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Immunotherapy for pediatric brain tumors: past and present

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Abstract

The field of cancer immunotherapy has progressed at an accelerated rate over the past decade. Pediatric brain tumors thus far have presented a formidable challenge for immunotherapy development, given their typically low mutational burden, location behind the blood–brain barrier in a unique tumor microenvironment, and intratumoral heterogeneity. Despite these challenges, recent developments in the field have resulted in exciting preclinical evidence for various immunotherapies and multiple clinical trials. This work reviews the history and advances in active immunotherapy, checkpoint blockade, and adoptive T-cell therapy for pediatric brain tumors, including ongoing clinical trials.

Keywords

brain tumor | immunotherapy | pediatric

Pediatric brain tumors encompass a wide array of histologic subtypes that occur throughout the central nervous system with varying degrees of aggressiveness. Despite many advancements in surgical, radiotherapeutic, and chemotherapeutic approaches over several decades, brain tumors represent the leading cause of disease-related death among children.[1] In addition, secondary toxicity and morbidity related to standard treatments leave the majority of surviving children affected by these tumors with lasting neurocognitive and physical deficits.[2] As the field of cancer immunotherapy continues to advance,[3] there has been renewed hope that a cure may come for these devastating tumors, potentially without the burden of toxicities associated with traditional therapies. In this review, we discuss the current state of immunotherapies in pediatric neuro-oncology, including vaccine approaches, checkpoint blockade, and adoptive cell therapy, as well as the future directions for the field.

Unique Considerations for Immunotherapy in the Brain

The central nervous system (CNS) is considered an immune-privileged site, separate from the complement of immune cells and signals that act on the remainder of the body. This immune privilege is derived from the blood–brain barrier (BBB), a highly specialized interface between the blood and CNS parenchyma created by capillary endothelia, pericytes, and astrocytes.[4] An additional barrier between the blood and cerebrospinal fluid (CSF) is

formed by adherent junctions between specialized epithelial cells. The BBB and CSF–brain barrier protect the brain from unwanted toxins, pathogens, and inflammation. Rather than being an absolute barrier penetrable only by disruption, the BBB is tightly regulated to allow for specific immune events to transfer from the periphery under precise endothelial cell signaling.[5,6] Routine immune surveillance occurs in the absence of neuroinflammation via draining lymphatics to deep cervical lymph nodes, with rare translocation of immune cells across the BBB.[7,8] Antigen presenting cells (APCs) are strategically positioned behind the blood–brain and blood–CSF barrier and their activation allows for changes to the signals, substances, and cells crossing these barriers.[4]

The tumor microenvironment (TME) has been shown to be unique for brain tumors compared with those found elsewhere in the body. As discussed, the blood–brain and CSF–brain barriers do not allow for many immune cells within the brain, and likewise, brain tumors have minimal immune cell infiltration. Macrophages and microglia predominate and tend to be pro-tumorigenic given their secretion of growth factors and cytokines[9] and lack of machinery for appropriate T-cell activation.[10] Furthermore, brain tumors have a unique extracellular matrix that has been shown to trap T cells and prevent migration into tumors.[11] The majority of studies on the TME in brain tumors have used adult glioblastoma multiforme (GBM) models, which may not accurately represent the TME of pediatric brain tumors. Several studies have indicated that immunophenotypes of pediatric brain tumors may be less immunosuppressive than adult brain tumors, and the TME tends to vary between types of pediatric brain tumors.[12–16] These findings suggest that immunotherapy strategies should be tailored based on the type of tumor being targeted and its associated microenvironment.

Importantly, inflammation secondary to immunotherapy can have significant consequences in the brain. The physical restriction of the cranial vault paired with mass effect from edema can cause damage to normal brain tissue or even herniation. Furthermore, tumor inflammation can make interpretation of clinical imaging challenging. Pseudoprogression refers to a transient increase in tumor size related to treatment effect rather than true progression. The term was originally used to describe increased enhancement and size of a lesion on imaging several weeks to months following radiotherapy, in particular when combined with temozolomide,[17] and more recently has been used in immunotherapy to describe transient increases in the size of lesions secondary to inflammation. In 2015, Okado et al set new guidelines for imaging interpretation post-immunotherapy, termed immunotherapy response assessment for neuro-oncology (iRANO),[18] incorporating pseudoprogression into the previously established RANO criteria. Accounting for the increased edema that can occur as a result of immune stimulation up to 6 months after immunotherapy, iRANO recommends close interval imaging follow-up to distinguish true progression from pseudoprogression. Unfortunately, there is no more specific modality to differentiate true progression from pseudoprogression, although immune biomarkers remain an area of intense interest.

Active Immunotherapy: Vaccines and Oncolytic Viruses

The concept of activating the immune system to treat cancer (often termed active immunotherapy) first arose in the late nineteenth century when William Coley injected bacteria into various tumors to induce an immune response.[19] Building upon this, researchers have since elucidated the ways in which the immune system is capable of interacting with tumor cells with the hope of harnessing these forces to develop active immunotherapies. In general, immune activation occurs through antigen presentation on major histocompatibility complex (MHC) molecules (also known as human leukocyte antigen [HLA]) or an MHC-independent fashion. For MHC-dependent activation, intracellular and extracellular proteins undergo digestion into short peptides for extracellular display on class I or class II MHC molecules.[20] Peptides on the MHC molecule can then be recognized as an antigen by T cells via their T-cell receptor (TCR) and elicit an immune response (Figure 1).[21] Peptide binding to the MHC is highly dependent on a patient's specific HLA genotype, a factor that becomes important when designing and implementing therapies based on this antigen type. Antigen epitopes can also result in immune activation in an MHC-independent fashion, typically through antibody-dependent B-cell activation.[22]

Tumor antigens fall into 2 general categories: tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs). As the name suggests, TSAs are defined by their uniqueness to the tumor, since they are completely absent in normal tissues. They can originate from nonsynonymous mutations found only in the tumor genome (ie, missense, nonsense point mutations, fusions, or frameshifts) or through other genetic alterations at the DNA, RNA, or protein level that result in the generation of novel proteins. These unique, mutation-derived proteins capable of immune stimulation are often termed "neoantigens."[23] TSAs can also arise through expression of virally encoded oncogenic proteins not found in the host genome. TAAs, unlike TSAs, are derived from proteins that lack any tumor-specific mutations or alterations. The antigenic potential of TAAs arises from their expression at significantly higher levels in tumor cells compared with host normal tissues.[20] A subset of TAAs, cancer-testis antigens, are antigens expressed by tumor cells that are also expressed only in host reproductive or fetal tissues.[20]

As targets for immunotherapy, TSAs and TAAs each have various advantages and disadvantages. TSAs, in particular neoantigens, are attractive given their ability to elicit a robust immune response, since they were exempt from central tolerance during immune system development. Combined with their tumor-specific nature that may limit off-target toxicities to normal host tissues, TSAs and neoantigens are promising targets.[23] The highly specific nature of neoantigen-based therapies can also bring

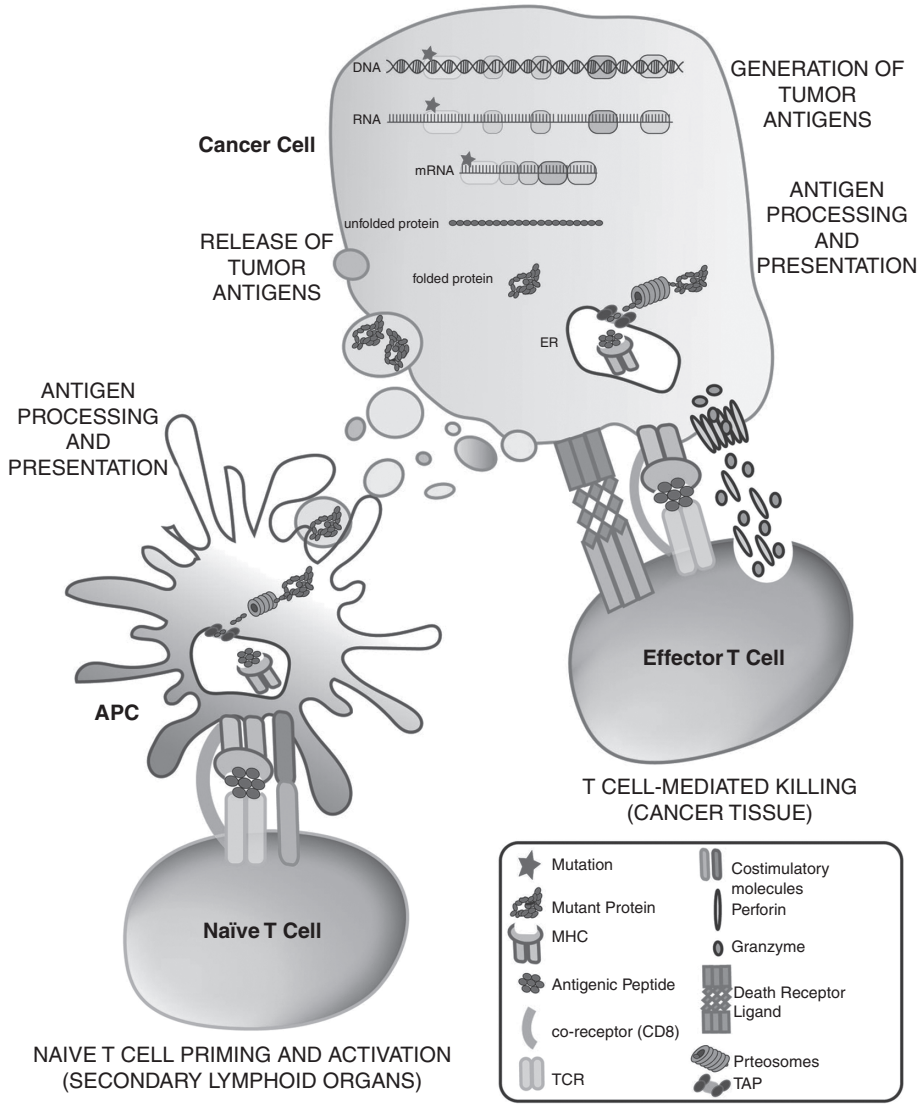

Figure 1. Immune activation by cancer cell TSAs. Mutations occurring in the cancer cell genome are transcribed and translated into mutant proteins ("neoantigen") that undergo digestion and processing onto MHC molecules for presentation to T cells via their specific TCR. Mutant proteins can also be released by the cancer cell for uptake and processing by APCs such as dendritic cells for immune activation. A similar process occurs for immune activation by TAAs except that self-antigens are being presented at high levels by tumor cells rather than novel or mutant proteins in the case of TSAs. Reproduced from Ajina et al,[21] with permission.

about challenges, as such therapies need to be tailored to individual tumor genomes and patient HLA genotypes, increasing the complexity of a therapy's design and implementation. Many of the difficulties that come with this need for personalization are diminishing given the rapid advances made in the field of functional genomics.[21] TAAs, on the other hand, are often shared among individuals within or even across cancer types, making therapies that target them easier to develop and more generalizable. The fact that TAAs derive from unmodified proteins, though, means they are viewed as self-antigens by the immune system and rely on a much smaller population of resting T cells for immune activation, potentially limiting the strength of immune response.[24] Their self-antigen nature also has the potential to bring about off-target effects such as autoimmunity.

Therapeutic Cancer Vaccines

One method of engaging the immune system to target cancer has been through active immunization against TSAs or TAAs with therapeutic cancer vaccines. To date, TAAs have been tested more often in vaccine development given their prevalence across patients and tumor types, a theme that holds true in the field of pediatric neuro-oncology. Identified TAAs are considered appropriate for vaccine development if they fulfill certain criteria: candidate TAAs

should have a tumor-specific expression pattern, be able to elicit a T-cell response similar to foreign antigens, and ideally should be derived from oncogenic proteins to limit the targeted cancer cell's ability to evade immune recognition through downregulation of the protein.[25] Following identification of antigens that fulfill these requirements, vaccines against TAAs can be generated utilizing DNA, RNA, or peptide delivery platforms, with peptide-based vaccines being the most frequently used to date. Peptide vaccines can be generated in vitro as either short (typically <15 amino acids) or long (>20 amino acids) synthetic peptides corresponding to the antigenic peptide.[24] These are then administered to the patient through injection into or near lymph nodes in hopes of directly supplying the peptides to dendritic cells (DCs) residing in lymph tissue that uptake and process the antigenic peptide for immune activation against the antigen. Uptake of the peptide vaccine is typically enhanced with the use of immunoadjuvants, such as polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose (poly-ICLC) or Montanide.[24]

Cancer vaccine strategies based on TSAs are similar to those that use TAAs, except that a personalized approach is required to identify tumor-specific mutations that underlie the TSAs. Advanced genomic techniques are often employed to computationally predict whether the peptides that result from the mutations bind to the patient's MHC alleles. The results of this prediction algorithm can be used to create personalized vaccines.[26] Occasionally, TSAs are shared across patients, allowing for a more generalizable vaccine approach that requires only basic identification of a previously discovered TSA within a patient's tumor. In the adult neuro-oncology field, the first promising attempt to target a TSA was rindopepimut, a peptide vaccine against epidermal growth factor receptor variant III (EGFRvIII), a shared TSA found in 30% of adult GBM patients.[27] Despite encouraging phase I and II data, the phase III randomized control trial of rindopepimut (ACT IV) unfortunately showed no difference in outcomes between the treatment and control arms, even in patients with robust anti-EGFRvIII humoral responses.[28]

A panel of peptide vaccines targeting TAAs have been developed at the University of Pittsburgh and tested in both high-grade and low-grade pediatric gliomas. Initial work was performed in patients with diffuse intrinsic pontine gliomas (DIPGs) and non-brainstem high-grade gliomas (HGGs), using peptide vaccines against glioma-associated TAAs ephrin type-A receptor 2 (EphA2), interleukin-13 receptor alpha 2 (IL13Rα2), and survivin with poly-ICLC.[29] Initial results showed promise, with 24 of 26 patients showing disease stabilization, partial response, or sustained disease-free survival. Notably, patients with brainstem gliomas that had evidence of pseudoprogression or tumor enlargement following vaccination had improved survival over those without pseudoprogression (19.5 vs 10.9 mo), suggesting that vaccination had incited inflammation that may be associated with a clinical response. A follow-up study was performed on 12 pediatric patients with relapsed HGG using the same vaccine targets with poly-ICLC.[30] Of 10 patients tested, 9 showed specific immune activation to the TAAs, most commonly against EphA2; however, only 2 of 12 patients appeared to have a clinical response. The study authors surmised that response rate may have been effected by TME factors, such as the presence of immunosuppressive molecules within the tumor, upregulation of checkpoint molecules, downregulation of antigen processing factors (MHC molecules), or increase in regulatory T cells.[30] Further investigation by this group in recurrent low-grade gliomas using the same peptide vaccines with poly-ICLC has shown more promising results.[31] Of 14 patients treated, 12 showed minor response, partial response, or stabilization of disease. One of the sustained partial responses occurred in a patient with metastatic disease who had a dramatic resolution of metastases that persisted for over 57 months at the time of publication. Similar to the relapsed HGG study, all 12 evaluable patients showed specific antitumor response, with EphA2 again being most common. Further investigation of this peptide vaccine cocktail is still ongoing, with a phase I clinical trial currently enrolling pediatric patients with gliomas (Table 1).

Another well-studied peptide vaccine that has moved from investigation in adult to pediatric brain tumor patients targets TAAs derived from Wilms tumor protein 1 (WT1). Initially studied in multiple adult malignancies, including GBM,[32,33] these vaccines have now been trialed in pediatric solid tumors, including brain tumors.[34,35] Results from early work have shown that the vaccines are well tolerated, but immune response to vaccination was variable. Given the heterogeneous populations and early results, efficacy of this approach in pediatric brain tumor patients has yet to be elucidated.

More recent work has been done to characterize the neoantigen generated by the H3K27M mutation found in many midline HGG and DIPG in children and young adults. The identified neoantigen has demonstrated immunogenic potential in preclinical models,[36] which led to the conceptualization and opening of a vaccine clinical trial utilizing the immunogenic peptide of this driver mutation administered with poly-ICLC (Table 1). Additionally, advances in the design and generation of personalized neoantigen vaccines are rapidly pushing forward clinical trials of these agents into the field of neuro-oncology. Two recent studies have shown safety and evidence of immune activation with suggestion of efficacy of personalized neoantigen vaccines in adult GBM.[37,38] These early studies will certainly pave the way for investigation into personalized neoantigen-based therapies in pediatric brain tumor patients, but work remains to be done to establish the feasibility of such a method in a cohort that is often thought of as having a low neoantigen burden.

Dendritic Cell Vaccines

DCs are professional APCs at the foundation of a robust immune response. They are capable of capturing, processing, and presenting antigens to naive T cells for immune activation against an antigen.[39] Antigen presentation by DCs involves digestion and binding of antigen to MHC molecules as previously described in relation to TAAs and TSAs (Figure 1), with the exception that DCs are focused on the uptake of antigen from their extracellular environment for presentation. This powerful antigen processing and immune activation can be harnessed through the use of DC

Table 1 List of current clinical trials for pediatric brain tumors utilizing immunotherapy

Clinical Trial	NCT Number	Status	Locations Offered	Therapeutic Mechanism
Vaccines				
A Pilot Study of Glioma Associated Antigen Vaccines in Conjunction With Poly-ICLC in Pediatric Gliomas	NCT01130077	Recruiting	Pittsburgh, PA	Peptide vaccine
Dendritic Cell (DC) Vaccine for Malignant Glioma and Glioblastoma	NCT01808820	Recruiting	Miami, FL	Dendritic cell vaccine
Trial of Heat Shock Protein Peptide Complex-96 (HSPPC-96) Vaccine	NCT02722512	Recruiting	Chicago, IL	Peptide vaccine
Dendritic Cell-Based Tumor Vaccine Adjuvant Immunotherapy of Human Glioblastoma Multiforme (WHO Grade IV Gliomas)	NCT02772094	Recruiting	China	Dendritic cell vaccine
A Trial of Poly-ICLC in the Management of Recurrent Pediatric Low Grade Gliomas (Poly-ICLC)	NCT01188096	Recruiting	San Diego, CA and Atlanta, GA	ICLC intramuscular injections
H3.3K27M Peptide Vaccine for Children With Newly Diagnosed DIPG and Other Gliomas	NCT02960230	Recruiting	Multiple US sites— PNOC	Peptide vaccine
Cytomegalovirus (CMV) RNA-Pulsed Dendritic Cells for Pediatric Patients With Newly Diagnosed WHO Grade IV Glioma, Recurrent Malignant Glioma, or Recurrent Medulloblastoma (ATTAC-P)	NCT03615404	Active, not recruiting	Durham, NC	CMV Dendritic cell vaccine
Oncolytic Virus				
HSV G207 Alone or With a Single Radiation Dose in Children With Progressive or Recurrent Supratentorial Brain Tumors	NCT02457845	Recruiting	Birmingham, AL	Oncolytic virus
A Phase I Study of AdV-tk + Prodrug Therapy in Combination With Radiation Therapy for Pediatric Brain Tumors	NCT00634231	Active, not recruiting	Boston, MA and Chicago, IL	Adenovirus carrying HSV-TK
Modified Measles Virus (MV-NIS) for Children and Young Adults With Recurrent Medulloblastoma or Recurrent ATRT	NCT02962167	Recruiting	Multiple US sites—PNOC	Oncolytic virus
Checkpoint Inhibitors				
Pembrolizumab in Treating Younger Patients With Recurrent, Progressive, or Refractory High-Grade Gliomas, Diffuse Intrinsic Pontine Gliomas, or Hypermutated Brain Tumors	NCT02359565	Recruiting	Multiple US sites—PBTC	Checkpoint blockade
A Study of Pembrolizumab (MK-3475) in Pediatric Participants With an Advanced Solid Tumor or Lymphoma (MK-3475-051/KEYNOTE-051)	NCT02332668	Recruiting	Multiple US sites—COG	Checkpoint blockade
Nivolumab in Combination With Metronomic Chemotherapy in Paediatrics Refractory / Relapsing Solid Tumors or Lymphoma	NCT03585465	Not yet recruiting	Belgium	Checkpoint blockade
Pilot Study of Nivolumab in Pediatric Patients With Hypermutant Cancers	NCT02992964	Recruiting	Multiple global sites—Philadelphia, PA	Checkpoint blockade
An Investigational Immuno-therapy Study of Nivolumab Monotherapy and Nivolumab in Combination With Ipilimumab in Pediatric Patients With High Grade Primary CNS Malignancies (CheckMate 908)	NCT03130959	Recruiting	Multiple US and global sites	Checkpoint blockade
Study of the IDO Pathway Inhibitor, Indoximod, and Temozolomide for Pediatric Patients With Progressive Primary Malignant Brain Tumors	NCT02502708	Recruiting	Atlanta, GA	Checkpoint blockade
Adoptive Cellular Therapy				
Multi-antigen T Cell Infusion Against Neuro-oncologic Disease (REMIND)	NCT03652545	Recruiting	Washington, DC	CTL directed against WT1, PRAME and/or survivin
Brain Stem Gliomas Treated With Adoptive Cellular Therapy During Focal Radiotherapy Recovery Alone or With Dose-intensified Temozolomide (Phase I) (BRAVO)	NCT03396575	Recruiting	Gainesville, FL	CTL expanded from ex vivo tumor RNA-pulsed DC
Adoptive Cellular Therapy in Pediatric Patients With High-grade Gliomas (ACTION)	NCT03334305	Recruiting	Gainesville, FL	CTL expanded from ex vivo tumor RNA-pulsed DC
CMV-specific Cytotoxic T Lymphocytes Expressing CAR Targeting HER2 in Patients With GBM (HERT-GBM)	NCT01109095	Recruiting	Houston, TX	HER2-CD28 CMV-T cells

Table 1 Continued

Clinical Trial	NCT Number	Status	Locations Offered	Therapeutic Mechanism
Fourth Ventricle Infusions of Autologous Ex Vivo Expanded NK Cells in Children With Recurrent Posterior Fossa Tumors	NCT02271711	Recruiting	Houston, TX	Expanded NK cells
Phase 2 STIR Trial: Haploidentical Transplant and Donor Natural Killer Cells for Solid Tumors (STIR)	NCT02100891	Recruiting	Madison, WI	Stem cell transplant plus donor NK cells
Genetically Modified T-cells in Treating Patients With Recurrent or Refractory Malignant Glioma	NCT02208362	Recruiting	Duarte, CA	1L13Ra2 CART cells
HER2-specific CART Cell Locoregional Immunotherapy for HER2-positive Recurrent/Refractory Pediatric CNS Tumors	NCT03500991	Recruiting	Seattle, WA	HER2 CART Cells
EGFR806-specific CART Cell Locoregional Immunotherapy for EGFR-positive Recurrent or Refractory Pediatric CNS Tumors	NCT03638167	Recruiting	Seattle, WA	EGFR806 CART Cells
Antibodies				
Nimotuzumab in Combination With Radio-chemotherapy for the Treatment of Brainstem Tumor in Children	NCT02672241	Recruiting	China	EGFR antibody
Radiolabeled Monoclonal Antibody Therapy in Treating Patients With Refractory, Recurrent, or Advanced CNS or Leptomeningeal Cancer	NCT00089245	Recruiting	New York, NY	4Ig-B7-H3 antibody
Iodine I 131 Monoclonal Antibody 3F8 in Treating Patients With Central Nervous System Cancer or Leptomeningeal Cancer	NCT00445965	Recruiting	New York, NY	GD2 antibody
Intrathecal Radioimmunotherapy, Radiation Therapy, and Chemotherapy After Surgery in Treating Patients With Medulloblastoma	NCT00058370	Recruiting	New York, NY	Radioactive iodine I 131 antibody 3F8

vaccines (DCVs). Generation of DCVs typically involves isolating DCs or DC precursors from a patient for maturation, priming (or "pulsing") with tumor antigens, and returning them to the patient for activation of the immune system against the tumor. Antigen priming can occur through the introduction of synthetic antigenic peptides, administration of DNA or mRNA encoding for antigens, exposure to tumor lysate that contains antigens, or introduction of extracted tumor mRNA that contains transcripts encoding antigens.[39]

DCVs have been utilized in multiple trials of adult patients with glioblastoma[40] and have now advanced into the pediatric neuro-oncologic population. Initial studies over a decade ago utilized pulsed tumor-derived mRNA as an antigen source for DCVs administered to 9 pediatric and young adult patients across a variety of relapsed brain tumors.[41] Response to vaccine was minimal, with only 1 patient showing partial radiographic response. Interestingly, the authors found that all patients had qualitatively impaired cellular immune responses at baseline, despite normal quantitative levels of immune cells, suggesting an inherent level of immunodeficiency in their population of pediatric patients with malignant brain tumors. In the same year, De Vleeschouwer et al described a case report of a child with a recurrent malignant glioma who underwent resection and vaccination with DC pulsed with whole tumor lysate.[42] The patient showed transient metabolic activity on PET imaging at the edges of the resection cavity after vaccination, thought to represent an inflammatory response, which then subsided and she remained without disease at 2 years of follow up. Following this remarkable report, De Vleeschouwer and colleagues went on to lead the HGG-IMMUNO trial, evaluating tumor lysate DCV in relapsed HGG in children and adults.[43] Among 56 patients aged 7 to 77 who were treated with at least 3 vaccinations, their group saw a trend toward improved progression-free survival (PFS) in the cohort receiving weekly vaccinations coupled with a boost of intradermal injections of tumor lysates. Importantly, younger age was associated with a trend toward improved overall survival at 15.4 months, although it is unclear whether this represented a better response to the vaccination or a different underlying biology and natural course of the tumors in younger patients. Total resection was the only independent predictor of improved PFS, emphasizing the strong difference in outcome when administering immunotherapy in a minimal residual disease state compared with when macroscopic disease is present. Expanding on the pediatric group, the HGG-IMMUNO trial continued to enroll a total of 45 patients, including patients with any relapsed malignant brain tumor.[44] Given the large variability in pathology and upfront therapy received, the study focused on feasibility, since outcomes would be difficult to interpret. All patients entered were successfully treated, and there appeared to be subgroups of patients that responded favorably, with 7 HGG patients and 2 atypical teratoid rhabdoid tumor patients still alive at up to 7 years of follow-up.

Finally, more recently Lasky et al reported on their use of tumor lysate pulsed DCVs in a small cohort of pediatric HGG patients.[45] Feasibility was difficult to achieve in their trial, as only 3 of 7 patients survived long enough after resection to receive vaccination. As in prior trials, all 3 patients received different chemotherapy and radiation therapy, making interpretation of the effect of vaccine difficult. Two of the 3 patients were still alive at the time of publication, but 1 had relapsed at 1 year and subsequently responded to chemotherapy and radiation. The third patient with subtotal

resection progressed and passed away within 9 months, again pointing toward a need for minimal residual disease at the time of vaccination. Currently, there are a number of clinical trials open and enrolling pediatric and young adult patients with brain tumors for DCV (Table 1).

Oncolytic Viruses

An additional method of activating the immune system to induce a response against a tumor is through the use of oncolytic viruses. With genetic modification of various viruses, often herpes simplex virus (HSV), adenovirus, measles virus, or poliovirus, it is possible to target tumor cells for infection. This selectivity can be achieved through incorporation of tissue-specific promoters to ensure replication only in certain tissues or deletion of various genes to allow for viral replication only in actively dividing cells.[46] Following infection, cancer cells can be killed through direct oncolysis, induction of apoptosis, and immune activation to an infected cell.[46] Tumor lysis further releases tumor antigens into the extracellular space for uptake by APCs (eg, DCs), leading to T-cell recognition and activation against the tumor. For CNS malignancies, targeting the virus to the tumor can often be a challenge given the limitations of the BBB, but with new advances in drug delivery, this is becoming less of a hurdle. Intratumoral delivery of virus via convection-enhanced catheter delivery has been utilized by a number of groups now and is becoming more widespread for delivery of virus, pharmaceuticals, or cell-based therapies as a means of bypassing the BBB.

Oncolytic viruses have been used in several adult oncology trials, with particular success seen in patients with melanoma.[46] More recently, a modified poliovirus delivered via intratumoral convection-enhanced delivery in adult patients with recurrent GBM has shown promising safety and efficacy results in a phase I clinical trial.[47] In the pediatric group, researchers from Spain have utilized a modified adenovirus with specificity for glioma cells (DNX-2401) in an ongoing clinical trial for patients with DIPG.[48] This study, in which the virus is injected directly into the tumor at the time of biopsy, was initiated based on the phase I trial using DNX-2401 in adults with relapsed HGG that showed tumor shrinkage in 75% of patients, and 5 of 25 patients living longer than 3 years.[49] Tejada et al recently published on the successful administration of the virus into the pontine tumor of an 8-year-old girl, showing feasibility without toxicity, with outcomes still pending.[50] Additional trials (Table 1) are also ongoing using modified HSV (NCT02457845) and modified measles virus (NCT02962167).

In summary, various methods of active immunotherapy for pediatric brain tumors—peptide vaccines, DCVs, oncolytic viruses—have been under investigation for over a decade, with small trials showing some promising results and multiple additional studies currently under way (Table 1). Further preclinical work is still needed to elucidate the interaction of these various interventions with the TME, to assess for methods of resistance, and to determine the most appropriate biomarkers to assess for treatment efficacy. To this end, further work is needed to determine the best immune correlates that can be used to gauge efficacy, detect the development of resistance, and monitor response to these various active immunotherapies. Future studies should also pursue the use of combinatorial strategies, which has been pointed out by many authors and investigators as a likely need for any immunotherapy strategy. Active immunotherapeutic strategies, in particular, will likely need to be coupled with checkpoint inhibitors (discussed below) to boost immune activation in response to vaccine administration. Combining these therapies with interventions beyond other immunotherapies, such as chemotherapy and radiation, will also likely be important. Radiation, in particular, may play an important role, as it has been shown to have the potential to increase the antigenicity of a tumor.[51] Finally, given the numerous types of active immunization and number of researchers in this field, it will become important for the pediatric neuro-oncology group to prioritize investigations, especially with the small number of patients available for enrollment in clinical trials.

Immune Checkpoint Blockade

Background

In 1996, James Allison and colleagues propelled immunotherapy to center stage by demonstrating that blocking negative checkpoint regulators on T cells allowed for immune eradication of solid tumors in mice.[52] T cells display several proteins on their surface known as "checkpoint regulators," which serve as a form of immune tolerance by downregulating immune functions when these receptors combine with ligands on APCs and other cells in the body (Figure 2). When engaged, these receptor–ligand pairs signal the T cell to shift away from a proliferative state and decrease effector functions. Blocking these receptor–ligand interactions disrupts the delicate balance of stimulatory and inhibitory signaling, so T cells are more easily stimulated, increasing the cytotoxic effect against tumor cells.[52] This preclinical work was confirmed in clinical trials for metastatic melanoma, and numerous adult cancers have benefited from incorporation of checkpoint blockade into therapy.

Work in adult brain tumors has focused on GBM, with the largest trial to date being CheckMate 143. Phase I of this trial investigated safety and tolerability of nivolumab (anti–programmed cell death protein 1 monoclonal antibody) with or without ipilumumab (anti–cytotoxic T lymphocyte antigen 4 monoclonal antibody) at 2 different dosing schedules.[53] Overall, treatment was well tolerated across the 40 enrolled patients, with no difference observed between nivolumab alone or with ipilumumab. However, the phase III trial comparing nivolumab with bevacizumab at first relapse showed no difference in survival or progression.[54] Numerous adult clinical trials are under way investigating additional combinations of checkpoint inhibitors, as well as combining radiotherapy with checkpoint blockade.

One important consideration in checkpoint blockade has been the utilization of biomarkers to identify tumors most likely to respond to this therapy. Expression of programmed cell death ligand 1 (PDL1) in adult tumors has been reported to correlate with response to checkpoint inhibition, although a recent meta-analysis showed that even patients with negative PDL1 tumors respond to checkpoint

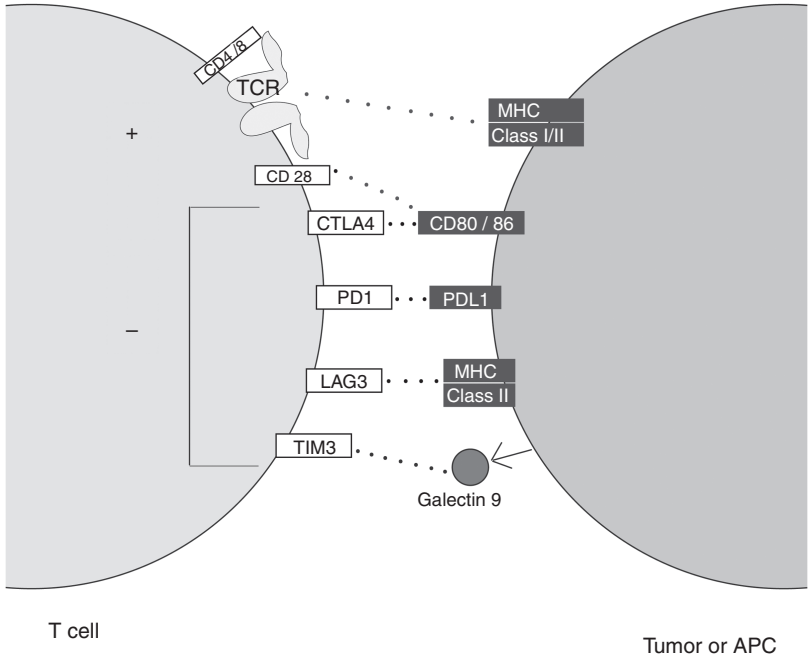

Figure 2. Negative checkpoint regulators. Simplified representation of stimulatory and inhibitor ligands and receptors at the T-cell surface. CTLA-4, PD1, Lag3, and Tim3 interact with ligands on tumor cells and antigen presenting cells (APCs) to produce an inhibitory effect on proliferation and cytotoxic effector function of the T cell. These regulators, as well as PDL1, are the targets blocked by checkpoint blockade therapeutics.

blockade.[55] PDL1 expression in pediatric cancers has been low in general, with pediatric brain tumors ranging 0–36% PDL1 positivity, depending on tumor type.[13] Alternative biomarkers that may better predict response to checkpoint blockade, such as tumor mutational burden, have been identified as discussed in more detail below.

Pediatric Clinical Trials

Pediatric literature investigating checkpoint blockade has been limited to case studies and small series. In 2016 a group in Israel reported retrospectively on their experience using pembrolizumab (anti-PD1 monoclonal antibody) in adult and pediatric patients with recurrent brain tumors, with a total of 5 pediatric patients in their cohort.[56] Unfortunately none of the patients showed clinical or histologic response, with a median overall survival in the children of 3.2 months. These initial reports curbed enthusiasm, although larger prospective trials were already under way (Table 1).

Meanwhile, with increased use of checkpoint inhibitors in adults, the importance of tumor mutational burden in the efficacy of checkpoint inhibition was discovered.[57] With a higher level of tumor mutations, there is an increased likelihood that neoantigens will be recognized by a patient's T cells, enhancing the efficacy of checkpoint blockade. Microsatellite instability (MSI), a surrogate for tumor mutational burden, has been found to be higher in pediatric gliomas than adult gliomas.[58] In addition, the group at Sick Kids in Toronto noted that patients with biallelic mismatch repair deficiency (bMMRD) have a predisposition for GBM with MSI and high mutational burden, making checkpoint blockade an attractive option for this group of patients. They reported on exome sequencing of 32 malignant tumors from bMMRD patients, including 21 GBM, all of which were hypermutant with >100 mutations per tumor exome.[59] Among the cohort, the researchers treated a pair of siblings with relapsed GBM using nivolumab every 2 weeks. Both patients presented with seizures and pseudoprogression on imaging after the first dose; however, after a short steroid taper, both patients resumed treatment and at 5–9 months had a dramatic clinical and radiographic response.[59] This report has generated enthusiasm for clinical trials using MSI or tumor mutational burden as a biomarker for eligibility. However, as a cautionary tale, Zhu et al reported on a pediatric patient with bMMRD and recurrent glioblastoma who was treated with nivolumab and developed extensive, fatal cerebral edema.[60] As with the cases from Sick Kids, this edema was presumed to be pseudoprogression from T-cell infiltration that escalated with subsequent doses, but the patient's autopsy also showed that progressive tumor growth clearly played a role.

To more rigorously test the efficacy and safety of checkpoint blockade therapy in pediatric brain tumors, there are several ongoing large studies (Table 1). The Pediatric Brain Tumor Consortium is conducting a phase I clinical trial with pembrolizumab among patients with DIPG, HGG, and a third stratum for brain tumors with hypermutation (NCT02359565). Early results from this trial describing 5 patients with DIPG were recently presented at the 2018 International Society for Pediatric Neuro Oncology meeting.

Unfortunately, the patients with DIPG progressed more rapidly than historical controls after initiation of checkpoint blockade, with a median PFS of 1 month, prompting an amendment to the study to exclude recurrent DIPG.[61] The Children's Oncology Group is participating in the phase I/II KEYNOTE-051 trial using pembrolizumab for relapsed/refractory pediatric solid tumors, including CNS tumors, with high PDL1 expression and high tumor mutational burden (ADVL1621, NCT02332668). A group in Belgium is investigating nivolumab in combination with metronomic chemotherapy (NCT03585465), and there is a pilot study of nivolumab in hypermutant cancers (NCT02992964). Finally, Children's Healthcare of Atlanta is investigating a newer checkpoint blockade agent, indoximod (IDO), which blocks indoleamine (2,3)-dioxygenase, a protein responsible for suppressing T-cell function and increasing the suppressive TME.[62] In their clinical trial, IDO is paired with temozolomide, or temozolomide with radiation therapy (NCT02502708).

Adoptive Cellular Immunotherapy

Adoptive cellular therapy (ACT) is an umbrella term for the ex vivo expansion and modification of human lymphocytes, most commonly T cells and natural killer (NK) cells, which are delivered back to a patient to attack neoplastic cells (Figure 3). This area of immunotherapy has felt a recent surge of interest after one form of ACT, chimeric antigen receptor (CAR) T-cell therapy directed against CD19, recently gained FDA approval for the treatment of pediatric relapsed and refractory acute lymphoblastic leukemia.[63] Efficacy in solid tumors has proven harder to achieve, with T-cell trafficking, the TME, and heterogeneous bulk of the tumors creating significant barriers.

Lymphocyte Activated Killers and NK Cell Infusions

Initial ACT studies in pediatric brain tumors date back to the 1980s, when Okamoto et al studied transfer of lymphocyte activated killer (LAK) cells to the CSF of patients with disseminated medulloblastoma.[64] LAK cells include CD8 T cells and NK cells that are stimulated by IL2 until they become indiscriminate killers. This group used patients' haploidentical relatives and stimulated production of LAK cells with administration of recombinant human IL2. The donor LAK cells were sorted and then delivered intrathecally to patients after completion of chemotherapy and radiotherapy. Of 6 patients treated, half showed improved neurologic symptoms and negative CSF cytology after treatment. One of the 6 patients showed a complete

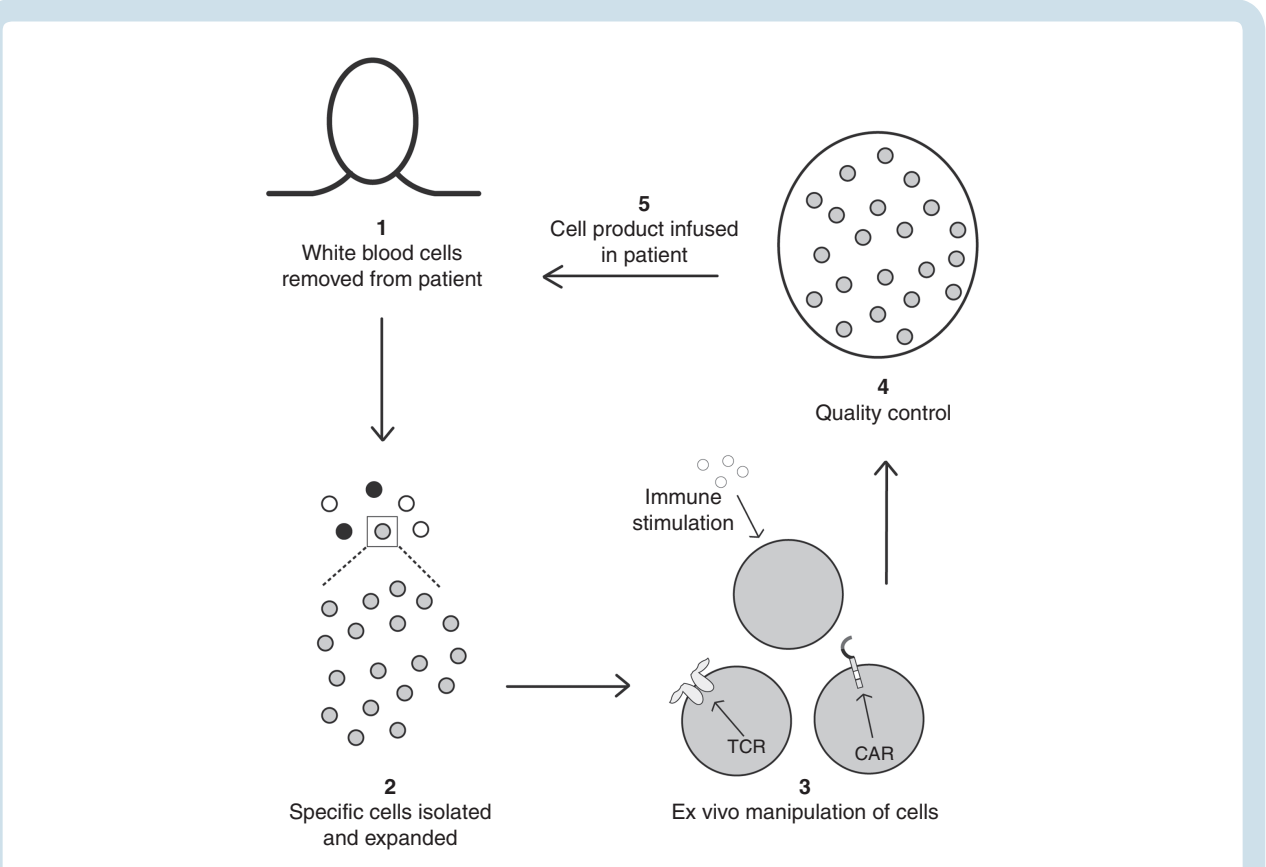

Figure 3. Schematic of adoptive T-cell therapy. Immune cells, often T cells or NK cells, are taken from a patient and separated for expansion. Immune cells can then be manipulated ex vivo, including selection for activity against a particular immune stimulus, or introduction of a TCR or CAR. After ensuring successful manipulation, these cells are then re-introduced into the patient where they can exert cytotoxic effects on a tumor.

response with no evidence of disease after 20 months. Several adult studies have looked at autologous LAK cell administration in combination with IL2 for GBM, delivered both intratumorally and systemically.[65–68] Both intratumoral and intracavity administration after resection showed a suggestion of improved survival[69,70]; however, no randomized trials have been done and no more pediatric studies have been pursued. LAK therapy has fallen out of favor with the advent of newer immunotherapeutic modalities, including preclinical advancement in NK cell immunotherapy.[71] Currently, there is one clinical trial investigating intrathecal delivery of expanded NK cells for relapsed posterior fossa tumors and one clinical trial exploring allogeneic transplant with donor infusion of NK cells for additional graft versus tumor effect (Table 1).

Cytotoxic T Lymphocytes and Engineered T-Cell Receptors

In addition to expanded polyclonal lymphocyte populations, T cells have also been selected and expanded for their specific antitumor properties. Cytotoxic T lymphocyte (CTL) therapy harnesses T cells that have been selected for their specificity in targeting a tumor or tumor antigen. These can be harvested from either tumor infiltrating lymphocytes (TILs) or peripheral lymphocytes and expanded ex vivo after exposure to a specific tumor antigen. These strategies have been utilized in adult gliomas[72,73] but have not yet been efficacious in pediatric patients. There are currently several open clinical trials investigating CTL therapy for pediatric brain tumors (Table 1). One study led by Children's National is investigating CTL directed against WT1, preferentially expressed antigen of melanoma (PRAME), and/or survivin, which was originally described and developed for acute lymphoblastic leukemia[74] but is now being used for brain tumors (NCT03652545). The University of Florida is investigating tumor-specific lymphocytes expanded ex vivo from total tumor RNA-pulsed DCs, which are being used in combination with vaccine therapy, radiotherapy, and temozolomide (NCT03396575 and NCT03334305).

An alternative but similar approach is engineered TCRs, where T cells have undergone genetic modification to create targeted cytotoxicity. Engineered TCRs are composed of cloned α- and β-chains capable of binding a tumor peptide presented on MHC molecules (Figure 1). These synthetic TCRs can be introduced into a patient's T cells through viral transduction and then reinfused back to the patient for homing to the tumor. Preclinical work for pediatric patients in this area has included development of a TCR directed against H3K27M mutation in DIPG and midline gliomas.[75] A group in Italy also recently published high expression of PRAME in medulloblastomas, and devised a TCR directed against PRAME with impressive preclinical efficacy.[76] No clinical trials of TCRs have been completed to date, but many will likely be starting within the next few years.

Chimeric Antigen Receptor T-Cell Therapy

One of the most successful areas of ACT has been CAR T-cell therapy. CARs are created by taking a binding domain, usually a single-chain variable fragment (scFv) derived from an antibody that targets the epitope of interest, which is then combined with the signal transduction domain of T cell receptors. Genetic material encoding CARs can be transduced into T cells via a variety of mechanisms, including viral, transposon, and mRNA transfer. Originally described in 1993,[77] the first generation CAR T cells lacked efficacy, and it was not until the introduction of a co-stimulatory endodomain into the CAR structure that meaningful cytotoxicity was achieved after adoptive transfer.[78,79] The combination of specific binding with co-stimulation of the T cells allows for CAR T cells to act as targeted killers without reliance on additional immune signaling for degranulation. This structure has been exploited with tremendous success for relapsed and refractory pediatric lymphoblastic leukemia.[80–84]

Treating solid tumors with CAR T cells has proven more difficult than hematologic malignancies,[85] but several major breakthroughs for brain tumors have emerged in the last 2 years. In 2016, Brown et al reported on the success of CAR T-cell therapy directed against IL13Rα2 in an adult with recurrent GBM. Following resection of several sites of disease, he received 6 doses of CAR T cells into the resection cavity. On follow-up imaging, he showed no progression at the site of delivery; however, he did develop progressive disease in other areas of the brain and spine. The patient then received 10 additional doses of IL13Rα2 CAR T cells delivered into the right lateral ventricle, allowing for distribution throughout the CSF. After 7.5 months from the start of CAR T-cell therapy, all prior metastatic disease, including spinal lesions, had completely resolved. Unfortunately, new disease emerged shortly thereafter in 4 new locations, with preliminary examination of the relapse specimens showing low IL13Rα2 expression. This report was the first notable example of efficacious CAR T-cell therapy in a brain tumor patient, and highlighted 3 important issues with using CAR T cells for brain tumors. First, the IL13Rα2 CAR T cells had limited T-cell expansion and persistence, and it is unclear if this was due to suppression from the TME or inherent limitations from the CAR construct itself. Second, the two delivery routes that were utilized highlight the difficulty in CAR T-cell trafficking in the brain for appropriate efficacy, with intraventricular regional delivery emerging as superior for this patient. Finally, the relapse specimen with low IL13Rα2 expression echoes the antigen-loss relapses which have been seen with CAR T-cell therapy in leukemia,[81,86] implying this resistance mechanism will likely also be important in brain tumor CAR T-cell therapy.

More recently, Texas Children's Hospital published on the safety and initial efficacy of human epidermal growth factor receptor 2 (HER2) directed CAR T-cell therapy in progressive GBM.[87] This CAR was used in virus-specific T cells, with the aim of increasing the persistence of T cells through co-stimulation through the native TCR by latent virus.[88] The FRP5 monoclonal antibody based HER2 CAR was developed for phase I clinical trial based on preclinical work showing potent antitumor activity in patient derived xenografts of GBM.[89] In this phase I trial, 10 adults and 7 pediatric patients were treated with CAR T cells, with 1 patient showing partial response for 9.2 months and 7 patients showing stable disease from 8 weeks to 29 months.[87] The patient with partial response was a 17-year-old boy, which was encouraging for pediatric patients; however, in this small phase I trial statistical analysis showed no associated improvement in outcome for younger patients. Interestingly, patients who

did not receive prior salvage therapy had an increased median overall survival, suggesting that prior therapy can affect CAR T-cell efficacy, likely related to T-cell manufacturing, as has been observed in CD19 CAR T-cell therapy.[90] Ongoing CAR T-cell trials for pediatric patients include those directed against IL13Rα2, HER2, and EGFR806 (Table 1).

Finally, preclinical advances are paving the way for additional CAR T-cell studies in pediatric patients, such as investigations targeting multiple epitopes to overcome tumor heterogeneity. The group at Texas Children's Hospital has demonstrated improved antitumor efficacy in preclinical models simultaneously targeting HER2 and IL13Rα2, using pooled CAR T-cells for each target,[91] T-cells expressing both CAR molecules,91 and a tandem CAR directed at both targets.[92] Stanford recently reported on the efficacy of GD2 directed CAR T-cell therapy for DIPG in murine patient derived xenograft models with excellent cytotoxicity but notably peritumoral edema causing a proportion of murine deaths following treatment.[93]

In summary, adoptive cell therapy has come a long way over the past three decades, with significant translation from the bench to the bedside. Pediatric brain tumors are now picking up this momentum, but it will be important to thoroughly investigate and vet all new tumor-specific targets, as both TCR and CAR T-cell therapy have produced significant, at times fatal, outcomes from unforeseen on-target off-tumor binding.[94–97]

Future Directions

Immunotherapy for pediatric brain tumors is a growing and exciting field with potential for therapeutic promise, with many new advances in recent years. However, there are still significant barriers to overcome, including optimizing delivery of immunotherapeutics to ensure BBB penetration and exposure to perfusion poor areas of the tumor. Combinatorial therapy will be key to help overcome tumor heterogeneity by targeting multiple antigens, as well as combined checkpoint blockade with other immunotherapeutics to help penetrate the highly immunosuppressive microenvironment. With a large number of trials already under way, and many more soon to follow based on impelling preclinical data, the cytotoxic power of the immune system is stepping closer to becoming effective therapy for these devastating tumors in children.

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