et al., Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota, *Science* 350 (2015) 1079–1084.
- [93] V Gopalakrishnan, CN Spencer, L Nezi, A Reuben, MC Andrews, TV Karpinet, PA Prieto, D Vicente, K Hoffman, SC Wei, et al., Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients, *Science* 359 (2018) 97–103.
- [94] M Smith, A Dai, G Ghilardi, KV Amelsberg, SM Devlin, R Pajarillo, JB Slingerland, S Beghi, PS Herrera, P Giardina, et al., Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy, *Nat. Med.* 28 (2022) 713–723.
- [95] D Davar, AK Dzutsev, JA McCulloch, RR Rodrigues, JM Chauvin, RM Morrison, RN Deblasio, C Menna, Q Ding, O Pagliano, et al., Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients, *Science* 371 (2021) 595–602.
- [96] H Jiang, W Zeng, X Zhang, Y Pei, H Zhang, Y Li, The role of gut microbiota in patients with benign and malignant brain tumors: a pilot study, *Bioengineered* 13 (2022) 7847–7859.
- [97] M Sun, W Wu, Z Liu, Y Cong, Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases, *J. Gastroenterol.* 52 (2017) 1–8.
- [98] Loris De Cecco VB, Elisabetta Schiavello, Andrea Carenzo, Maria Federica Iannò, Armando Licata, Manuela Marra, Maria Carollo, Luna Boschetti, Maura Massimino, DIPG-36. The brain-gut-microbiota axis to predict outcome in pediatric diffuse intrinsic pontine glioma, *Neuro-Oncol.* 24 (2022) i26.

- [99] C Mirzayi, A Renson, C Genomic Standards, A Massive, S Quality Control, F Zohra, S Elsafoury, L Geistlinger, LJ Kasselmann, K Eckenrode, et al., Reporting guidelines for human microbiome research: the STORMS checklist, *Nat. Med.* 27 (2021) 1885–1892.
- [100] ML Persson, AM Douglas, F Alvaro, P Faridi, MR Larsen, MM Alonso, NA Vitanza, MD Dun, The intrinsic and microenvironmental features of diffuse midline glioma; implications for the development of effective immunotherapeutic treatment strategies, *Neuro. Oncol.* (2022).
- [101] NAP Lieberman, K DeGolier, HM Kovar, A Davis, V Høglund, J Stevens, C Winter, G Deutsch, SN Furlan, NA Vitanza, et al., Characterization of the immune microenvironment of diffuse intrinsic pontine glioma: implications for development of immunotherapy, *Neuro-Oncol.* 21 (2019) 83–94.
- [102] GL Lin, S Nagaraja, MG Filbin, ML Suva, H Vogel, M Monje, Non-inflammatory tumor microenvironment of diffuse intrinsic pontine glioma, *Acta Neuropathol. Commun.* 6 (2018) 51.
- [103] HL Kinkead, A Hopkins, E Lutz, AA Wu, M Yarchoan, K Cruz, S Woolman, T Vithayathil, LH Glickman, CO Ndubaku, et al., Combining STING-based neoantigen-targeted vaccine with checkpoint modulators enhances antitumor immunity in murine pancreatic cancer, *JCI Insight* 3 (2018).
- [104] JP Antonios, H Soto, RG Everson, J Orpilla, D Moughon, N Shin, S Sedighim, WH Yong, G Li, TF Cloughesy, et al., PD-1 blockade enhances the vaccination-induced immune response in glioma, *JCI Insight* 1 (2016).
- [105] Y Song, Q Liu, T Zuo, G Wei, S Jiao, Combined antitumor effects of anti-EGFR variant III CAR-T cell therapy and PD-1 checkpoint blockade on glioblastoma in mouse model, *Cell. Immunol.* 352 (2020) 104112.
- [106] RJ Duchatel, A Mannan, AS Woldu, T Hawtrey, PA Hindley, AM Douglas, ER Jackson, IJ Findlay, ZP Germon, D Staudt, et al., Preclinical and clinical evaluation of German-sourced ONC201 for the treatment of H3K27M-mutant diffuse intrinsic pontine glioma, *Neurooncol. Adv.* 3 (2021) vdab169.
- [107] JE Romaguera, HJ Lee, R Tarapore, V Prabhu, J Allen, L Schalop, A Zloza, CY Ok, ET Sadimin, J Schenkel, et al., Integrated stress response and immune cell infiltration in an ibrutinib-refractory mantle cell lymphoma patient following ONC201 treatment, *Br. J. Haematol.* 185 (2019) 133–136.
- [108] J Wagner, CL Kline, L Zhou, KS Campbell, AW MacFarlane, AJ Olszanski, KQ Cai, HH Hensley, EA Ross, MD Ralf, et al., Dose intensification of TRAIL-inducing ONC201 inhibits metastasis and promotes intratumoral NK cell recruitment, *J. Clin. Invest.* 128 (2018) 2325–2338.
- [109] M Ruckert, AS Flohr, M Hecht, US Gaip, Radiotherapy and the immune system: More than just immune suppression, *Stem Cell.* 39 (2021) 1155–1165.
- [110] MM Ahmed, JW Hodge, C Guha, EJ Bernhard, B Vikram, CN Coleman, Harnessing the potential of radiation-induced immune modulation for cancer therapy, *Cancer Immunol. Res.* 1 (2013) 280–284.
- [111] EA Reits, JW Hodge, CA Herberts, TA Groothuis, M Chakraborty, EK Wansley, K Camphausen, RM Luiten, AH de Ru, J Neijssen, et al., Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful anti-tumor immunotherapy, *J. Exp. Med.* 203 (2006) 1259–1271.
- [112] A Omuro, DA Reardon, JH Sampson, J Baehring, S Sahebjam, TF Cloughesy, A-G Chalamandaris, V Potter, N Butowski, M Lim, Nivolumab plus radiotherapy with or without temozolomide in newly diagnosed glioblastoma: Results from exploratory phase I cohorts of CheckMate 143, *Neuro-Oncol. Adv.* 4 (2022).
- [113] X Wan, M Fang, T Chen, H Wang, Q Zhou, Y Wei, L Zheng, Y Zhou, K Chen, The mechanism of low-dose radiation-induced upregulation of immune checkpoint molecule expression in lung cancer cells, *Biochem. Biophys. Res. Commun.* 608 (2022) 102–107.
- [114] X Liu, Y Pu, K Cron, L Deng, J Kline, WA Frazier, H Xu, H Peng, YX Fu, MM Xu, CD47 blockade triggers T cell-mediated destruction of immunogenic tumors, *Nat. Med.* 21 (2015) 1209–1215.
- [115] RJ Duchatel, ER Jackson, F Alvaro, B Nixon, H Hondermarck, MD Dun, Signal transduction in diffuse intrinsic pontine glioma, *Proteomics* 19 (2019) e1800479.
- [116] IJ Findlay, GN De Iuliis, RJ Duchatel, ER Jackson, NA Vitanza, JE Cain, SM Waszak, MD Dun, Pharmacoproteomic profiling of pediatric diffuse midline glioma to inform future treatment strategies, *Oncogene* 41 (2022) 461–475.
- [117] S Chandrasekaran, CR Funk, T Kleber, CM Paulos, M Shanmugam, EK Waller, Strategies to overcome failures in T-cell immunotherapies by targeting PI3K-delta and -gamma, *Front. Immunol.* 12 (2021) 718621.
- [118] CR Funk, S Wang, KZ Chen, A Waller, A Sharma, CL Edgar, VA Gupta, S Chandrasekaran, JT Zoine, A Fedanov, et al., PI3Kdelta/gamma inhibition promotes human CART cell epigenetic and metabolic reprogramming to enhance antitumor cytotoxicity, *Blood* 139 (2022) 523–537.
- [119] R Abu-Eid, RN Samara, L Ozbun, MY Abdalla, JA Berzofsky, KM Friedman, M Mkrtichyan, SN Khleif, Selective inhibition of regulatory T cells by targeting the PI3K-Akt pathway, *Cancer Immunol. Res.* 2 (2014) 1080–1089.
- [120] LS Carnevali, C Sinclair, MA Taylor, PM Gutierrez, S Langdon, AML Coenen-Stass, L Mooney, A Hughes, L Jarvis, A Staniszewska, et al., PI3Kalpha/delta inhibition promotes anti-tumor immunity through direct enhancement of effector CD8(+) T-cell activity, *J. Immunother.* 36 (2018) 158.
- [121] S Zhu, AH Ma, Z Zhu, E Adib, T Rao, N Li, K Ni, V Chittepu, R Prabhala, J Garisto Risco, et al., Synergistic antitumor activity of pan-PI3K inhibition and immune checkpoint blockade in bladder cancer, *J. Immunother.* 9 (2021).
- [122] Z Miklja, VN Yadav, RT Cartaxo, R Siada, CC Thomas, JR Cummings, B Mullan, S Stallard, A Paul, AK Bruzek, et al., Everolimus improves the efficacy of dasatinib in PDGFRalpha-driven glioma, *J. Clin. Invest.* 130 (2020) 5313–5325.
- [123] AE Schade, GL Schieven, R Townsend, AM Jankowska, V Susulic, R Zhang, H Szpurka, JP Maciejewski, Dasatinib, a small-molecule protein tyrosine kinase inhibitor, inhibits T-cell activation and proliferation, *Blood* 111 (3) (2008) 1366–1377.
- [124] K Mestermann, T Giavridis, J Weber, J Rydzek, S Frenz, T Nerretter, A Mades, M Sadelain, H Einsele, M Hudecek, The tyrosine kinase inhibitor dasatinib acts as a pharmacologic on/off switch for CAR T cells, *Sci. Transl. Med.* 11 (2019).
- [125] EW Weber, KR Parker, E Sotillo, RC Lynn, H Anbunathan, J Lattin, Z Good, JA Belk, B Daniel, D Klysz, et al., Transient rest restores functionality in exhausted CAR-T cells through epigenetic remodeling, *Science* (2021) 372.
- [126] O Khan, JR Giles, S McDonald, S Manne, SF Ngwi, KP Patel, MT Werner, AC Huang, KA Alexander, JE Wu, et al., TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion, *Nature* 571 (2019) 211–218.
- [127] JA Fraietta, CL Nobles, MA Sammons, S Lundh, SA Carty, TJ Reich, AP Cogdill, JJD Morrisette, JE DeNizio, S Reddy, et al., Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells, *Nature* 558 (2018) 307–312.
- [128] B Prinzing, CC Zebly, CT Petersen, Y Fan, AA Anido, Z Yi, P Nguyen, H Houke, M Bell, D Haydar, et al., Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity, *Sci. Transl. Med.* 13 (2021) eab0272.
- [129] CC Kloss, J Lee, A Zhang, F Chen, JJ Melenhorst, SF Lacey, MV Maus, JA Fraietta, Y Zhao, CH June, Dominant-negative TGF-beta receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication, *Mol. Ther.* 26 (2018) 1855–1866.
- [130] X Li, M Wenes, P Romero, SC Huang, SM Fendt, PC Ho, Navigating metabolic pathways to enhance antitumor immunity and immunotherapy, *Nat. Rev. Clin. Oncol.* 16 (2019) 425–441.
- [131] K DePeaux, GM Delgoffe, Metabolic barriers to cancer immunotherapy, *Nat. Rev. Immunol.* 21 (2021) 785–797.
- [132] A Biederstadt, GS Manzar, M Daher, Multiplexed engineering and precision gene editing in cellular immunotherapy, *Front. Immunol.* 13 (2022) 1063303.
- [133] DM O'Rourke, MP Nasrallah, A Desai, JJ Melenhorst, K Mansfield, JJD Morrisette, M Martinez-Lage, S Brem, E Maloney, A Shen, et al., A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma, *Sci. Transl. Med.* 9 (2017).
- [134] N Hilf, S Kuttruff-Coqui, K Frenzel, V Bukur, S Stevanovic, C Gouttefangeas, M Platten, G Tabatabai, V Dutoit, SH van der Burg, et al., Actively personalized vaccination trial for newly diagnosed glioblastoma, *Nature* 565 (2019) 240–245.
- [135] M Hegde, A Corder, KKH Chow, M Mukherjee, A Ashoori, Y Kew, YJ Zhang, DS Baskin, FA Merchant, VS Brawley, et al., Combinational targeting offsets antigen escape and enhances effector functions of adoptively transferred T cells in glioblastoma, *Mol. Cell. Ther.* 21 (2013) 2087–2101.
- [136] M Hegde, M Mukherjee, Z Grada, A Pignata, D Landi, SA Navai, A Wakefield, K Fousek, K Bielamowicz, KK Chow, et al., Tandem CAR T cells targeting HER2 and IL13Ralpha2 mitigate tumor antigen escape, *J. Clin. Invest.* 126 (2016) 3036–3052.
- [137] K Bielamowicz, K Fousek, TT Byrd, H Samaha, M Mukherjee, N Aware, MF Wu, JS Orange, P Sumazin, TK Man, et al., Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma, *Neuro. Oncol.* 20 (2018) 506–518.
- [138] M Yarmarkovich, QF Marshall, JM Warrington, R Premaratne, A Farrel, D Groff, W Li, M di Marco, E Runbeck, H Truong, et al., Cross-HLA targeting of intracellular oncoproteins with peptide-centric CARs, *Nature* (2021).