

Review

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# Immunotherapy for pediatric brain tumors

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## ABSTRACT

Immunotherapy, while effective against lymphoid cancers and some solid tumors, has shown less benefit against pediatric brain tumors. Tumor heterogeneity, a suppressive immune microenvironment, and the blood-brain barrier have the potential to diminish any immune-based approach and limit efficacy. More importantly, most pediatric brain tumors are immunologically quiescent, stemming from a low mutational burden. This review focuses on innate *vs.* adaptive immunotherapeutic approaches and describes how the immunologic context of pediatric brain tumors can help identify well-suited immunotherapies for our patients. In this framework, we will discuss past and current approaches using virotherapy, immunoconjugates, monoclonal antibodies, active immunization, and adoptive cellular therapy, and share our thoughts on how immunotherapy can cure children with brain tumors.

**Keywords:** Immunotherapy, brain tumor, pediatrics, virotherapy, active immunization, adoptive cellular therapy

## INTRODUCTION

The incidence of pediatric brain tumors varies by country and ranges between 1-5 cases/100,000 persons, with about 4600 primary central nervous system tumors diagnosed in the United States annually<sup>[1,2]</sup>. There are over 100 histologic subtypes of brain tumors, but the most common diagnoses in children are low-grade gliomas, particularly pilocytic astrocytoma (incidence roughly 0.8/100,000) and medulloblastoma (incidence roughly 0.4/100,000)<sup>[2]</sup>. Outcomes for recurrent malignant brain tumors in children remain poor, and brain tumors are the leading cause of cancer death in children<sup>[3]</sup>. Even when effective, surgery, radiation, and chemotherapy cause neurologic and neurocognitive morbidity. Many children with brain tumors who survive



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their disease have significant cognitive disability that limits their ability to live independently, progress fully in their education, or pursue a vocation<sup>[4]</sup>.

Immunotherapy attempts to leverage the high specificity of the immune system to target and eliminate cancer cells while leaving healthy cells undamaged. Chimeric antigen receptor (CAR) T cells and PD-1/PD-L1 monoclonal antibodies are the most impactful immunotherapies to date and have cured patients who otherwise had no curative option. Unfortunately, these successes have not significantly improved outcomes for most children with brain tumors. Understanding the immune environment in which pediatric brain tumors exist is requisite for identifying effective immune-based therapies for these diseases.

Historically, the blood-brain barrier and perceived sensitivity of deep midline structures to manipulation have limited investigators' ability to develop and deliver therapies for children with brain tumors. In the modern era, direct delivery methods, improved drug design, and surgical intervention involving brainstem and deep midline tumors drive the field forward. This review will indicate the routes whereby immunotherapies are delivered and mechanisms through which they selectively target the tumor, but will not unduly focus on the blood-brain barrier or tumor delivery methods.

### **IMMUNOLOGICALLY “HOT” VS. “COLD” TUMORS AND MUTATIONAL LOAD**

“Hot” vs. “cold” tumors are distinguished by whether significant numbers of tumor infiltrating lymphocytes (TILs), most notably T cells, are present<sup>[5]</sup>. Functionally, T cells are potently cytolytic and are important for immunologic memory and surveillance to maintain an anti-tumor immune response<sup>[6]</sup>. T cell homing is influenced by activating cytokines, the tumor vasculature, integrins, and the presence of tumor-specific proteins, called “neoantigens”<sup>[5]</sup>. Hot tumors supply inflammatory cytokines and allow T cells permissive access within the tumor bed. Cold tumors lack T cell infiltration either because of a harshly immunosuppressive tumor microenvironment, or because the tumor is not inflammatory or exists in a strictly immune-privileged site. Whereas the central nervous system (CNS) was formerly regarded as immune-privileged, this notion has been dispelled; immune cells are highly adept at reaching the CNS, even without blood brain barrier disruption<sup>[7]</sup>.

In addition to inflammatory cytokines and permissive vasculature, hot tumors tend to exhibit a high number of neoantigens, which are novel peptide epitopes caused by mutations in the cancer genome. Non-synonymous mutations are changes in the cancer genome that produce an altered amino acid sequence that can drive tumorigenesis by altering cellular pathways or lead to expression of neoantigens<sup>[8]</sup>. Synonymous mutations do not change the amino acid sequence of an expressed gene but are not necessarily silent mutations. Synonymous mutations can serve as driver mutations by influencing translation, transcription, splicing, and mRNA transport<sup>[9]</sup>.

Melanoma and lung cancer are hot tumors that sometimes respond to immune checkpoint blockade<sup>[10,11]</sup>. Ultraviolet light in melanoma and smoke carcinogens in lung cancer induce DNA damage, and these tumors in older adults have accumulated higher numbers of non-synonymous mutations<sup>[8]</sup>. Pediatric cancers harbor few somatic mutations compared to adult tumors, and this is particularly true for pediatric brain tumors, which are almost always immunologically cold<sup>[12,13]</sup>. The lower tumor mutational load in pediatric brain tumors produces few neoantigens to stimulate T cell activation and proliferation within the tumor bed. Accordingly, an immunotherapy aimed at promoting an existing T cell immune response, such as checkpoint blockade, will be ineffective.

### **DAMAGE RESPONSE AND TUMOR IMMUNITY**

Inflammation is an important component of an immune response. While the CNS is not an immune-priv-

ileged site, regulatory immune cells and cytokines protect against excessive inflammation that would cause unacceptable inflammation involving the brain<sup>[14]</sup>. As brain tumors expand, local tissue damage and hypoxia induce regulatory cytokines and immune cells to quell inflammation and promote healing<sup>[15,16]</sup>. These factors contribute to the immunosuppressive behavior of the tumor itself and can blunt an anti-tumor immune response.

A typical endogenous immune response occurs in two phases. Pathogens, damaged DNA, cellular debris from apoptosis or necrosis, and inflammatory cytokines attract phagocytes, natural killer cells, and antigen-presenting cells as part of the innate immune response. Antigen-presenting cells then display peptide epitopes on MHC molecules, which engage T cells through their T cell receptor as part of the adaptive immune response.

Cells employ sophisticated DNA maintenance machinery to monitor and repair the genome, and damage-sensing pathways are important for eliminating pre-cancerous and cancerous cells<sup>[17]</sup>. Conventional chemotherapy and radiation, as well as innate-based immunotherapies, induce DNA damage and cell death by either apoptosis or non-apoptotic pathways<sup>[14,18,19]</sup>. Cell death and DNA degradation produce molecules called damage-associated molecular patterns (DAMP), which are recognized by the innate immune system and promote an immune response. DNA damage-sensing machinery within the nucleus transmits this signal to the cytoplasm and activates stimulator of interferon genes (STING) to induce proinflammatory interferon signals, which can shift the immunosuppressive tumor bed toward a more inflammatory, anti-tumor state<sup>[17]</sup>. DNA damage sensors also induce cell-surface ligand expression to recruit natural kill cells, natural killer T cells, and phagocytes to eliminate damaged cells and prime an adaptive immune response against the tumor<sup>[20,21]</sup>.

In health, damage-sensing pathways preserve the integrity of the genome and recruit the immune system to eliminate damaged cells when needed. In many instances, tumors deactivate the cellular damage-sensing machinery, which allows immune evasion and can dampen the response to conventional therapies like radiation or immunotherapies that are directly cytotoxic<sup>[22]</sup>. In addition, mutations within the damage-sensing machinery itself can contribute to tumorigenesis<sup>[22]</sup>. In this way, tumors with high mutational loads are more likely to harbor deleterious mutations within damage-sensing genes. This explains, in part, why hypermutated tumors are often resistant to radiation and alkylating chemotherapy<sup>[23]</sup>.

Defective damage response pathways have implications for immunotherapy as well. Innate-based immunotherapies are typically inflammatory and attempt to kill target cells to increase tumor antigen exposure. Tumor cells that lack damage-sensing machinery and have defective death pathways will be less amenable to many innate-based immune responses. The ultimate goal of any immunotherapy is to create a T cell response targeting the entire tumor and generates immunologic memory to protect against recurrence. With this understanding how mutational load, tumor neoantigens, and DNA damage machinery affect tumor immunology, we will discuss approaches in each of the main areas of immunotherapy for pediatric brain tumors.

## **VIROTHERAPY**

Virotherapy broadly refers to the use of viruses as therapeutic agents. Oncolytic viruses, which cause tumor cell death and can stimulate the immune system, are the most prominent clinical branch of virotherapy. Viruses are also useful as vectors for gene therapy, whereby viruses induce expression of a transgene that modifies the immune environment to promote an anti-tumor response. Retroviruses are used to genetically modify immune cells, most notably to express chimeric antigen receptors (CAR) on primary human T cells, and this will be discussed subsequently under adoptive cellular therapy. The last clinical branch of virotherapy, termed viral immunotherapy, uses viruses to introduce antigens that sensitize the host immune system

to the tumor through cross-reactivity or as an adjuvant. Currently, clinical applications of virotherapy in pediatric brain tumors are limited to approaches using oncolytic viruses or viruses as gene transfer platforms.

### Oncolytic viruses

Oncolytic viruses show promise in treating pediatric brain tumors. At least 50 clinical trials are ongoing using oncolytic viruses to treat cancer patients, mostly adults with non-CNS solid tumors. The oncolytic virus talimogene laherparepvec (TVEC), a modified type I herpes simplex virus for adults with advanced melanoma, is the first oncolytic virus to receive FDA approval<sup>[24]</sup>. Mechanistically, oncolytic viruses predominantly function through a combination of tumor cell lysis and stimulation of the innate immune system through DAMP and pathogen-associated molecular patterns (PAMP)<sup>[25]</sup>. Initially, oncolytic viruses were engineered for selective tumor tropism and direct cytotoxicity. However, oncolytic viruses are now regarded as immunotherapy for which efficacy depends on activating an endogenous anti-tumor immune response<sup>[26]</sup>.

TVEC enters tumor cells through nectin adhesion molecules and replicates within tumor cells that have dysfunctional anti-viral pathways<sup>[24]</sup>. The virus induces tumor cell death and causes DAMP and PAMP expression within the tumor. Additionally, the viral genome is genetically modified to increase MHC class I expression and to secrete GM-CSF, which promotes dendritic cell accumulation and antigen presentation to prime an adaptive immune response<sup>[24]</sup>. Taken together, this first-in-class agent represents how oncolytic viruses can be modified to stimulate innate immunity and readily combined with conventional and immune-based therapies. TVEC is undergoing clinical evaluation with checkpoint inhibitors and agents that target the MAP kinase pathway, which is activated in melanoma.

To date, at least five oncolytic viruses have been evaluated clinically in children with brain tumors: recombinant poliovirus<sup>[27,28]</sup>, adenovirus<sup>[29]</sup>, reovirus<sup>[30]</sup>, herpesvirus<sup>[31,32]</sup>, and new castle disease virus<sup>[33,34]</sup>.

PVSRIP0, is a recombinant, live attenuated, nonpathogenic oncolytic virus containing the oral poliovirus Sabin type 1 in which the internal ribosomal entry site (IRES) is replaced with the IRES from human rhinovirus. PVSRIP0 is administered intratumorally for adults with recurrent glioblastoma via convection-enhanced delivery and enters cells expressing the poliovirus receptor, CD155, which is ubiquitously expressed on malignant glioma. PVSRIP0 is directly cytotoxic and induces a marked inflammatory response. Interestingly, dendritic cells express CD155 and are infected by PVSRIP0. Whereas PVSRIP0 lyses tumor cells expressing CD155, the virus induces interferon-dominant activation of dendritic cells and tumor-specific CD8+ T cells<sup>[35]</sup>. PVSRIP0 was well-tolerated in adults with recurrent glioblastoma (GBM) with some encouraging responses<sup>[36]</sup> and is being evaluated in a phase II trial in adults with recurrent GBM in combination with lomustine. PVSRIP0 is also being used to treat children with recurrent supratentorial malignant glioma as a phase Ib trial<sup>[28]</sup>.

Currently, three other early-phase trials using oncolytic viruses are ongoing for children with brain tumors. HSV G207, a modified type 1 herpesvirus, is delivered intratumorally to children with recurrent or progressive supratentorial malignant brain tumors. HSV G207 is cytotoxic and replicates within infected cells, then infects neighboring cells following cell lysis. Subsequent cohorts of patients will receive a single dose of radiation, which has been shown to increase viral activity in pre-clinical studies and was well-tolerated in an adult phase I trial<sup>[37]</sup>. Wild-type reovirus preferentially infects and kills cancer cells in its unmodified form<sup>[38]</sup> and induces an interferon-dominant immune response following intravenous administration in adults with brain tumors<sup>[39]</sup>. Reovirus is being evaluated in combination with GM-CSF in children with recurrent malignant brain tumors<sup>[30]</sup>. Newcastle disease virus (NDV) also induces selective tumor cell death and stimulates the innate immune system. NDV has been used clinically in cancer patients for decades with scattered clinical responses<sup>[40]</sup>. Currently, NDV is administered intratumorally in children with diffuse intrinsic brainstem glioma to induce tumor lysis. Tumor antigens are then harvested systemically and used to prime autologous

**Table 1. Virotherapy trials for pediatric brain tumors**

Trial/therapy	Description	NCT/reference
Recombinant poliovirus, PVSRIPO	Phase Ib trial evaluating PVSRIPO in children with recurrent supratentorial malignant glioma	NCT03043391 [27]
Modified type I herpesvirus, HSV G207	Phase I trial evaluating HSV G207 alone or with single radiation dose in children with recurrent supratentorial malignant brain tumors	NCT02457845 [32]
Wild-type reovirus	Phase I trial evaluating reovirus in combination with GM-CSF in children with recurrent malignant brain tumors	NCT02444546 [30]
Newcastle disease virus	Phase I trial evaluating newcastle disease virus in combination with autologous DC in children with brainstem glioma	[33]
Modified adenovirus, Ad-RTS-hIL-12	Phase I trial evaluating Ad-RTS-hIL-12, a vector for viral gene therapy, in children with progressive supratentorial tumors and diffuse intrinsic pontine glioma	NCT03330197 [47]

dendritic cells<sup>[34]</sup>.

### Viral gene therapy

Viruses are highly adept at introducing foreign genes and utilizing host machinery for protein expression. The human genome contains a large number of endogenous retroviral sequences, and roughly 8% of the human genome derives from infectious retroviruses<sup>[41]</sup>. Retroviruses, which predominantly infect dividing cells and stably integrate into the host genome, are useful for viral gene therapy. The lentivirus genus of retroviruses can transduce slowly-dividing or quiescent cells, overcoming in part the limitation that retroviruses must transduce dividing cells<sup>[42]</sup>. In cancer immunotherapy, retroviral transduction is typically performed *ex vivo* to genetically modify immune cells and not to deliver a direct therapeutic benefit.

Toca-511, however, uses a retroviral replicating vector to selectively transduce cancer cells with a yeast-derived cytosine deaminase gene following intratumoral administration. The prodrug 5-fluorocytosine is given systemically and selectively converted fluorouracil (5-FU) in tumor cells expressing cytosine deaminase. This platform illustrates how viruses are useful as a form of gene therapy and has shown clinical efficacy in adults with glioblastoma. Toca-511 has been studied preclinically in medulloblastoma models and has a strong rationale for clinical evaluation in children<sup>[43]</sup>.

Compared to retroviruses, adenoviruses more readily transduce non-dividing cells<sup>[44]</sup>. Adenovirus vectors typically have smaller DNA capacity and are more immunogenic, which can limit gene expression *in vivo*<sup>[45]</sup>. One adenovirus gene therapy platform is being evaluated clinically in children with brain tumors: The modified adenovirus, Ad-RTS-hIL-12, is injected intratumorally and uses an oral activator ligand to toggle IL-12 expression within the tumor. In early phase adult studies in recurrent GBM, this platform was well-tolerated, and preliminary data showed correlation between tumor response and IL-12 secretion<sup>[46]</sup>. Ad-RTS-hIL-12 is being evaluated in children with progressive supratentorial tumors and diffuse intrinsic pontine glioma<sup>[47]</sup>. Table 1 lists notable virotherapy trials for pediatric brain tumors.

### MONOCLONAL ANTIBODIES AND IMMUNOCONJUGATES

Similar to oncolytic viruses and tumor-directed viral gene therapy, monoclonal antibodies (mAb) and immunoconjugates can be used as immunotherapy to elicit an innate immune response. MAb directed against tumor-specific antigens have varied mechanisms of action, which are often incompletely understood, even in clinically effective products. For example, the HER2-specific mAb trastuzumab improves survival in patients with advanced HER2-positive breast cancer and is FDA approved in this disease<sup>[48]</sup>. Several anti-tumor mechanisms of trastuzumab have been identified, including antibody-dependent cell-mediated cytotoxicity (ADCC) involving Fc receptors on phagocytes<sup>[49]</sup>, inhibition of HER2 signaling<sup>[50,51]</sup>, and downregulation of HER2 cell-surface expression<sup>[51]</sup>.

There are two main ways mAbs can promote an immune response: ADCC and immune modulation. MABs can trigger ADCC, in which cells of the innate immune system, specifically NK cells and phagocytes, lyse a tumor cell coated with antibodies containing Fc regions. There are no mAbs for primary brain tumors that function primarily by ADCC. Bevacizumab is a mAb that inhibits angiogenesis by binding to vascular endothelial growth factor (VEGF), a pro-angiogenesis cytokine. Bevacizumab is not strictly an immunotherapy but may have immunomodulatory effects, such as enhanced T cell recruitment and dendritic cell maturation and migration related to an inhibitory VEGF effect<sup>[52]</sup>. Bevacizumab is effective in some children with recurrent low-grade glioma<sup>[53]</sup> and FDA approved for recurrent GBM in adults<sup>[54]</sup>, but is not immunotherapy in the sense that it does not elicit an anti-tumor immune response. Similarly, monoclonal antibodies recognizing epidermal growth factor receptor (EGFR), block EGFR signaling in tumor cells and may, to a lesser extent, promote ADCC. Erlotinib, an anti-EGFR mAb, is ineffective against recurrent pediatric malignant glioma<sup>[55]</sup> and ependymoma<sup>[56]</sup>. Newer generation EGFR mAbs have been developed with improved ADCC characteristics but have not been evaluated in pediatric brain tumors<sup>[57]</sup>.

### Immunomodulatory monoclonal antibodies

MABs can also promote anti-tumor immunity through immune modulation. Checkpoint inhibitors are an example of this and will be discussed separately. CD40 is a TNF receptor superfamily member expressed broadly on dendritic cells, B cells, and monocytes, as well as some tumor cells. Binding to the natural ligand CD40L expressed on T helper cells causes immune cell activation<sup>[58]</sup>. Agonistic CD40 mAbs activate antigen presenting cells and cytotoxic myeloid cells<sup>[59]</sup> and have induced clinical responses in adults with lymphoid tumors<sup>[58]</sup>. The CD40 agonistic antibody APX005M, delivered intravenously, is being evaluated in a phase I trial for children with recurrent malignant brain tumors and newly diagnosed diffuse intrinsic pontine glioma<sup>[60]</sup>.

CD47 is an integrin-associated protein ubiquitously expressed on human cells. In the context of tumor immunology, CD47 serves as a “don’t-eat-me” signal for macrophages<sup>[61]</sup>. The anti-CD47 mAb Hu5F9-G4 currently is in early phase trials for adults with lymphoid and non-CNS solid tumors. While not yet used clinically for primary brain tumors, Hu5F9-G4 has shown promising preclinical activity *in vivo* against orthotopic xenograft models of malignant pediatric brain tumors<sup>[62]</sup>.

### Immunoconjugates

Several immunoconjugates have reached the clinic in pediatric brain tumors as a form of immunotherapy. Immunoconjugates consist of an antibody fragment joined to some sort of effector molecule. Examples of effector molecules include immunotoxins, radioisotopes, and immune ligands. Immunoconjugates using immunotoxins are most prevalent and are typically comprised of a toxin coupled to a single-chain variable-region antibody fragment (scFv) that binds a tumor antigen. As a class, immunoconjugates typically have a short half-life following administration and are given directly into the tumor by convection enhanced delivery.

The EGFR gene is frequently amplified in adult GBM but not in pediatric GBM<sup>[63]</sup>. However, most pediatric glial tumors overexpress EGFR, making it an attractive target for immunotherapy for pediatric brain tumors<sup>[63]</sup>. D2C7-IT is an immunoconjugate comprised of a scFv that recognizes for both wild-type EGFR and the deletion variant EGFRvIII fused to the pseudomonal exotoxin PE38KDEL<sup>[64]</sup>. Upon binding EGFR, D2C7-IT is internalized and inhibits protein synthesis and causes tumor cell death. In a phase 1 trial in adults with malignant glioma, D2C7-IT induces inflammation within the tumor bed and has produced some clinical responses<sup>[65]</sup>. D2C7-IT will be evaluated in a phase I trial in children with recurrent, EGFR-positive malignant glioma at Duke.

Podoplanin is a tumor-associated glycoprotein highly expressed on pediatric malignant glioma and medulloblastoma<sup>[66]</sup>. A recombinant anti-podoplanin immunotoxin containing the pseudomonal exotoxin is effec-

**Table 2. Immunomodulatory mAb and immunoconjugate trials in pediatric brain tumors**

Trial/therapy	Description	NCT/reference
Agonistic CD40 mAb, APX005M	Phase I trial for children with recurrent malignant brain tumors and newly diagnosed diffuse intrinsic pontine glioma	NCT03389802 [60]
B7-H3 directed radioisotope, 124I-8H9	Phase I trial for children with diffuse intrinsic pontine glioma	NCT01502917 [71]

tive in preclinical pediatric brain tumor models but has not reached the clinic<sup>[66]</sup>. Immunoconjugates bearing a pseudomonas exotoxin that the IL-4 receptor<sup>[67]</sup>, IL-13 receptor<sup>[68]</sup>, or tumor growth factor alpha (TGF $\alpha$ )<sup>[69]</sup> were safe following direct administration into the tumor and intermittently effective in early-phase studies but have not been evaluated in children.

Immunoconjugates can also induce tumor death and anti-tumor immunity using radioisotopes, referred to as radioimmunotherapy. To date, only one radioisotope immunoconjugate has been used to treat primary pediatric brain tumors. <sup>124</sup>I-8H9 contains a scFv recognizing the B7-H3 antigen, expressed on glial tumors but not healthy cells, coupled to a radioactive iodine isotope<sup>[70]</sup>. <sup>124</sup>I-8H9 is being evaluated as a phase I trial for children with diffuse intrinsic pontine glioma and is administered intratumorally<sup>[71]</sup>.

Lastly, immunoconjugates incorporating an immune-activating ligand, also called bispecific antibodies, are being explored. Blinatumomab is a bispecific molecule that recognizes CD19, expressed on immature B cells, and CD3, which engages T cells. This T cell-engaging molecule induces remission in relapsed, immature B-lineage leukemia and is FDA approved for this disease<sup>[72]</sup>. Investigators at Duke developed a fully human, bispecific antibody (hEGFRvIII-CD3 bi-scFv) that redirects human T cells to kill EGFRvIII-positive malignant glioma cells<sup>[73]</sup>. This product is being evaluated in adults but is less useful for children with malignant glioma, as less than 5% of malignant glioma in children expresses EGFRvIII<sup>[74]</sup>. Table 2 lists current immunomodulatory mAb and immunoconjugate trials in pediatric brain tumors.

## IMMUNE CHECKPOINT INHIBITORS

Initial clinical evaluation of PD-1 and PD-L1 monoclonal antibodies demonstrated response rates of around 25% in adults with relapsed/refractory solid tumors<sup>[75,76]</sup>. Patients rarely had complete or sustained responses, but these encouraging results prompted evaluation checkpoint inhibitors in brain tumors. Unfortunately, these agents have been largely ineffective for most patients with brain tumors<sup>[77]</sup>.

A growing number of immune checkpoints are being targeted clinically, but mAbs targeting the PD-1/PD-L1 immune checkpoint remain the most widely used. Activated T cells express PD-1, a member of the CD28 family which impairs T cell activation and promotes T cell anergy and apoptosis<sup>[78]</sup>. PD-L1, which is expressed ubiquitously on solid tumors and also on regulatory immune cells within the tumor bed, binds PD-1 to dampen an anti-tumor T cell response<sup>[79,80]</sup>. Blocking this interaction using a mAb binding either PD-1 or PD-L1 can promote anti-tumor T cell activity.

In order for checkpoint inhibition to be optimally effective, the tumor must be immunologically hot, with T cell infiltration and tumor antigens that can be recognized by T cells. Tumor mutational load and T cell infiltration within the tumor are highly predictive for response with checkpoint inhibitors<sup>[81,82]</sup>. In melanoma and lung cancer, tumor mutational burden, which impacts the number of neoantigens and T-cell immunogenicity, correlate with response to checkpoint blockade<sup>[8,83]</sup>.

Hypermutant pediatric GBM, while rare, responds to anti-PD-1 checkpoint blockade. Two children with biallelic mismatch repair deficiency and hypermutated recurrent GBM responded to nivolumab<sup>[13]</sup>. These data are similar to those reported in adults with hypermutated colorectal carcinoma who received pembrolizumab, a PD-1 checkpoint inhibitor<sup>[84]</sup>.

Most often, pediatric brain tumors harbor fewer mutations compared to adult tumors, which have a lower mutational load than most solid tumors<sup>[13,85]</sup>. In a large analyses from over 300 adult glioma samples, less than 4% of tumors had a high tumor mutational load<sup>[86]</sup>. Even rare tumors that were hypermutated did not have significant T cell infiltration within the tumor. Taken together, these data explain at least in part why checkpoint blockade as monotherapy is unlikely to be impactful for pediatric brain tumors.

CheckMate 143 was a phase III randomized trial to evaluate efficacy of nivolumab, an anti-PD-1 monoclonal antibody compared to bevacizumab in adults with recurrent GBM. Nivolumab did not improve overall survival compared to bevacizumab<sup>[77]</sup>. Two additional trials of combining nivolumab and radiation with or without temozolomide in patients with newly-diagnosed, MGMT-unmethylated<sup>[87]</sup> and MGMT-methylated<sup>[88]</sup> GBM are ongoing.

While there have been no completed studies evaluating efficacy of checkpoint inhibitors in pediatric brain tumors, a number of trials are ongoing, including PD-1 antibodies as monotherapy or in combination with a CTLA-4 antibodies, and another checkpoint inhibitor against indoleamine (2,3)-dioxygenase (IDO). However, based on the disappointing results in CheckMate 143 and more recently for an IDO inhibitor in large phase III trial<sup>[89]</sup>, these agents are likely to be more effective in combination with immunotherapies which cause inflammation and promote T cell infiltration and activation first.

## ACTIVE IMMUNIZATION

Active immunization therapies deliver an immune stimulus to trigger an endogenous anti-tumor response. Typically, a vaccine is administered to stimulate and direct the host immune system to target antigens on the tumor. Cancer vaccines are a promising area of immunotherapy and are typically well tolerated. Vaccines containing tumor antigens, such as peptides, tumor lysate, or nucleic acids, and autologous dendritic cells are the most common approaches used clinically for patients with brain tumors. The intent of any active immunization strategy is to trigger an anti-tumor T cell response. T cell activation optimally occurs when T cells recognize antigen displayed on MHC molecules of antigen presenting cells in the setting of inflammation. Accordingly, active immunization approaches are designed to cause inflammation and antigen uptake by antigen presenting cells in lymphoid tissues, most often in lymph nodes.

### Dendritic cell vaccines

Dendritic cells (DC) are a critical link between the innate and adaptive immune systems. Upon encountering foreign antigens, specifically pathogen-associated molecular patterns, DC release inflammatory cytokines that activate the innate immune system. DC also process and present antigens to T cells and B cells, thereby activating naïve, effector, and memory immune cells or maintaining tolerance against self-antigens<sup>[90]</sup>.

Most commonly, DC for active immunization are generated by isolating monocytes from cancer patients that are expanded and activated *ex vivo*. These DC are loaded with either tumor lysate, peptides, nucleic acids, or viral epitopes that are expressed by the tumor. DC are usually matured with GM-CSF, then administered as a vaccine. Adjuvants such as tetanus toxoid are important to improve inflammation and immunogenicity in the host<sup>[90]</sup>.

Clinical testing of DC vaccines has demonstrated modest yet encouraging results in patients with advanced cancers<sup>[91,92]</sup>. There is general consensus that DC vaccines can induce tumor-specific T cell responses and immunological memory, and this is a promising platform for pediatric brain tumors<sup>[92]</sup>. To date, there have been several trials using autologous DC vaccines loaded with tumor RNA<sup>[93]</sup> or tumor lysate<sup>[94-96]</sup> for children with brain tumors. At this juncture, DCs are reliably manufactured and extremely well-tolerated. However, to improve efficacy, strategies to improve targeting, antigen loading, and migration *in vivo* are needed.

One of the central challenges for any active immunization approach is how to elicit an immune response against relatively weak “self” tumor antigens. Interestingly, cytomegalovirus (CMV) nucleic antigens are ubiquitously expressed in human malignant glioma<sup>[97]</sup>, and an adult patient treated with a DC vaccine pulsed with GBM tumor lysate developed a robust T cell response against the CMV antigen pp65<sup>[98]</sup>. The relative ease of eliciting an immune response against viral antigens contrasts with the difficulty of immunization against “self” tumor antigens and makes CMV an attractive target for immunotherapy. Dendritic cells targeting pp65 lead to long-term survival in small numbers of adults with newly diagnosed GBM<sup>[99]</sup>, and survival correlated with DC migration in a CCL3-dependent fashion<sup>[100]</sup>. This DC platform targeting CMV antigens will be evaluated in children with malignant glioma and recurrent medulloblastoma at Duke.

### Peptide vaccines

Manufacturing DC vaccines is costly, and poor DC migration following administration remains a challenge. Accordingly, active immunization strategies that stimulate endogenous DC activation are appealing, such as peptide vaccines, which inject tumor peptides with adjuvants, usually adjacent to lymph nodes. A few peptide vaccines for children with brain tumors are in early phase testing. One trial using a peptide vaccine targeting the H3.K27M neoantigen for HLA-A2+ children with H3K27M mutated glioma is underway<sup>[101]</sup>. A second peptide trial targeting the CMV epitopes pp65 and glycoprotein B is also underway for children with recurrent malignant glioma and medulloblastoma<sup>[102]</sup>. Additionally, a peptide trial using glioma-associated antigens for HLA-A2+ children with malignant brainstem and non-brainstem gliomas, including low-grade glioma, is underway<sup>[103]</sup>. This platform has been well tolerated and effective at generating an anti-tumor immune response<sup>[104]</sup>. At least four children with progressive, low-grade glioma have had sustained partial responses, providing evidence that peptide vaccines, typically given with Montanide adjuvant, can generate an endogenous anti-tumor response<sup>[105]</sup>. Montanide is a water-in-oil emulsion that acts as an adjuvant in these vaccines by enhancing CD4+ and CD8+ T cell response against antigens in the vaccine<sup>[106]</sup>.

Recently, highly personalized, neoantigen vaccines are gaining momentum. Initial clinical studies with cancer vaccines used whole tumor lysates, which contain a mixture of self-antigens and undefined neoantigens. These vaccines elicited broad immune responses but were generally ineffective. Using next-generation sequencing to identify DNA and RNA sequences of neoantigens and advanced algorithms to predict MHC I and MHC II loading, vaccines can be created that target specific neoantigens and hold promise for improving outcomes<sup>[92]</sup>. This personalized neoantigen approach was effective in some advanced melanoma patients, and combination with checkpoint blockade expanded the repertoire of neoantigen-specific T cells and further improved efficacy<sup>[107]</sup>. Table 3 lists notable past and current active immunization trials for pediatric brain tumors.

### ADOPTIVE CELLULAR THERAPY

Adoptive cellular therapy (ACT) involves manipulating effector immune cells *ex vivo* before transfer back to a patient with cancer. Initially, ACT for brain tumors used tumor-infiltrating lymphocytes (TIL) harvested from the tumor bed or immune cells isolated from peripheral blood or lymph nodes. Following collection, autologous lymphocytes were stimulated with cytokines or tumor antigen and infused back into patients. Overall, ACT using TILs or peripheral lymphocytes was well-tolerated but clinically ineffective, although immune activation and some responses were reported<sup>[108,109]</sup>. Natural killer T cells, which are specialized, CD1d-restricted T cells, recognize lipid antigens and have been tested in melanoma, but not brain tumors<sup>[110]</sup>.

By far, the most prominent type of adoptive cellular therapy involves cytotoxic T cells that are genetically modified to express a chimeric antigen receptor (CAR). CARs are synthetic receptors containing an antigen-binding domain, typically derived from the short chain variable fragment (scFv) of an antibody, coupled to the zeta chain and cytolytic machinery of a T cell receptor. Using retroviral vectors, primary human T cells

**Table 3. Notable past and ongoing active immunization trials for pediatric brain tumors**

Trial/therapy	Description	Reference
Monocyte-derived DCs loaded with tumor RNA	Phase I trial evaluating DCs pulsed with tumor RNA in children with brain tumors	[93]
Monocyte-derived DC loaded with whole tumor lysate	Phase I trial evaluating DCs pulsed with whole tumor lysate in children and adults with relapsed malignant glioma	[94]
Monocyte-derived DC loaded with whole tumor lysate	Phase I trial with DC pulsed with tumor lysate for children with newly diagnosed or recurrent high-grade gliomas	[95]
Monocyte-derived DC loaded with whole tumor lysate	Phase I trial with postoperative DC loaded with tumor lysate for children and adults with recurrent GBM	[96]
Peptide vaccine targeting H3.K27M	Phase I trial evaluating peptide vaccine targeting H3.K27M neoantigen for HLA-A2+ children with H3K27M mutated glioma	NCT02960230 [101]
Peptide vaccine targeting CMV epitopes pp65 and glycoprotein B	Phase I trial evaluating peptide vaccine targeting CMV pp65 and glycoprotein B for children with recurrent malignant glioma and medulloblastoma	NCT03299309 [102]
Peptide vaccine targeting glioma antigens	Phase I peptide trial using glioma-associated antigens for HLA-A2+ children with malignant brainstem and non-brainstem gliomas, including low-grade glioma	NCT01130077 [103]

are genetically modified to express the CAR molecule, which is designed to bind a tumor-restricted antigen and cause tumor cell death.

The CD19 CAR, which is effective against B-lineage lymphoid malignancies<sup>[111,112]</sup>, is FDA approved and induces remission in most patients with relapsed CD19-positive leukemia. CAR T cells targeting HER2<sup>[113]</sup>, IL13 $\alpha$ 2<sup>[114]</sup>, EGFRvIII<sup>[115]</sup>, and EphA2<sup>[116]</sup> have been used to treat adults with GBM. A trial involving CMV-specific cytotoxic T lymphocytes expressing a HER2 CAR treated seven children with GBM. There were no serious adverse events or instances of cytokine release syndrome, and at least one child had a partial response<sup>[113]</sup>. Transient responses following adoptive CAR T cell therapy are not infrequent, but almost all patients ultimately suffer disease progression.

There are multiple reasons the success of the CD19 CAR for B-lymphoblastic leukemia has not been duplicated by CAR T cells for brain tumors. The CD19 CAR targets an antigen that is ubiquitous and expressed solely on tumor cells or non-essential B cells without a strongly immunosuppressive tumor bed. Additionally, the CD19 single chain variable fragment (scFv) that guides the CAR T cell imparts an optimal activation profile and supports continued T cell killing<sup>[117]</sup>. This characteristic of the scFv is a key and unique distinction in this T cell product. ScFvs for other CAR T cells cause tonic signaling, which can cause T cell exhaustion and limits anti-tumor activity in patients following adoptive transfer<sup>[117]</sup>.

Antigen escape, tumor heterogeneity, and a harshly immunosuppressive immune microenvironment also contribute to treatment failure by CAR T cells. In a recently completed phase I trial for adults with recurrent GBM, EGFRvIII CAR T cells reliably reached the tumor bed following peripheral administration. However, *ex vivo* analyses from resected tumor showed dramatic adaptive resistance, with markedly increased PD-L1 expression and an influx of regulatory T cells, as well as decreased expression of the targeted EGFRvIII antigen<sup>[115]</sup>.

## CONCLUSIONS

Immunotherapy holds tremendous promise for improving outcomes for children with brain tumors. While checkpoint inhibitors and CAR T cells are well suited for hypermutated, immunologically hot tumors and B-cell malignancies, respectively, these modalities are less of a fit for pediatric brain tumors. Rather, immunotherapy approaches that induce inflammation and an innate immune response may be a better starting point, on which checkpoint agents and other T cell-directed agents can build.

While we are optimistic about immunotherapy in pediatric neuro-oncology, it is important to recognize that conventional chemotherapy and radiation will likely retain a role in treatment, particularly as both of these

modalities can be immunomodulatory and useful for shifting the immune balance toward anti-tumor immunity. Advanced surgical practice, radiation, and chemotherapy, including novel, targeted agents, remain important tools for treating our pediatric patients. It is important to point out that the most impactful treatment for brain tumors in the last decade is probably not an immunotherapy; BRAF and MEK inhibitors targeting the MAP kinase pathway, which is constitutively overactive in pilocytic astrocytoma and a fraction of other glial tumors, are radically changing how these diseases are treated and improving outcomes<sup>[118]</sup>. Taken together, the immunological context and molecular pathogenesis of each child's tumor must be considered on a case-by-case basis in determining any therapy, particularly in deciding what type of immunotherapy is most likely to add benefit.

## DECLARATIONS

### Authors' contributions

Design: Landi D, Ashley D

Literature research, manuscript writing, editing, and revision: Landi D, Thompson E, Ashley D

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None.

### Conflicts of interest

The authors declare that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

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