

# Oncolytic virotherapy for pediatric malignancies: future prospects

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**Abstract:** Pediatric solid tumors remain a major health concern, with nearly 16,000 children diagnosed each year. Of those, ~2,000 succumb to their disease, and survivors often suffer from lifelong disability secondary to toxic effects of current treatments. Countless multimodality treatment regimens are being explored to make advances against this deadly disease. One targeted treatment approach is oncolytic virotherapy. Conditionally replicating viruses can infect tumor cells while leaving normal cells unharmed. Four viruses have been advanced to pediatric clinical trials, including herpes simplex virus-1, Seneca Valley virus, reovirus, and vaccinia virus. In this review, we discuss the mechanism of action of each virus, pediatric preclinical studies conducted to date, past and ongoing pediatric clinical trials, and potential future direction for these novel viral therapeutics.

**Keywords:** oncolytic virus, herpes simplex virus, Seneca Valley virus, reovirus, vaccinia

## Introduction

Cancer is rare in the pediatric population but remains the second leading cause of death in the 0- to 18-year age range, behind trauma. Cancer occurrence rates vary by age, but the overall most common cancers are acute lymphoblastic leukemia, brain and central nervous system tumors, neuroblastoma, and non-Hodgkin's lymphoma.<sup>1</sup> Prognosis of most cancer subtypes has improved significantly; however, long-term survival for some remains poor. Consequently, >2,000 children in the US die each year from this disease.<sup>2</sup> Additionally, many patients who survive often suffer from life-altering morbidities secondary to treatment.

Innovative therapies and strategies are being explored for children diagnosed with solid tumors. One such targeted solution is the development of human recombinant viruses designed to attack rapidly dividing tumor cells while leaving normal healthy cells unharmed. Concerns exist as to the safety of viral therapy, especially in children, and most of the viruses currently in use have been genetically altered to be nonpathogenic or the parent virus is not pathogenic in humans. To date, four viral constructs have been shown to be safe with some evidence of efficacy in the treatment of a number of pediatric cancers in the laboratory and in early Phase I trials. In this review, we focus on these four viruses because of their proven efficacy in childhood cancer, but obviously there are other viruses in development that will likely be advanced to the pediatric population in the future.

## Herpes simplex virus

Herpes simplex virus-1 (HSV-1) is a large, double-stranded DNA virus that is 152 kb in length and encodes 84 different genes.<sup>3</sup> The genome was successfully mapped in the

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1980s, and researchers began investigating HSV-1 as a possible vector for gene therapy.<sup>4</sup> The double-stranded genome is composed of a long ( $U_L$ ) and short ( $U_S$ ) genomic segment with accessory genes that can be deleted without interfering with viral replication.<sup>3</sup> Due to the ability to easily manipulate the genome, HSV-1 has been genetically engineered to function as an oncolytic virus.

Multiple viral constructs are being investigated in pre-clinical studies but have yet been advanced to pediatric clinical trials. G207 is a genetically engineered HSV-1 strain F that lacks the  $\gamma_134.5$  gene and contains an inactivation insertion of lacZ within the  $U_L39$  gene that stops lytic activity in nondividing cells (summary of viruses discussed in the text that have been or are being advanced to clinical trial is given in Table 1). NV1020 is a similar construct but has only one of the  $\gamma_134.5$  loci deleted. The gene product of  $\gamma_134.5$  plays a role in inhibiting viral replication. To avoid viral infection, RNA-dependent protein kinase (PKR) is activated. PKR phosphorylates alpha subunit of translation initiation factor 2 (eIF-2 $\alpha$ ) and subsequently blocks translation of viral messenger RNA. ICP 34.5 counteracts this pathway by dephosphorylating eIF-2 $\alpha$ , which prevents protein synthesis shutoff.<sup>5</sup> Bharatan et al<sup>6</sup> studied the sensitivity of multiple sarcoma cell lines to both G207 and NV1020. All cell lines tested demonstrated efficient viral entry and replication but were not all equally sensitive to virus killing. The rhabdomyosarcoma (RMS) and malignant fibrous histiocytoma cell

lines were most sensitive followed by osteosarcoma cells. Least sensitive to both viruses were the Ewing sarcoma cells. Additionally, Parikh et al compared the efficacy of wild-type adenovirus Type 5 to the HSV-1 mutant, NV1066, on multiple neuroblastoma cell lines. The recombinant virus NV1066 lacks the  $\gamma_134.5$  gene but carries a transgene for enhanced green fluorescent protein, which is a visual marker of infectivity. NV1066 was able to successfully replicate and induce apoptosis in all cell lines tested. The oncolytic potency of NV1066 was much higher than adenovirus in neuroblastoma.<sup>7</sup> Wang et al recently demonstrated that the viral construct HSV1716 was able to infect and had an antitumor response in pediatric neuroblastoma cell lines and that this response could not be predicted by the presence or absence of oncolytic engineered HSV (oHSV) entry receptors. Further, they demonstrated complete responses in six of ten animals bearing CHP-134 neuroblastoma tumors.<sup>8</sup>

Pediatric brain tumor models have been shown to be sensitive to several oHSVs. Both G207 and M002 have been studied in these models. G207 was described earlier. M002 lacks the  $\gamma_134.5$  gene found within the inverted repeats of the  $U_L$  segment of the parent recombinant virus HSV-1(F), rendering it unable to replicate in postmitotic cells, while the virus can replicate and rapidly lyse tumor cells. Additionally, M002 expresses both subunits of murine interleukin-12 (mIL-12) under the direct transcriptional control of the murine early-growth response-1 promoter.<sup>9</sup> As the virus

**Table 1** Oncolytic viruses that have had applications to pediatric tumors

Virus	Deletions/ mutations <sup>a</sup>	Foreign gene/ promoter insertions <sup>a</sup>	Pediatric tumor targets	References
HSV-1(F)				
M002	$\gamma_134.5$ both copies	mIL-12	Neuroblastoma, hepatoblastoma, malignant rhabdoid renal tumor, renal sarcoma, rhabdomyosarcoma, medulloblastoma, glioblastoma	9–15
G207	$\gamma_134.5$ both copies	lacZ	Rhabdomyosarcoma, osteosarcoma, medulloblastoma, glioblastoma	6, 10–11
HSV-1(I7)				
HSV1716 (Seprehvir)	RL1 both copies		Neuroblastoma	8
Seneca Valley			Retinoblastoma, glioma, neuroblastoma, rhabdomyosarcoma	19, 21–22
Reovirus			Medulloblastoma, rhabdomyosarcoma, Ewing sarcoma, synovial sarcoma, lymphoma	34, 36–37
Vaccinia				
Western Reserve, vvDD	Both copies TK and vaccinia growth factor		Atypical teratoid rhabdoid tumor, neuroblastoma, osteosarcoma, rhabdomyosarcoma	53
JX-594	TK gene both copies	Human GM-CSF and lacZ insertion into the TK gene region	Pediatric solid tumors	54

**Note:** <sup>a</sup>Blank spaces in the table are because they do not have deletions or foreign gene insertions.

**Abbreviations:** HSV, herpes simplex virus; mIL-12, murine interleukin-12; vvDD, double-deleted vaccinia virus; TK, thymidine kinase; GM-CSF, granulocyte macrophage colony-stimulating factor.

replicates, mIL-12 is produced and augments the oncolytic effect of the virus through the stimulation of cytotoxic T-cells and natural killer cells and the antiangiogenic properties of mIL-12. Friedman et al<sup>10</sup> examined the effects of M002 and G207 on medulloblastoma (MB), including group 3 tumors that are highly resistant to conventional therapies and MB cancer stem cells with the surface proteins CD15 and CD133. All four human pediatric MB xenografts tested were highly sensitive to G207 and M002. Additionally, mice bearing these tumors intracranially lived significantly longer after treatment with the viruses.<sup>10</sup> Friedman et al<sup>11,12</sup> also demonstrated that the CD133+ cancer stem cells in the pediatric glioblastoma xenograft D456 were equally sensitive to virus when compared to CD133 tumor cells.

M002 has also been studied in pediatric extracranial solid tumors with encouraging results. Gillory et al demonstrated that M002 inhibited neuroblastoma growth in vitro and in vivo.<sup>13</sup> Using two neuroblastoma cell lines, one with and another without MCYN amplification, they showed that M002 could successfully infect, replicate, and kill tumor cells. Immunocompromised mice had an increased lifespan when treated with low-dose radiation (3 Gy) in addition to M002. In vitro experiments with hepatoblastoma, malignant rhabdoid renal tumor, and renal sarcoma cell lines were likewise sensitive to treatment with M002. Viral titer assays demonstrated that the virus was able to enter and use the cell's machinery to

make progeny virus. In vivo studies demonstrated decreased tumor growth and prolonged survival in an immunocompromised xenograft mouse model.<sup>14</sup> Furthermore, M002 was able to infect and diminish survival of both alveolar (SJCRH30) and embryonal (RD) RMS cell lines including the CD133+ cancer stem cells, and other investigators have shown encouraging results in vivo.<sup>15</sup> The humanized version of the virus that produces human IL-12, M032, is in clinical trial in adults with recurrent glioblastoma (<https://clinicaltrials.gov/ct2/show/NCT02062827>). Future pediatric trials with this virus are planned.

Based on the promising preclinical data, several pediatric clinical trials in children using oHSVs are currently being conducted including two studies utilizing HSV1716 and one using G207 (summary of pediatric clinical trials based on virus type is given in Table 2). Cripe at Nationwide Children's Hospital is currently enrolling children and young adults (7–30 years old) with relapsed solid, noncentral nervous system tumors in a Phase I dose escalation study with HSV1716 (<https://clinicaltrials.gov/ct2/show/NCT00931931>). This study is designed to determine the safety of intratumoral or intravenous delivery of the virus and to measure any antiviral immune response. HSV1716 is also being tested at Cincinnati Children's Hospital in children 12–21 years old with refractory or recurrent high-grade glioma that can be removed by surgery (<https://clinicaltrials.gov/ct2/show/NCT02031965>).

**Table 2** Clinical trials involving oncolytic viruses and pediatric tumors

Virus	Completed studies <sup>a</sup>	Current studies <sup>a</sup>	Patient age range (years)	ClinicalTrials.gov Identifier	References <sup>b</sup>
HSV-1(F) G207		Phase I, recurrent malignant supratentorial brain tumors	3–18	NCT02457845	
HSV-1(17) HSV1716 (Seprehvir)		Phase I, recurrent gliomas Phase I, non-CNS solid tumors	12–21 7–30	NCT02031965 NCT00931931	
Seneca Valley SVV-001	Phase I, relapsed neuroblastoma, rhabdomyosarcoma, or other tumors with neuroendocrine features		3–21	NCT01048892	23
Reovirus Reolysin	Phase I, relapsed or refractory extracranial solid tumors		3–20	NCT01240538	40
Reolysin		Phase I, high-grade recurrent or refractory brain tumors	10–21	NCT02444546	
Vaccinia JX-594 (Pexa-Vex)	Phase I, refractory solid tumors; hepatoblastoma, neuroblastoma, Ewing sarcoma		4–18	NCT01169584	54

**Notes:** <sup>a</sup>Blank areas indicate there are no completed studies, no current studies, and no clinical trials. <sup>b</sup>Blank areas indicate these are ongoing phase I studies that are not completed and the data are not yet published.

**Abbreviations:** HSV, herpes simplex virus; CNS, central nervous system; SVV, Seneca Valley virus.

In this study, patients undergo maximal surgical resection of the tumor followed by injection of HSV1716 into the resection cavity. Friedman at the University of Alabama at Birmingham has a Phase I study open to enrollment evaluating G207 alone or combined with a single low dose of irradiation for children with recurrent or progressive supratentorial brain tumors (any malignant histological subtype) ([www.clinicaltrials.gov/ct2/show/NCT02457845](http://www.clinicaltrials.gov/ct2/show/NCT02457845)). The primary goal of this study is to determine safety, and the secondary aims are to obtain preliminary information on the effectiveness of and immune response to G207. In this study, patients will receive a 6-hour infusion of G207 through one to four catheters placed into the enhancing regions of the tumor. If this is proven safe, subsequent patients will receive a single 5 Gy dose of radiation to the tumor bed within 24 hours of G207 infusion. Preclinical studies indicate that low-dose radiation enhances HSV replication, and this approach was safely used in adult studies with radiographic evidence of responses.<sup>16,17</sup> The increasing abundance of favorable preclinical trials using oHSV endorses the need for more clinical trials in this area. HSV virotherapy has the potential to target and treat rare and aggressive pediatric solid tumors.

## Seneca Valley virus

Nonhuman viruses have antitumor potential due to their lack of immunogenicity and capacity to cause disease in humans.<sup>18</sup> Seneca Valley virus (SVV, SVV-001) is a small, replication-competent, single-stranded, nonenveloped RNA picornavirus, with a natural host in pigs and cows. SVV is the first oncolytic picornavirus to be tested in humans and can penetrate solid tumors through the vascular system unlike many other oncolytic viruses. Interestingly, SVV was first discovered incidentally in 2002 as a contaminant introduced in cell cultures via fetal bovine serum or porcine trypsin.<sup>19</sup>

Preclinical studies by Reddy et al<sup>19</sup> demonstrated that SVV could be successfully delivered systemically to mice bearing retinoblastoma xenografts resulting in a remarkable decrease in tumor growth compared to control animals. This important study proved that SVV was effective via a systemic approach without deleterious effects on normal replicating cells. Viral replication assays proved that SVV was able to invade and replicate over time in tumor cells but not in normal cells such as hepatocytes. Additionally, viral levels were almost completely cleared from the blood, while high viral titers were found selectively in the tumors.

Additional preclinical studies demonstrated that SVV successfully passed the blood–brain barrier and infected and replicated in MB after a single intravenous injection, leading

to further studies with SVV and pediatric gliomas.<sup>20</sup> Liu et al<sup>21</sup> examined the sensitivity of patient glioma tumor cells and a panel of orthotopic xenograft mouse models of pediatric malignant gliomas to SVV using Rag2/severe combined immunodeficient (SCID) mice. SVV was able to directly lyse tumor cells cultured from patient tumors and was able to infect glioblastoma cells grown as neurospheres. In the murine studies, SVV was able to eliminate 80% of xenograft tumors after 4 weeks of treatment. Animal survival was also significantly prolonged in three permissive cell types identified by *in vitro* studies, but no survival benefit was noted in the *in vitro* resistant cell lines.

Morton et al<sup>22</sup> evaluated SVV against 23 pediatric cancer cell lines in the Pediatric Preclinical Testing Program using *in vitro* and *in vivo* models. They found that neuroblastoma and alveolar RMS were the most sensitive tumor types. These studies led to a Children's Oncology Group study of SVV in children with solid tumors. Twenty-two children with either relapsed neuroblastoma, RMS, or other tumors with neuroendocrine features were enrolled to evaluate SVV without or with cyclophosphamide therapy in a Phase I clinical trial (<https://clinicaltrials.gov/ct2/show/NCT01048892>).<sup>23</sup> SVV was successfully administered intravenously and well tolerated with only a single patient developing a dose-limiting toxicity (grade 3 pain at dose level 1). All patients cleared the virus from their blood and stool within 3 weeks of infusion. Despite the administration of low-dose cyclophosphamide, intravenous infusion of SVV did elicit the formation of neutralizing antibodies against the virus within 3 weeks.<sup>23</sup> Further studies are needed to investigate techniques to reduce these neutralizing antibodies as well as anti-inflammatory cells such as T regulatory cells that deter a promising antitumor response. Despite promising preclinical data, no pediatric central nervous system tumor trials with SVV have been conducted to date.

## Reovirus

Respiratory enteric orphan virus, or reovirus, is a double-stranded, nonenveloped RNA virus that is a member of the Reoviridae family. The cell surface receptor for reovirus is sialic acid, which is universally expressed on mammalian cells, allowing the virus to bind to most cells; however, binding and internalization do not lead to a productive infection in normal human cells.<sup>24</sup> Cellular activation of PKR and subsequent phosphorylation of eIF-2 $\alpha$  lead to translational arrest and inhibition of viral protein production, resulting in an unproductive infection.<sup>25</sup> Therefore, asymptomatic infection in humans is common with an

estimated 60%–100% of adults containing anti-reovirus antibodies indicating a prior infection. When reovirus causes disease, it typically results in mild respiratory or gastrointestinal symptoms, thus making the wild-type virus attractive as an oncolytic agent.<sup>26</sup> In children located in the US, an age-specific increase in reovirus-specific antibodies was seen with a seroprevalence of 8.2%, 32.8%, and 50% seen in children 1–2 years old, 4–5 years old, and 5–6 years old, respectively.<sup>27</sup>

Strong et al<sup>25</sup> demonstrated that reovirus-resistant mouse fibroblast cells became susceptible when transformed with activated Ras, which prevented PKR activation and allowed for viral protein synthesis to ensue. Activated Ras signaling is common in many tumor types and has been shown to mediate tumorigenesis, thereby providing reovirus with tumor specificity.<sup>28</sup> Coffey et al<sup>29</sup> initially demonstrated the potential of reovirus (serotype 3, Dearing strain) as an oncolytic therapeutic agent in preclinical studies against human malignant glioma, and soon thereafter, other preclinical studies demonstrated that reovirus could target a variety of adult malignancies including lung, ovarian, colon, breast, lymphoid, and pancreatic cancers.<sup>30–35</sup> These studies revealed that reovirus could be safely injected both intratumorally with evidence of systemic antitumor effects against distant disease and systemically to target local and metastatic disease in murine models. While intravenous reovirus effectively targeted metastatic cancer in immunocompetent mice, preexisting immunity to reovirus did limit responses. Immunosuppressive agents such as cyclosporine A or anti-CD-4/anti-CD8 antibodies could be combined with the virus to try to overcome this obstacle.<sup>31</sup>

Several preclinical studies demonstrated the effectiveness of reovirus at targeting pediatric malignancies. In MB, the most common pediatric malignant brain tumor, five of seven human cell lines, two mouse cell lines, and three primary cultures derived from surgical specimens were sensitive *in vitro* to reovirus infection.<sup>36</sup> Susceptibility of the MB cells to infection was associated with levels of activated Ras. Furthermore, a single intrathecal injection of reovirus dramatically prolonged survival in an *in vivo* Daoy orthotopic animal model, and multiple injections reduced spinal and leptomeningeal metastases. The *in vivo* study was limited by the model chosen since Daoy is desmoplastic, a favorable, low-risk feature in MB. Similarly, reovirus inhibited proliferation and viability of several pediatric human sarcomas lines, including RMS, osteosarcoma, Ewing sarcoma, and synovial sarcoma.<sup>37</sup> When injected in the tail vein every other day for three doses every 21 days, the virus inhibited xenograft tumor growth and prolonged survival in mice. The therapeutic benefit

was enhanced in some of the sarcoma models when reovirus was combined with radiation and/or cisplatin. While many primary lymphoid neoplasms were sensitive to reovirus in preclinical studies, reovirus efficiently infected and lysed only two of five Burkitt lymphoma cell lines, suggesting that some tumor types may be less susceptible to killing by reovirus.<sup>34</sup>

Based on the promising preclinical pediatric data and the safety of reovirus alone and combined with chemotherapy in several adult human extracranial solid tumor clinical trials, the Children's Oncology Group studied reovirus (Reolysin) in children with relapsed or refractory extracranial solid tumors in a Phase I study (<https://clinicaltrials.gov/ct2/show/NCT01240538>).<sup>38–40</sup> A standard 3+3 design was used, and 24 patients were treated with intravenous reovirus for 5 consecutive days of a 28-day cycle alone or combined with oral cyclophosphamide (50 mg/m<sup>2</sup>/d × 21 days). Both approaches were safe, and reovirus was well tolerated up to 5 × 10<sup>8</sup> tissue culture inhibitor dose 50% per kg. A grade 5 respiratory failure and a grade 5 thromboembolic event were reported in the setting of disease progression, but no dose-limiting toxicities related to reovirus occurred. Correlative studies demonstrated an average time of reovirus viremia clearance of 6.5 days with no viremia seen in any patient after 17 days. Additionally, no virus was detected in the saliva or stool. While the Phase I study was not designed to demonstrate efficacy, no objective responses were seen. Three patients had stable disease and received a second cycle. Based on the lack of responses, the authors concluded that combination approaches with reovirus will be needed for efficacy.

At this time, there are no additional studies of reovirus in extracranial pediatric solid tumors, but there is an active Phase I study of reovirus in children 10 years and older with high-grade recurrent or refractory brain tumors. The study combines reovirus with sargramostim, recombinant granulocyte macrophage colony-stimulating factor, designed to enhance immune cell tumor killing induced by reovirus (<https://clinicaltrials.gov/ct2/show/NCT02444546>). Patients will receive sargramostim subcutaneously on days 1 and 2 and reovirus intravenously on days 3–5 of a 28-day cycle. Based on the safety profile of reovirus, opportunities exist for future studies combining reovirus with upfront therapy, other chemotherapeutic agents and/or radiation, and even other oncolytic viruses or new immune modulating agents.

## Vaccinia virus

Vaccinia virus (VV), a double-stranded, nonintegrating, enveloped DNA virus in the Poxviridae family, was initially used as a vaccination to eradicate smallpox worldwide in

1979. More recently, the virus gained interest as an oncolytic agent because scientists identified nonessential genes that may be deleted to provide protection for normal cells while maintaining the oncolytic ability of the virus. Similar to HSV, these nonessential genes can be replaced with foreign DNA to augment tumor cell cytotoxicity. Several engineering approaches to attenuate VV have been used. Deletion of both copies of the thymidine kinase (TK) gene results in decreased pathogenicity for normal cells and preferential replication in tumor cells because thymidine triphosphate, which is required by a TK-deleted virus for DNA synthesis, is provided by replicating tumor cells but not normal cells.<sup>41,42</sup> Similarly, deletion of vaccinia growth factor or hemagglutinin genes reduces VV virulence in normal cells and has been used in combination with TK deletions for additional protection of normal cells.<sup>42–45</sup> Another mechanism to protect normal cells is through deletions in the *B18R* gene, the product of which neutralizes secreted type-I interferons.<sup>46</sup> Without the gene product, the mutant virus is inactivated by interferon production in normal cells, but infection occurs in tumor cells that are often resistant to the antiviral effects of type-I interferons. Recombinant virus JX-795, which contains double deletions of *B18R* and TK and expresses  $\beta$ -interferon, demonstrated tumor-specific replication and efficacy following systemic delivery in preclinical cancer models.<sup>47</sup> Viruses with protective gene deletions can also be delivered directly to tumors by pre-infecting cytokine-induced killer cells that are attracted to tumors and exert their cytotoxic effects following intravenous delivery.<sup>48</sup>

In addition to the  $\beta$ -interferon gene, several other foreign genes have been added to VV to enhance tumor cell killing. JX-594, which has been used both intratumorally and intravenously in multiple adult human solid tumor clinical trials, provides immunostimulation with increased intratumoral cytotoxic lymphocytes through the production of granulocyte macrophage colony-stimulating factor. Two viruses have been designed to target the tumor microenvironment through insertion of an anti-vascular endothelial growth factor (VEGF) foreign gene. VV GLV-1h68, a mutant virus with deletions in TK and hemagglutinin genes, was engineered to encode the anti-VEGF protein GLAF-1. The GLAF-1-producing virus demonstrated enhanced therapeutic efficacy in targeting adult human pancreatic, lung, and prostate cancer xenografts compared to the GLV-1h68 parent virus, and the treatment effect was comparable to that seen with the parent virus combined with bevacizumab.<sup>49</sup> Similarly, vvDD, a Western Reserve VV with deletions in TK and VV growth factor, inhibited enhanced antitumor efficacy with an antiangiogenic

effect in renal cell cancer models when armed with soluble VEGF receptor 1 protein.<sup>50</sup> Other approaches for enhanced cell killing or molecular imaging have been used including the cytosine deaminase gene as suicide gene therapy with 5-fluorocytosine or the gene expressing the human somatostatin receptor type 2.<sup>51,52</sup>

Limited preclinical data exist on the efficacy of VV in pediatric tumor models. Lun et al<sup>53</sup> demonstrated that the double-deleted VV, vvDD, effectively infected and killed nine of eleven pediatric tumor cell lines tested (three atypical teratoid rhabdoid tumors, four neuroblastomas, two osteosarcomas, and two RMSs). A neuroblastoma and an osteosarcoma cell line were more resistant to killing with ~40%–60% of the cells still alive after treatment with a high dose of the virus after 4 days. In xenograft models of intracranial atypical teratoid rhabdoid tumor, flank neuroblastoma, and metastatic osteosarcoma, intravenous vvDD decreased tumor size and prolonged survival in SCID mice supporting further evaluation of VV in pediatric brain and extracranial solid tumors.

To date, a single pediatric VV trial has been conducted (<https://clinicaltrials.gov/ct2/show/NCT01169584>).<sup>54</sup> The Phase I study tested the safety of intratumoral JX-594 (Pexa-Vex) at either  $10^6$  or  $10^7$  plaque-forming units (PFU) per kg in six children (three hepatocellular carcinoma, two neuroblastoma, and a Ewing sarcoma). Five of the patients received a one-time injection in up to three tumor sites and one patient received two injections. The virus treatment was safe and tolerable; toxicities were mild ( $\leq$  grade 3) with fever and sinus tachycardia being the most common symptoms. The three patients who received the  $10^7$  PFU/kg dose all developed grade 1 treatment-related skin pustules that resolved within 3–4 weeks. A strong cellular immunoreactivity to vaccinia antigens was detected in two patients who were tested. One of the six patients had radiographic evidence suggestive of antitumor activity. While a few adult cancer trials are examining VV in combination with small molecule inhibitors, there are no forthcoming pediatric trials at this time. Similar to reovirus, combination approaches with VV will likely be needed for efficacy.

## Conclusion

Oncolytic recombinant viruses are promising treatments for pediatric malignancies. Several Phase I studies have been completed, and each virus was deemed safe and tolerable in the studies. Additional studies with other viruses are ongoing. Future studies of these viruses will likely include complementary combination approaches with standard chemotherapy, radiation, monoclonal antibodies or other

small molecule inhibitors, or even other viruses. New viruses continue to be designed to increase tumor selectivity while enhancing virus-mediated tumor cell killing. These new viruses have likewise proven safe and efficacious in preclinical and animal studies and require testing in human pediatric clinical trials. While more clinical trials are needed to test new viruses and novel combination approaches, oncolytic virotherapy has the potential of becoming an important targeted modality of cancer treatment.

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## Disclosure

The authors report no conflicts of interest in this work.

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