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Clinical Advances in Oncolytic Virotherapy for Pediatric Brain Tumors

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Abstract

Malignant brain tumors constitute nearly one-third of cancer diagnoses in children and have recently surpassed hematologic malignancies as the most lethal neoplasm in the pediatric population. Outcomes for children with brain tumors are unacceptably poor and current standards of care—surgical resection, chemotherapy, and radiation—are associated with significant long-term morbidity. Oncolytic virotherapy has emerged as a promising immunotherapy for the treatment of brain tumors. While the majority of brain tumor clinical trials utilizing oncolytic virotherapy have been in adults, five viruses are being tested in pediatric brain tumor clinical trials: herpes simplex virus (G207), reovirus (pelareorep/Reolysin), measles virus (MV-NIS), poliovirus (PVSRIPO), and adenovirus (DNX-2401, AloCELYVIR). Herein, we review past and current pediatric immunovirotherapy brain tumor trials including the relevant preclinical and clinical research that contributed to their development. We describe mechanisms by which the viruses may overcome barriers in treating pediatric brain tumors, examine challenges associated with achieving

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effective, durable responses, highlight unique aspects and successes of the trials, and discuss future directions of immunovirotherapy research for the treatment of pediatric brain tumors.

Keywords

pediatric; oncolytic; virotherapy; immunotherapy; brain tumors; glioma

1. Introduction

Primary central nervous system (CNS) tumors account for nearly one-third of all childhood and adolescent cancer diagnoses (Siegel, et al., 2021). Roughly 30% of primary CNS tumors are malignant and, as such, have recently surpassed leukemia as the most common cause of childhood cancer-related deaths (Ostrom, et al., 2020). Treatment regimens for malignant brain tumors centered on surgical resection, chemotherapy, and radiation have only modestly improved outcomes for decades. Additionally, current treatments are associated with significant adverse effects and long-term treatment sequelae including neurocognitive and neurosensory impairment and endocrine dysfunction, which are of particular concern for the pediatric patient population (Plant-Fox, et al., 2021). Immunotherapies, including oncolytic virotherapy, offer novel, targeted approaches for adult and pediatric patients with brain tumors and may be beneficial as adjuvant therapy, allowing for lower doses and reduced toxicity from traditional therapies (Cripe, et al., 2015; Friedman, et al., 2015).

Most oncolytic viruses (OVs) are engineered to selectively lyse and replicate in cancer cells, sparing healthy cells from the adverse effects of antitumor treatments. These OVs may be inoculated intratumorally or intracranially to bypass the blood-brain barrier which allows them to overcome this significant challenge in the successful treatment of pediatric brain tumors. Following infection and lysis of tumor cells, released viral progeny spread to adjacent tumor cells propagating oncolysis throughout heterogeneous tumor tissue. Accompanying the release of viral progeny, tumor-associated antigens, which are typically limited in pediatric brain tumors due to low somatic mutational burden, are released and made available for immune recognition (Mackay, et al., 2017). Furthermore, molecules containing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) are also released, leading to inflammation and innate immune responses that increase tumor antigen presentation and result in T cell priming and activation (Zhang, et al., 2021). OVs can be further engineered or combined with other therapies to amplify the release of tumor antigens and stimulate an antitumor immune response, turning immunologically inert tumor microenvironments into antitumor immune hotspots (Jones & Baker, 2014).

While OVs differ in their mechanisms of tumor specificity and extent of immune modulation, they are all used with the intent to selectively kill tumor cells and/or trigger durable antitumor immune responses while minimizing toxicities. Of the many viruses being studied as potential oncolytic virotherapy agents, five have been used in children with brain tumors. Herein, we review pediatric brain tumor clinical trials using OVs. The timeline in which these therapies have been tested in adults and subsequently children is detailed in

Figure 1. In discussing selected adult clinical trials and preclinical evidence, we aim to provide context and rationale for the design of pediatric brain tumor trials. Additionally, we highlight unique features and successes of the trials and discuss implications for the future of oncolytic virotherapy for the treatment of pediatric brain tumors.

2. Herpes Simplex Virus-1 (HSV-1)

HSV-1 is an enveloped double-stranded DNA virus and member of the *Herpesviridae* family. The first OV to receive U.S. Food and Drug Administration (FDA) approval as an immunotherapeutic agent was talimogene laherparepvec (T-Vec), an engineered oncolytic HSV-1 (oHSV) that produces granulocyte-macrophage colony-stimulating factor (GM-CSF) during replication, for the treatment of advanced melanoma (Andtbacka, et al., 2015); see Table 1 for a summary of viruses discussed in the text. Currently, there are six oHSVs (G207, HSV1716, G47Delta, M032, C134, and rQNestin) that have been used in clinical trials for brain tumors (Cassady, et al., 2017; Chiocca, et al., 2020; Friedman, et al., 2021; Markert, et al., 2000; Patel, et al., 2016; Rampling, et al., 2000; Todo, et al., 2001). These clinically relevant oHSVs are engineered to include deletions of the $\gamma_1$34.5 neurovirulence gene (Mineta, et al., 1995; Whitley, et al., 1993), thereby preventing productive infection in normal cells through protein kinase R (PKR)-mediated translational arrest (Liu, et al., 2003). Tumor cells often contain signaling alterations such as Ras overactivity that prevent effective shutdown of translation, allowing for viral replication and lysis of tumor cells (Farassati, et al., 2001).

Most oHSVs used in clinical trials have taken advantage of the large HSV genome to include additional modifications. For example, to further reduce the risk of infection in normal cells and enhance safety, G207 and G47Delta contain a disabling *lacZ* insertion within U_L39, the viral gene that encodes the large subunit of ribonucleotide reductase (Mineta, et al., 1995; Todo, et al., 2001). Additionally, oHSVs have an intact or reinserted thymidine kinase gene and, therefore, maintain sensitivity to anti-viral agents already in clinical use such as acyclovir, which serves as an additional safety mechanism should viral replication cause unexpected toxicity and need to be halted (Coen, et al., 1989; Mineta, et al., 1994). oHSVs use a variety of mechanisms to increase viral replication in tumors. G47Delta has an additional deletion in the $\alpha47$ gene which results in immediate early expression of *US11* (Todo, et al., 2001); C134 expresses the human cytomegalovirus (HCMV) *IRS1* gene (Cassady, et al., 2017); and rQNestin restores one copy of ICP34.5 under transcriptional control of the promoter/enhancer element of nestin, which is overexpressed in many cancers including high-grade glioma (HGG) (Chiocca, et al., 2020). Each of these alterations allow improved viral replication in tumor cells without compromising safety in normal tissues. To enhance antitumor immune responses, G47Delta contains the $\alpha47$ gene deletion that downregulates the transporter associated with antigen presentation (TAP) in host cells and sequesters major histocompatibility complex I (MHC I) in the endoplasmic reticulum thereby increasing antigen presentation (Todo, et al., 2001); and M032 contains the human interleukin-12 (IL-12) gene which results in physiologically relevant amounts of IL-12 during replication (Patel, et al., 2016) and produces a Th1 immune response and activation of natural killer (NK) cells (Gately, et al., 1998).

oHSV works through the dual mechanism of action described above, which involves an initial virus-induced direct oncolysis followed by stimulation of a consequent antitumor immune response. The virus attaches to and enters cells through several cell surface receptors shown to be expressed on pediatric brain tumors including the cell adhesion molecule nectin-1 (CD111) (Friedman, et al., 2018; Krummenacher, et al., 2004). In preclinical studies, oHSV has been shown to infect and kill a heterogeneous population of pediatric brain tumor histotypes including HGG, ependymoma, medulloblastoma, primitive neuroectodermal tumors (PNET), and atypical teratoid/rhabdoid tumors (AT/RT) (Bernstock, et al., 2020b; Friedman, et al., 2018; Friedman, et al., 2009; Friedman, et al., 2016; Studebaker, et al., 2017). Therefore, oHSV is an attractive therapeutic for overcoming intratumoral and intertumoral cell heterogeneity found in brain tumors. In addition, oHSV has been shown to kill chemotherapy- and radiotherapy-resistant cancer stem cells in HGG and medulloblastoma (Friedman, et al., 2009; Friedman, et al., 2016). Interestingly, as a whole, pediatric brain tumor patient-derived xenograft (PDX) cells express more nectin-1 than adult HGG PDX cells and are 11- to 37-fold more sensitive to oHSVs suggesting that pediatric brain tumors may be an ideal target for oHSV (Friedman, et al., 2018).

The time required for an antitumor immune response to occur following oHSV administration is an important consideration, particularly in aggressive recurrent malignant brain tumors like HGG for which median survival in children is less than 6 months (Jakacki, et al., 2016). While data from brain tumor trials are limited and additional data are needed, a study utilizing GM-CSF-producing oHSV T-Vec (Imlygic®) in melanoma patients highlights the importance of accounting for late responses to oncolytic immunovirotherapy even after an apparent initial tumor progression; the researchers demonstrated that delayed regional and systemic antitumor responses may occur after treatment, ranging from approximately 18 weeks for directly injected lesions to 23 or more weeks for uninjected lesions (Kaufman, et al., 2016). Therefore, when evaluating response to OVs in brain tumor clinical trials, investigators may need to consider additional parameters beyond the standard criteria defined by immunotherapy Response Assessment in Neuro-Oncology (iRANO) or Response Assessment in Pediatric Neuro-Oncology (RAPNO) (Erker, et al., 2020; Okada, et al., 2015). In addition, testing OVs upfront when patients have more time to respond and a more functional immune system prior to receiving myelosuppressive chemotherapy is likely important to fully evaluate the clinical efficacy of OVs.

The first oHSV tested in a pediatric brain tumor clinical trial was HSV1716, but the trial only enrolled two patients and was subsequently terminated in 2016 (NCT02031965). Outcomes from the trial have not been published, and HSV1716 has not been tested in any additional pediatric brain tumor clinical trials. G207 has been the most extensively studied in humans and is the only oHSV currently being studied and advanced in pediatrics. The design of the G207 pediatric brain tumor trials, including a completed trial in supratentorial tumors (NCT02457845) and an ongoing trial in cerebellar tumors (NCT03911388) (Bernstock, et al., 2020a; Waters, et al., 2017), is largely based on the results from three early-phase clinical trials (NCT00157703, US0235, NCT00157703) of G207 in adults with recurrent HGG (Markert, et al., 2009; Markert, et al., 2000; Markert, et al., 2014). G207 was safely inoculated into adults with up to 3×10^9 plaque-forming units (PFU) in up to two doses. Three methods were used for these intracranial inoculations:

directly into the tumor and by controlled-rate infusion via intratumoral catheters, into the brain surrounding a resection cavity, and combined with a single 5 Gy dose of radiation within 24 hours of virus inoculation. The use of radiation was based on preclinical studies that demonstrated a 5 Gy dose within 24 hours of virus optimally increased viral replication and spread throughout the tumor (Advani, et al., 2011; Mezhir, et al., 2005). These adult trials demonstrated the safety of G207, and a maximum tolerated dose (MTD) was not reached. Furthermore, evidence of radiographic and neuropathologic responses was seen in approximately half of the patients. Interestingly, while doses up to 3×10^9 PFU were safely administered without dose-limiting toxicities, responses were not dose-dependent and occurred at doses as low as 1×10^6 PFU, suggesting that the direct oncolytic effect, which depends on virus dose, is likely not as important as the secondary immune response engendered against the tumor to achieve clinical responses.

The Phase 1 trial of G207 alone and combined with a 5 Gy dose of radiation in children with recurrent or progressive supratentorial HGG (NCT02457845) was the first completed oncolytic virotherapy brain tumor trial in children; see Table 2 for summary of pediatric oncolytic virotherapy brain tumor clinical trials. The trial included 12 children with an age range of 7–18 years old and demonstrated the safety of G207 at a maximum planned dose of 1×10^8 PFU delivered by controlled rate infusion through up to four intratumoral catheters alone and when combined with radiation dose (Friedman, et al., 2021). All patients had isocitrate dehydrogenase (IDH)-wild type HGG without known favorable mutations or H3.3 mutations. There were no serious adverse events (AEs) attributable to G207. Related AEs were mild, grade 1 and infrequent with a total of 20 events reported. Fever, which was seen in 33% of patients, was the most common AE attributable to G207. There was no evidence of G207 shedding in the blood, conjunctiva secretions or saliva in any patient.

Matched pre-treatment and 2–9 months post-treatment resection tissue from several patients conclusively demonstrated that G207 created an inflammatory tumor microenvironment and resulted in a shift from immunologically ‘cold’ to ‘hot’ with a significant increase in tumor-infiltrating lymphocytes ($CD4^+$ and $CD8^+$ T cells) within tumor areas both adjacent to and several centimeters away from the site of virus inoculation. Radiographic changes consistent with antitumor activity, including pseudoprogression or the development of progressively enlarging benign-appearing intratumoral cystic spaces were seen in most patients. The median overall survival of 12.2 months (95% confidence interval, 8.0 to 16.4) compared favorably to historical survival of 5.6 months in children with recurrent HGG (Jakacki, et al., 2016; Kline, et al., 2018). Of note, four of five patients without pre-existing HSV-1 antibodies seroconverted between 1–5 months after receiving G207 at the 1×10^8 PFU dose. Those that seroconverted survived on average longer than patients with pre-existing neutralizing antibodies; however, the number of patients was small and thus no definitive conclusion could be drawn. Whether the presence of pre-existing neutralizing antibodies or seroconversion after receiving an OV affects outcomes is unclear and may ultimately depend on tumor location, route of administration, and dosing regimen (e.g. single dose versus multiple doses). Based on these promising results, a Phase 2 multi-institutional trial in children with recurrent HGG at first relapse is expected to open in 2022 (NCT04482933). The trial will use the Recommended Phase 2 Dose (RP2D) of 1×10^8 PFU with a 5 Gy dose of radiation and the primary objective of the trial will be efficacy as evaluated by

post-progression survival. Planned secondary objectives include safety, virologic shedding, radiographic response, and changes in lymphocyte infiltration within tumor sites.

Based on preclinical data suggesting that embryonal tumors, such as medulloblastoma that arises in the cerebellum, may be even more sensitive to oHSV than pediatric HGG (Friedman, et al., 2018), a second Phase 1 trial with G207 was initiated and is ongoing in children 3–18 years old with recurrent or refractory malignant cerebellar brain tumors (NCT03911388) (Bernstock, et al., 2020a). Similar to the supratentorial pediatric trial, the study is testing G207 alone and then combined with a 5 Gy dose of radiation. The starting dose chosen was lower (1×10^6 PFU) than the supratentorial trial secondary to unique challenges of inoculating tumors in the cerebellum: the posterior fossa is less amendable to placement of catheters and swelling from pseudoprogression; and the cerebellum is adjacent to the brainstem, which is responsible for central autonomic functions and potentially vulnerable to smaller degrees of inflammation (Bernstock, et al., 2020a). If lower doses are proven safe, the trial will test a maximum planned dose of 1×10^8 with 5 Gy dose of radiation, similar to the completed Phase 1 trial.

3. Adenovirus

Adenovirus (AdV) is a nonenveloped, double-stranded DNA virus that causes mild upper respiratory symptoms in healthy individuals. AdVs have been exploited both as vectors for gene transfer therapies and as OVs in the treatment of brain tumors (Castro, et al., 2014; Kiyokawa & Wakimoto, 2019). While virally-delivered gene therapies are beyond the scope of this review, they have shown some promise in clinical trials. To date, six oncolytic AdVs derived from human AdV serotype 5 (ONYX-015, DNX-2401, DNX-2440, CRAd-S-pk7, ICOVIR-5, and Ad-TD-nsIL12) have been used in HGG clinical trials. Some of these trials use neural stem cell (NSC) or mesenchymal stem cell (MSC)-based carrier systems to deliver the AdVs. Oncolytic AdVs have been engineered to conditionally replicate in tumor cells through various mechanisms. For example, ONYX-015 lacks the *E1B* gene, leading to loss of E1B-mediated late viral RNA export and restriction of replication in normal cells, while replication is supported in tumor cells that provide the RNA export function of E1B (Bischoff, et al., 1996; O'Shea, et al., 2004). A 24-base pair deletion in the *E1A* gene of DNX-2401, DNX-2440, and ICOVIR-5 limits viral replication to malignant cells that have defective Rb tumor suppressor protein (Whyte, et al., 1989). To further increase tumor tropism and bioavailability while limiting potential damage to surrounding brain parenchyma or off-target tissues, DNX-2401, DNX-2440, and ICOVIR-5 are further modified to include the arginine-glycine-aspartate (RGD) peptide. RGD directs the engineered virus to bind integrins, which are expressed at much higher levels than the natural AdV receptor on glioma cell surfaces (Fueyo, et al., 2003). In addition, DNX-2440 expresses the immune modulator OX40 ligand, which is designed to enhance antitumor T cell responses (Jiang, et al., 2017). NSC-CRAd-S-pk7 is a conditionally replicative AdV delivered by NSCs that contains the human survivin promoter to drive *E1* expression and a polylysine modification of the fiber knob to enhance the ability to transduce glioma (Ulasov, et al., 2007). Ad-TD-nsIL12 harbors protective deletions in both *E1A* and *E1B* as well as a deletion in *E3gp-19K* and an insertion of non-secreting IL-12 gene driven by

the endogenous E3gp-19K promoter to enhance antitumor immune responses (Wang, et al., 2017; Wang, et al., 2003).

In two completed Phase 1 adult trials for recurrent malignant gliomas, oncolytic AdVs were safe with evidence of efficacy when administered up to 10^{10} PFU directly into 10 sites of the tumor resection cavity (ONYX-015) (Chiocca, et al., 2004) and as a single dose up to 3×10^{10} intratumorally (DNX-2401; NCT00805376) (Lang, et al., 2018). Five of 25 patients (20%) that received DNX-2401 survived over three years after treatment and three patients (12%) had a ≥ 95% reduction in enhancing tumor (Lang, et al., 2018). The latter trial included an additional group of patients that received intratumoral DNX-2401 via an implanted catheter followed by *en bloc* resection of the tumor and catheter, and second dose of virus 14 days later. Viral replication and spread within the tumor was documented and histopathology demonstrated tumor infiltration by $CD8^+$ and T-bet^+ cells suggesting that responses were likely due to direct oncolysis and generation of an antitumor immune response. A single Phase 1/2 dose-escalation study in the Netherlands designed to test up to 10^{11} viral particles of DNX-2401 in adults with recurrent brain tumors was completed in 2014 (NCT0158251648), but results have not yet been published. Additionally, combining therapies with OVs to maximize an immune response is of great interest, and several drugs including temozolomide (NCT01956734), pembrolizumab (NCT02798406), and interferon gamma (IFN-γ) (NCT02197169) have been tested in combination with DNX-2401 in early phase trials for adults with recurrent HGG. These trials have completed enrollment, and results are forthcoming.

Another important area of oncolytic virotherapy research is novel delivery approaches. Delivery of virus via infected NSCs, or allogeneic or patient-derived bone marrow-derived MSC carriers is of interest due to their tumor tropism, and ability to evade neutralizing antibodies, cross the blood-brain barrier, distribute throughout a tumor and its margins, and migrate in brain parenchyma to target tumor cells (Fares, et al., 2021; Ruano, et al., 2020; Shimizu, et al., 2021). Recently, a first-in-human Phase 1 trial using NSC-CRAd-S-pk7 was conducted in adults with newly diagnosed HGG (NCT03072134) (Fares, et al., 2021). Patients received the virus into the walls of the resection cavity at a maximum planned dose of 1.875×10^{11} viral particles administered by 1.5×10^8 NSCs, and subsequently received standard of care with temozolomide and radiation. The therapy was deemed safe, with a single grade 3 event, viral meningitis due to inadvertent injection in the lateral ventricle. Immunological and histopathological evidence of responses was seen to support further investigation of the virus and delivery technique.

Additionally, a first-in-human trial of autologous bone marrow-derived MSCs as carriers for oncolytic AdV ICOVIR-5 (CELYVIR) in children and adults with relapsed/refractory solid tumors (NCT01844661), including a child with medulloblastoma and three adults with HGG, demonstrated safety of six weekly intravenous (IV) infusions of 2×10^6 cells/kg in children and $0.5–1 \times 10^6$ cells/kg in adults at a dose of 2×10^4 viral particles/MSC (Ruano, et al., 2020). Adenoviral replication was detected by PCR in 7 of 9 pediatric patients but none of the six adult patients. There are two ongoing clinical trials of allogenic MSCs infected with AdVs: a Phase 1b/2 trial with ICOVIR-5 (AloCELYVIR) in children and young adults with newly diagnosed diffuse intrinsic pontine glioma (DIPG) combined with

radiation or in relapsed medulloblastoma as monotherapy (NCT04758533); and a Phase 1 trial with DNX-2401 administered via intra-arterial injection in adults with recurrent HGG (NCT03896568).

Based on preclinical data demonstrating efficacy of DNX-2401 in models of pediatric HGG and DIPG, the most deadly form of pediatric HGG (Martinez-Velez, et al., 2019a; Martinez-Velez, et al., 2019b), and the established safe doses of intratumoral DNX-2401 from the adult trials, a first-in-human Phase 1 trial was initiated for children 1–18 years old with newly diagnosed DIPG that have not received previous treatment (NCT03178032) (Tejada, et al., 2018a; Tejada, et al., 2018b). After stereotactic biopsy, patients received intratumoral infusion of $1–5 \times 10^{10}$ viral particles in 1 mL delivered over approximately 70 minutes through a cannula, and subsequently, received standard radiotherapy within 1 month of DNX-2401 administration. While final study results have not been reported, 12 patients received the virus and no dose-limiting toxicities were observed (Garcia-Moure, et al., 2021). Efficacy evaluations and correlative analyses of tumor biopsy and blood samples including pre- and post-treatment titers of neutralizing antibodies are ongoing; however, the investigators recently reported an increased clonal T cell diversity following treatment with virus when comparing paired pre- and post-treatment samples (Iñigo-Marco, et al., 2022). Since patients also received standard radiation and the timing of the post-treatment samples in relation to DNX-2401 and radiation was not provided, it is unclear if these changes are due specifically to the virus. Nevertheless, this proof-of-principle study demonstrates the ability to directly treat a tumor in a very challenging location with an OV as part of initial therapy, which is an important paradigm shift towards upfront immunovirotherapy.

4. Poliovirus

Poliovirus (PV) is a non-enveloped, single-stranded positive-sense RNA virus of the *Picornaviridae* family. PV has an inherent tropism for lower motor neurons and can cause paralytic poliomyelitis. Its neuropathogenicity and CNS tropism, while not entirely understood, depend on tissue-specific function of its internal ribosomal entry site (IRES) element and differential expression of integral membrane protein CD155, which is the viral attachment and entry receptor. CD155 is known to play a role in cell adhesion and immune response (Takai, et al., 2008) and is upregulated in malignant cells and stromal components of many solid tumors including gliomas (Merrill, et al., 2004). Recombination with human rhinovirus type 2 ablates the inherent neuropathogenicity of PV while preserving its oncolytic properties, and preclinical studies have demonstrated that HGG were highly susceptible to oncolysis by the recombinant PV (Gromeier, et al., 1996; Gromeier, et al., 2000; Merrill, et al., 2004).

The first Phase 1 trial of an attenuated polio-rhinovirus chimera, PV (Sabin)-Rhinovirus IRES PV Open reading frame (PVSRIPO) was in 61 adults with recurrent glioblastoma who received a dose between 10^7-10^{10} median tissue culture infectious dose ($TCID_{50}$) infused via intratumoral convection-enhanced delivery (CED) (NCT01491893) (Desjardins, et al., 2018). Pre-clinical studies using tagged viruses have demonstrated that CED has the potential to improve volume distribution and tissue penetration of viral doses in animal models (White, et al., 2011a; White, et al., 2011b). Seven of the tumors were IDH-mutant

and 9 had unknown IDH status. Patients were given a boost immunization with trivalent inactivated PV vaccine at least one week prior to PVSRIPO in an effort to generate a robust immune response against PVSRIPO and further limit the possibility of neurovirulence in normal brain cells. In the dose expansion phase, 19% of the participants had a ≥ grade 3 AE. Due to locoregional inflammation after treatment resulting in prolonged glucocorticoid use, the dose was deescalated to the RP2D of 5×10^7 $TCID_{50}$. Overall survival was reported at 21% at 24 months (95% confidence interval, 11 to 33); based on the results, the therapy was granted Breakthrough Therapy designation by the FDA in 2016. When patients were stratified by median tumor mutational burden (TMB; 1.3 mutations/megabase), those with a lower TMB had a significantly longer survival (Gromeier, et al., 2021), which may be attributable to an inverse relationship between TMB and enrichment of inflammatory gene signatures discovered in cohorts of recurrent but not newly diagnosed glioblastoma. These findings may have important implications for treating pediatric brain tumors with OVs since most pediatric brain tumors have a very low TMB and generally have a lower TMB than adult brain tumors (Jones & Baker, 2014).

The promising results of the Phase 1 trial of PVSRIPO in adults prompted the initiation of a Phase 1b trial for recurrent pediatric HGG in 2017 designed to determine safety of the approach in children 12–17 years of age (NCT03043391) (Ashley, et al., 2018). Based on preclinical data demonstrating susceptibility of both pediatric glial and embryonal brain tumors to PVSRIPO, the pediatric trial was subsequently amended to include any recurrent malignant glial tumor (e.g. malignant glioma, anaplastic oligodendroglioma, ependymoma), medulloblastoma, or AT/RT, and to include patients up to 21 years of age (Thompson, et al., 2018). Similar to the adult study design, patients receive a boost immunization with inactivated PV vaccine at least one week prior to PVSRIPO delivery. Immediately following stereotactic biopsy and histopathologic confirmation of recurrent tumor, patients then receive 5×10^7 $TCID_{50}$ of PVSRIPO via catheters over 6.5 hours. The study is no longer recruiting and results are forthcoming.

5. Reovirus

Reovirus is a non-enveloped double-stranded RNA (dsRNA) virus that typically results in asymptomatic or mild symptoms including fever, vomiting or diarrhea in healthy individuals, as the PKR pathway in non-malignant cells recognizes the presence of dsRNA and blocks active viral translation (Muller, et al., 2020). In many tumor types, including gliomas, Ras upregulation inhibits PKR activation and renders wild-type reovirus naturally oncoselective in a manner similar to engineered oHSV; sensitivity of tumor cells to reovirus, however, is likely dependent on both molecular and cellular determinants that are still being elucidated (Gong & Mita, 2014; Muller, et al., 2020). In the first U.S. clinical trial utilizing reovirus for brain tumors, escalating doses of 10^8 to 10^{10} $TCID_{50}$ of pelareorep (Reolysin), a live, replication-competent wild-type reovirus serotype-3-Dearing strain, were infused intratumorally over a 72-hour period in 15 adults with recurrent HGG (NCT00528684) (Kicielinski, et al., 2014). Pelareorep was safe without dose-limiting toxicities, and while the trial was the first to show that the presumed approach of viral delivery by CED was safe in recurrent HGG, the volume of distribution of the virus was not assessed secondary to financial limitations and difficulty of demonstrating the distribution of the viral dose versus

that of the vehicle in humans. Similar to the completed adult and pediatric oHSV G207 trials, a MTD was not reached. In addition, evidence of antitumor activity was seen in some patients but did not appear to be dose-dependent.

Prior to testing pelareorep in pediatric brain tumors, safety of the virus alone and combined with low-dose oral cyclophosphamide was confirmed in children and young adults with relapsed or refractory non-CNS, non-lymphoma solid tumors in a multi-institutional Children's Oncology Group Phase 1 trial (NCT01240538) (Kolb, et al., 2015). Twenty-four evaluable patients 3–21 years of age received IV reovirus at 5×10^8 $TCID_{50}$/kg daily for 5 days every 28 days, and five of those patients also received low-dose oral cyclophosphamide at 50 mg/m^2 × 21 days (Kolb, et al., 2015). Cyclophosphamide was used to test the hypothesis that reovirus could be safely administered with an immunosuppressive agent, as this combination was shown to inhibit both NK cells and T-regulatory cells and facilitate antitumor efficacy in murine models (Ghiringhelli, et al., 2007; Qiao, et al., 2008). The trial demonstrated the virus was rapidly cleared from the serum with a median time of 6.5 days, and there was no difference in the duration of viremia or peak viremia in nine patients without pre-existing reovirus antibodies compared to the 14 patients with baseline antibodies. In addition, there was no shedding of virus in the saliva or stool. Cyclophosphamide did not appear to affect viral clearance or peak anti-reovirus antibody level. While there were no objective responses, three patients had stable disease and received a second cycle.

Similar to the pediatric non-CNS trial, the ongoing Phase 1 trial of pelareorep in patients 10–21 years of age with high-grade relapsed or refractory brain tumors (NCT02444546) utilizes an IV delivery route; however, the virus is given on days 3–5 of a 28-day cycle after subcutaneous GM-CSF daily on days 1 and 2 of each cycle for up to 12 cycles in the absence of disease progression or unacceptable toxicity. The use of GM-CSF for this trial was based on preclinical murine melanoma model data demonstrating that IV reovirus is rapidly neutralized by pre-existing reovirus antibodies but functional virus bound by antibody was present in $CD11b^+$ monocytes/macrophages, which effectively chaperoned the virus to tumor cells (Ilett, et al., 2014). Mobilization of monocytes/macrophages with GM-CSF prior to reovirus treatment resulted in improved tumor control in the melanoma model, and conditioning with GM-CSF was most efficacious when there were pre-existing neutralizing antibodies. The primary objective of the pediatric Phase 1 study is to determine the MTD and associated toxicities of the combination therapy, and secondary objectives are to assess median progression-free survival and overall survival. Exploratory objectives are to determine whether there is a correlation between antibody responsiveness to the virus or an increase in number of circulating monocytes with tumor response. This study completed accrual with 6 patients, and results are pending.

6. Measles Virus

Measles virus (MV) belongs to the *Paramyxoviridae* family of single-stranded negative-sense RNA viruses. Oncolytic modified MV, derived from the attenuated Edmonston B vaccine strain, displays tropism for CD46, a cofactor of complement inactivation (Dorig, et al., 1993). Tumor cells, which express higher densities of CD46 surface receptors than

normal surrounding tissue as a possible mechanism to overcome complement-dependent cytotoxicity, are selectively infected by MV (Anderson, et al., 2004; Fishelson, et al., 2003). There are two Edmonston lineage MVs in clinical trials that have been genetically engineered to express trackable proteins: MV-CEA expresses human carcinoembryonic antigen (CEA), an inert peptide that is widely used as a tumor marker (Peng, et al., 2002); and MV-NIS expresses a sodium iodide symporter (NIS), which enables non-invasive monitoring of viral accumulation and propagation (Dingli, et al., 2004). MV-CEA, showed no dose limiting toxicities following direct intratumoral or resection cavity administration of up to 10^7 $TCID_{50}$ in a Phase 1 clinical trial of adults with HGG (NCT00390299). Full results have not been published to date, and this virus has not been used in pediatric trials.

Safety of MV-NIS has been demonstrated in adult extracranial solid tumors (Galanis, et al., 2015), and while the virus has not been used in clinical trials for adult brain tumors, there is an active trial of MV-NIS (NCT02962167) for children and young adults (12 months to 39 years old) with recurrent medulloblastoma or AT/RT. The trial design was based on preclinical studies showing expression of CD46 in medulloblastoma tissue specimens and cells lines, and the effectiveness of intratumoral, IV, or intraventricular MV in prolonging survival in mice with localized or disseminated medulloblastoma or AT/RT (Hutzen, et al., 2012; Studebaker, et al., 2012; Studebaker, et al., 2015; Studebaker, et al., 2010). The primary objective of this ongoing Phase 1 trial is to determine AE frequency and a RP2D. Secondary and exploratory objectives include objective response rate and progression-free survival, and distribution of MV-NIS via single-photon emission computed tomography (SPECT) imaging after technetium-99m. Patients with locally recurrent tumors receive MV-NIS directly into the tumor bed following local resection. Those with disseminated disease will receive either one or two doses of MV-NIS via lumbar puncture into the subarachnoid space. This study is the first and only OV trial in pediatric brain tumors to utilize inoculation of virus into the cerebrospinal fluid via this route, which enables the direct targeting of leptomeningeal disease and spinal metastases.

7. Future Perspectives

Undoubtedly, significant progress has been made in the clinical use of OVs for pediatric brain tumors. Based on the results of completed clinical trials, OVs generally have favorable safety profiles, particularly in comparison to standard-of-care chemotherapy and radiation. Despite the predominance of infratentorial tumors amongst children with brain tumors, the majority of pediatric clinical trials to date have enrolled children with supratentorial brain tumors, mirroring the patient populations enrolled in counterpart adult trials. With increasing evidence of the safety of OVs in both adult and pediatric patients, trials focused on infratentorial tumors have been initiated. oHSV, MV, and AdV-based therapies are being evaluated in Phase 1 clinical trials specifically enrolling children with infratentorial tumors. Notably, the latter therapies are being explored as upfront therapy, potentially foreshadowing the implementation of immunovirotherapy as a frontline adjuvant treatment.

Several challenges exist and must be overcome to maximize immunovirotherapy and achieve durable responses in brain tumor patients. Improved preclinical models of disease are needed to gain a better understanding of therapeutic mechanisms in order to optimize

oncolytic virotherapy. PDX models of brain tumors recapitulate many of the histological and genetic features of patient tumors, but the necessity to inoculate PDX lines into immunocompromised mice precludes comprehensive evaluation of immune responses. Alternatively, syngeneic murine models of brain tumors enable study of immune responses in immunocompetent mice, but there is significant heterogeneity in the infectivity and replicative capacity of OVs to various murine tumor models. Defining biomarkers to predict response from oncolytic virotherapy is critical so that patients most likely to benefit are included in trials and those unlikely to benefit can receive an alternative therapy. In addition, delivery of OVs to the intracranial compartment typically necessitates a neurosurgical procedure. While intracranially inoculated OVs can minimize concerns of systemic toxicity and ensure adequate delivery to the tumor, these procedures can be technically challenging and not without risk. Developing increasingly novel methods of viral delivery, such as the use of nanoparticle or cell-based carriers, may eliminate the need for a neurosurgical procedure and open new therapeutic avenues for patients with lesions in locations that are not surgically accessible or pose significant operative risks.

Currently, there are inadequate response assessment tools available after a patient receives immunovirotherapy, which makes it challenging to make subsequent treatment decisions. Improved imaging modalities to define responses from immunovirotherapy are critical. OVs can cause inflammation within brain tumors and surrounding brain parenchyma, which can lead to neurologic symptoms from increased edema. Corticosteroids are typically used in brain tumor patients to control neurologic symptoms; however, corticosteroids can cause immunosuppression. Bevacizumab has been used off-label as an alternative anti-inflammatory agent, but the ideal approach to managing inflammation is unknown. Lastly, an improved understanding of the tumor microenvironment and mechanisms of resistance to oncolytic virotherapy is needed to design next generation OVs and rational combination therapies to maximize antitumor immune responses. In addition to the combination approaches currently being tested in clinical trials, future immunovirotherapy brain tumor trials will likely include small molecule inhibitors, monoclonal antibodies, adoptive cellular therapies, radioimmunotherapy, cancer vaccines, and checkpoint inhibitors in conjunction with OVs. Oncolytic immunovirotherapy clinical trial results to date have been very promising in children, who may be ideal candidates for the therapy based on both their immune responsiveness and tumor biology. If the challenges described above can be overcome, oncolytic virotherapy has the potential to change the clinical landscape for the treatment of pediatric brain tumors.

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Conflict of Interest Statement:

JDB has an equity position in Avidea Technologies, Inc., which is commercializing polymer-based drug delivery technologies for immunotherapeutic applications. JDB has an equity position in Treovir LLC, an oHSV clinical

stage company and is a member of the POCKiT Diagnostics Board of Scientific Advisors. JMM received payments from a structured buyout of Catherex, Inc., completed March 2021 (Catherex no longer exists); he has an equity position in and has received royalties from Aettis, Inc., which holds frozen oncolytic viral stocks, and in Treovir, Inc., which holds a small business innovation research fund to execute a clinical trial of G207 in pediatric patients; he has served as a consultant for Imugene; finally, he holds intellectual property for an oncolytic virus which has been licensed to Mustang Bio, Inc. but is blinded to the specifics of the relationship. GKF receives support from Eli Lilly and Company and Pfizer through contracts to UAB for clinical trials unrelated to OVs.

The remaining authors (GG, KK, SKT, SG, AR, SB, KK, MRC, RL, EB, AB, and JMJ) declare no conflict of interest exists.

Abbreviations:

AdV	Adenovirus
AE	adverse event
AloCELYVIR	allogenic mesenchymal stem cells loaded with ICOVIR-5
AT/RT	atypical teratoid/rhabdoid tumor
CEA	carcinoembryonic antigen
CED	convection-enhanced delivery
CELYVIR	autologous bone marrow-derived mesenchymal stem cells loaded with ICOVIR-5
CNS	central nervous system
DIPG	diffuse intrinsic pontine glioma
GBM	glioblastoma
GM-CSF	granulocyte-macrophage colony-stimulating factor
HGG	high-grade glioma
HSV	Herpes Simplex Virus-1
IDH	isocitrate dehydrogenase
IFN-γ	interferon gamma
IL-12	interleukin-12
IRES	internal ribosomal entry site
IT	intratumoral
IV	intravenous
LP	lumbar puncture
MB	medulloblastoma
MSC	mesenchymal stem cell

MTD	maximum tolerated dose
MV-CEA	engineered oncolytic measles virus expressing carcinoembryonic antigen
MV-NIS	engineered oncolytic measles virus expressing sodium iodide symporter
MV	Measles virus
NIS	sodium iodide symporter
NSC	neural stem cell
oHSV	engineered oncolytic HSV-1
OV	oncolytic virus
Peds	pediatric
PFU	plaque-forming units
PKR	protein kinase R
PNET	primitive neuroectodermal tumors
PV	Poliovirus
PVSRIPO	PV (Sabin)-Rhinovirus IRES PV Open reading frame
Rad	radiation
RGD	arginine-glycine-aspartate
RP2D	Recommended Phase 2 Dose
SQ	subcutaneous
T-Vec	talimogene laherparepvec
$TCID_{50}$	median tissue culture infectious dose
TMB	tumor mutational burden

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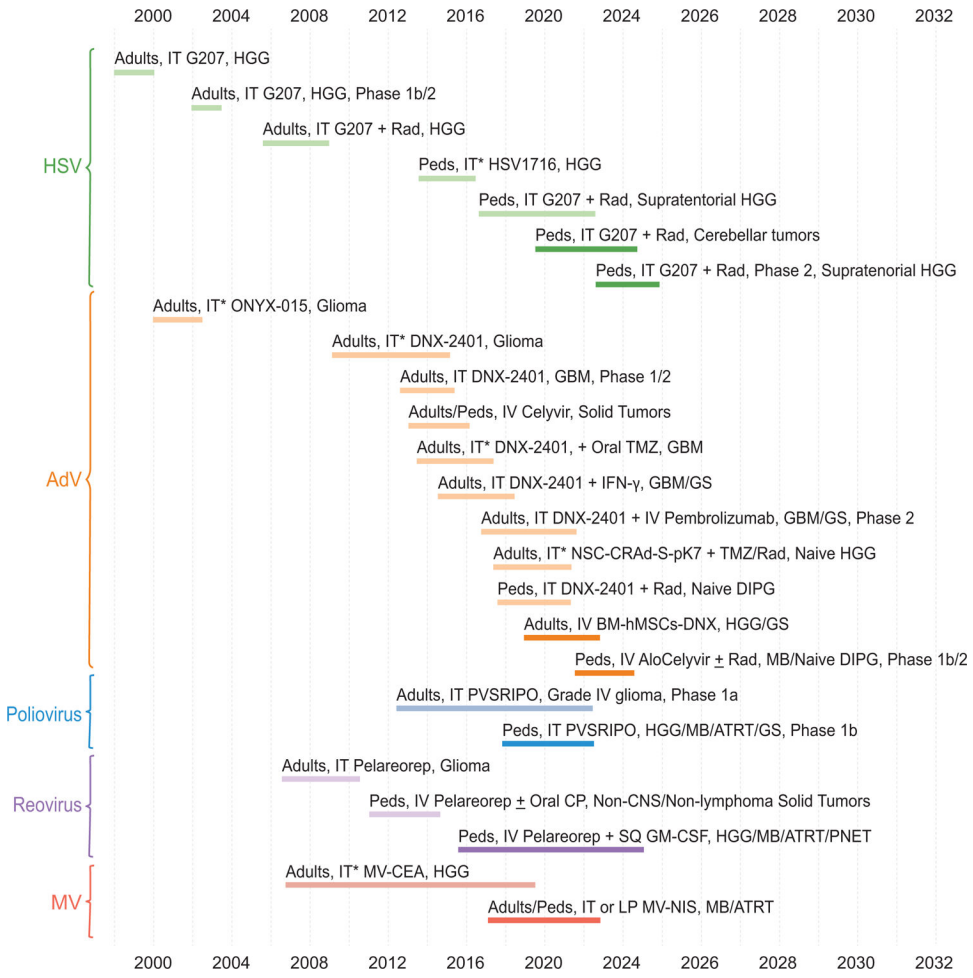

Figure 1: Timeline of clinical trials contributing evidence to the design and rationale of current clinical trials of oncolytic viruses for the treatment of pediatric brain tumors.

Each block represents a clinical trial. Lighter colored blocks represent trials that have been completed or terminated. Darker colored sections indicate active trials with estimated start and end dates as reflected on Clinicaltrials.gov, Gene Therapy Clinical Trials Worldwide (http://www.abedia.com/wiley/index.html), and (Chiocca, et al., 2004)as of January 2022. Unless otherwise noted, all trials are Phase 1. Age groups up to 21 years are designated pediatric. AdV adenovirus; ATRT atypical teratoid/rhabdoid tumor; CNS central nervous system; CP cyclophosphamide; DIPG diffuse intrinsic pontine glioma; GBM glioblastoma; GM-CSF granulocyte-macrophage colony-stimulating factor; GS gliosarcoma; HGG high-grade glioma; HSV herpes simplex virus; IFN-γ interferon-gamma; IT intratumoral (*indicates treatment may have been delivered peritumorally or into resection cavity); IV intravenous; LP lumbar puncture; MB medulloblastoma; MV measles virus; Peds pediatric; PNET primitive neuroectodermal tumor; Rad radiation; SQ subcutaneous; TMZ temozolomide. Created with BioRender.com

Table 1.

Summary of viruses

Virus	Deletion/Mutation	Foreign gene/promoter insertion	Reference
HSV-1			
C134	Deletion in both copies of $\gamma_1$34.5 gene	IRS1 gene under control of an HCMV immediate early promoter	(Cassady, et al., 2017)
G207	Deletion in both copies of $\gamma_1$34.5 gene and disabling lacZ insertion in U_L39	None	(Mineta, et al., 1995)
G47Delta	Deletion of the $\gamma_1$34.5 and α47 genes and a disabling lacZ insertion within U_L39	None	(Todo, et al., 2001)
HSV1716	Deletion in both copies of $\gamma_1$34.5 gene	None	(Streby, et al., 2017)
M032	Deletion in both copies of $\gamma_1$34.5 gene	Human IL-12 gene insertion	(Patel, et al., 2016)
rQNestin	Deletion in $\gamma_1$34.5 gene and U_L39	ICP-34.5 under control of synthetic nestin promoter	(Chiocca, et al., 2020)
T-Vec	Deletions of the ICP34.5 and ICP47 genes	Granulocyte-macrophage colony-stimulating factor, CMV promoter	(Andtbacka, et al., 2015)
Adenovirus			
Ad-TD-nsIL12	Deletions in E1A, E1B, E3gp-19K genes	Non-secretory IL-12 gene under control of E3gp-19K promoter	(Wang, et al., 2017)
CRAd-S-pk7	Deletion of native E1 promoter	E1A expression under control of human survivin; pk7 encoding polylysine	(Ulasov, et al., 2007)
DNX-2401	Deletion of Rb-binding region from E1A	RGD peptide motif insertion	(Fueyo, et al., 2003)
DNX-2440	Deletion of Rb-binding region from E1A	RGD peptide motif insertion; OX40 ligand expression cassette replacing E3 region	(Jiang, et al., 2017)
ICOVIR-5	Deletion of Rb-binding region from E1A	RGD peptide motif insertion; Substitution of the E1A promoter for E2F1-responsive elements	(Majem, et al., 2006)
ONYX-015	E1B gene deletion preventing production of E1B-55kDa protein	none	(Bischoff, et al., 1996)
Poliovirus			
PVSRIPO	Native IRES	Native IRES substituted with IRES from human rhinovirus type 2	(Gromeier, et al., 2000)
Reovirus			
Pelareorep	None relative to wild type reovirus Type 3 Dearing strain	None relative to wild type reovirus Type 3 Dearing strain	(Kicielinski, et al., 2014)
Measles Virus			
MV-CEA	None relative to Edmonston vaccine strain	Expresses soluble extracellular domain of human CEA	(Peng, et al., 2002)
MV-NIS	None relative to Edmonston vaccine strain	Expresses human thyroidal NIS	(Dingli, et al., 2004)

Table 2.

Pediatric Oncolytic Virotherapy Clinical Trials

Class	Virus (Dose)	Phase; Status	Delivery/ Location	Combinations	Age (Years)	Disease	Novel Aspects; Key findings	NCT
Herpes Simplex Virus	HSV1716 (10^5 PFU)	1; terminated	Peri- and intratumoral after tumor resection	IV dexamethasone prior to and 6 and 12 hrs post-surgery	12–21	Refractory/ recurrent HGG	Well tolerated but trial retracted after 2 pts enrolled	NCT02031965
	HSV G207 (10^7 or 10^8 PFU)	1; completed	IT infusion over 6 hrs via 3–4 catheters	5 Gy radiation to tumor within 24 hrs after virus	3–18	Progressive/ recurrent supratentorial malignant glioma	First completed trial of OV for pediatric brain tumor; 12.2 month median overall survival. 5/12 patients lived ≥18 months post treatment. 10^8 PFU safe and tolerable. Marked increase in TILs	NCT02457845
	HSV G207	1; recruiting	IT via catheter	5 Gy radiation to tumor within 24 hrs of virus	3–18	Refractory/ recurrent malignant cerebellar tumors; includes LMD	First OV delivered via catheter to the cerebellum; first trial of oHSV for infratentorial tumors	NCT03911388
	HSV G207 (10^8 PFU)	2; not yet recruiting	IT infusion over 6 hrs via ≤4 catheters	5 Gy radiation to tumor	3–21	Progressive/ recurrent malignant HGG	First phase 2 trial of OV in children	NCT04482933
Adenovirus	DNX-2401 (≤ 5×10^{10} viral particles in 1mL)	1; completed	IT infusion via catheter in cerebellar peduncle	Standard radiation and/or chemotherapy 3–4 wks after virus	1 −18	Naïve DIPG	Including OV as upfront therapy; Safe and tolerable. All patients showed reduced tumor volume	NCT03178032
	AloCELYVIR (500 cells/kg)	1b/2; recruiting	IV infusion; weekly × 8	Radiotherapy for naïve DIPG	1 – 21	Naïve DIPG; Relapsed/refractory MB	Cellular therapy	NCT04758533
Poliovirus	PVSRIPO (5×10^7 $TCID_{50}$)	1b; active, not recruiting	IT infusion via intracerebral CED		12 – 21	Recurrent HGG/MB/ATRT	Disease involving cerebellum, pituitary, leptomeninges, brainstem, spinal cord, or requiring ventricular access can be included at discretion of neurosurgeon	NCT03043391
Reovirus	Pelareorep	1; active, not recruiting	IV infusion over 60 min on days 3–5 of 28-day cycle × ≤ 12	SQ GM-CSF on days 1 and 2 of 28 day cycle. Total cycles ≤ 12	10–21	Refractory/relapsed HGG/MB/ATRT/ PNET	IV virus delivery	NCT02444546
Measles Virus	MV-NIS	1; recruiting	Locally for recurrent tumors; Lumbar puncture for disseminated disease		1 – 39	Disseminated or locally recurrent MB; refractory ATRT	NIS allows noninvasive spatial and temporal virus tracking Lumbar puncture delivery	NCT02962167

ATRT, atypical teratoid/rhabdoid tumor; CED, convection enhanced delivery; DIPG, diffuse intrinsic pontine glioma; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGG, high-grade glioma; hrs, hours; IT, intratumoral; IV, intravenous; LMD, leptomeningeal disease MB, medulloblastoma; oHSV, oncolytic herpes simplex virus therapy; OV, oncolytic virotherapy; PNET, primitive neuroectodermal tumor; SQ, subcutaneous; wks, weeks

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