Pediatric cancer gone viral. Part I: strategies for utilizing oncolytic herpes simplex virus-1 in children

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Abstract

Progress for improving outcomes in pediatric patients with solid tumors remains slow. In addition, currently available therapies are fraught with numerous side effects, often causing significant lifelong morbidity for long-term survivors. The use of viruses to kill tumor cells based on their increased vulnerability to infection is gaining traction, with several viruses moving through early and advanced phase clinical testing. The prospect of increased efficacy and decreased toxicity with these agents is thus attractive for pediatric cancer. In part I of this two-part review, we focus on strategies for utilizing oncolytic engineered herpes simplex virus (HSV) to target pediatric malignancies. We discuss mechanisms of action, routes of delivery, and the role of preexisting immunity on antitumor efficacy. Challenges to maximizing oncolytic HSV in children are examined, and we highlight how these may be overcome through various arming strategies. We review the preclinical and clinical evidence demonstrating safety of a variety of oncolytic HSVs. In Part II, we focus on the antitumor efficacy of oncolytic HSV in pediatric tumor types, pediatric clinical advances made to date, and future prospects for utilizing HSV in pediatric patients with solid tumors.

Despite advances in medical and surgical modalities, pediatric cancer remains a major public health concern. Over 12,000 children and adolescents are diagnosed with cancer each year in the United States and approximately 2,300 succumb to their disease yearly.1 While death rates from childhood cancer have declined, outcomes remain poor for children with...
high-grade, disseminated, or recurrent solid tumors despite multimodality therapy including surgery, chemotherapy, and radiation, which can all be very damaging to a developing child. Five-year survival for patients with the most common extracranial solid tumor, neuroblastoma, an embryonal malignancy of primordial neural crest cells, is only 83% for infants, 55% for children 1–4 years old and less than 40% for older children. Overall survival for children with metastatic Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, and Wilms tumor is just 30.6, 29.3, 27.5, and 76.6%, respectively. Many of the patients that are cured have significant morbidity secondary to their therapy. Patients with central nervous system tumors, the most common solid tumor in children accounting for approximately 20% of malignancies, frequently develop long-term sequelae such as hormone dysfunction, neurosensory impairment, and neurocognitive changes that are attributed to the treatment. Novel targeted therapies are desperately needed to improve outcomes for children with recurrent or refractory disease, and importantly, may be useful as an adjuvant to standard therapies resulting in lower doses, and subsequently, less toxicity from the treatments.

One innovative strategy for treating pediatric cancer is oncolytic virotherapy. Oncolytic viruses can be harnessed to attack tumor cells while leaving normal cells unharmed; they can directly infect and replicate in cancer cells, express therapeutic gene products, or alter signaling pathways. Multiple DNA and RNA viruses are currently being studied to target pediatric cancers including herpes simplex virus (HSV), adenovirus, pox virus, reovirus, Seneca Valley virus, vaccinia virus, Newcastle disease virus, myxoma virus, and vesicular stomatitis virus. Various strategies are being employed based on the type of virus being utilized. For example, conditionally replicating adenoviruses have been genetically engineered to target tumor cells with mutations in the p53 and RB tumor suppressor pathway. Cancer cells with an activated Ras pathway, which has been implicated in tumor progression and metastasis, are targeted by the native, wild-type reovirus. Furthermore, adenovirus, HSV, and vaccinia virus have all been shown to become potent oncolytic viruses with the deletion of antiapoptosis viral genes.

ONCOYTIC HERPES VIRUS

HSV type 1, an enveloped, double-stranded linear DNA virus, is among the largest DNA viruses developed for gene transfer. The viral genome is 152 kb in length that consists of unique long (UL) and short (US) regions each flanked by inverted repeat sequences (RL and RS) (Figure 1). There is an approximately 19 kb nonessential joint region of the HSV genome that can be removed without substantially affecting viral potency, making it attractive for developing viral vectors that harbor large foreign sequences. Other features making HSV appealing as an oncolytic virus include the fact that it is nonintegrating so there is no risk of insertional mutagenesis, it is a human virus with a defined and well-known clinical spectrum, so potential side-effects can be predicted, and there are FDA-approved antitherpetic drugs available in the event of a pathologic infection. It is also very potent as a lytic virus, with the ability to infect and kill a cell in approximately 18 hours and spread rapidly, such that only one infectious particle per 1000 cells is required to kill an entire monolayer in a culture dish in 5–6 days. Although it is best known as a neurotropic
virus due to its latency in neurons, HSV-1 actually can infect a wide variety of cell types and thus tumor types.

The wide range of cell tropism of HSV-1 is in part due to four known cell surface molecules that are recognized as major HSV entry receptors, at least one of which is present on most cell types. HSV attaches to host cell membrane through the binding of its surface glycoproteins B and C (gB and gC) to heparan sulfate proteoglycans. Following the binding of gD to the entry receptor, nectin-1, it undergoes a conformational change that allows gH/gL to interact with gB and further triggers the fusion of the virus envelope to the host cellular membrane and then releases the naked virion into the host cell cytosol. The fusion process can happen at the plasma membrane of the cell surface via the direct fusion pathway and/or in the endocytic vesicle via the endocytic pathway. The amount of virus entry can be measured via PCR for detecting viral genome copy or by checking the expression level of viral capsid proteins in the host cells to determine the susceptibility of the cells. Having successfully entered the cell, the virion will either undergo degradation mainly mediated by lysosome or traffic along the microtubule toward the cell nucleus. Once the viral genome is delivered into the nucleus, though it remains as a nonintegrating episome even at the latent stage, virus replication takes place leading to host cell destruction. Thus, cellular permissivity to the virus is measured by the production of infectious particles via plaque assay.

Oncolytic versions of HSV-1 have been constructed by mutating critical metabolic viral genes including thymidine kinase (tk), DNA polymerase, and ribonucleotide reductase (RR, the large subunit of which is encoded by the ICP6 gene). Currently, most oHSVs were developed by abrogating the expression of the virulence factor ICP34.5 (encoded by the RL1 or γ134.5 gene). ICP34.5 is essential for virus to combat the antiviral innate immunity PKR pathway by redirecting cellular protein phosphatase 1 to dephosphorylate the transcription factor e-IF2α and allow productive virus replication. Many tumors have a defective PKR pathway, making ICP34.5-mutant viruses tumor-selective. Current vector designs with an intact tk gene also permit antiherpetic agents such as acyclovir or ganciclovir to serve as safeguard to prevent viral outbreak, and these drugs are commonly used in children.

**ROUTES OF DELIVERY**

Oncolytic HSVs can be delivered to the tumor through various routes of administration. Determining the ideal route of delivery for targeting pediatric solid tumors remains an ongoing challenge for researchers. For the majority of ongoing and completed adult clinical trials, the predominant method of oHSV delivery is via a direct injection of the virus into the bulk of the tumor or the resected tumor bed. Intratumoral delivery provides the obvious advantage of delivering a highly concentrated dose of virus directly to the neoplasm. As such, the amount of oHSV that can potentially be lost to host immune system neutralization and off-target absorption is greatly reduced. Intratumoral oHSV delivery is an invasive procedure by its nature (especially when preceded by surgical debulking), but the use of ultrasound, CT or MRI needle guidance can facilitate this process to some degree. Accessing deep tissue sites for diagnostic or therapeutic purposes using imaging guidance...
by interventional radiology is commonplace in pediatric hospitals. In cases where the tumor is inaccessible or is widely metastatic, which frequently occurs in pediatric high-grade malignancies, systemic delivery of the oHSV may be the preferred route of administration.

Systemic oHSV delivery can be accomplished by administering the virus through an intravenous (IV), intra-arterial or intraperitoneal route. Virus delivered in this fashion can conceivably access the primary tumor and any overt or undiagnosed metastatic nodules simultaneously.\(^{23-25}\) In order to be effective, a systemically delivered oHSV must successfully circulate through the systemic vasculature, exit the intratumoral vascular space, and traverse the interstitium before it can infect neoplastic cells.\(^{26,27}\) Each of these stages can present significant physiological and immunological barriers to virus delivery (for a recent review, see ref. 27), and indeed preclinical biodistribution studies have shown that only a small fraction of the input oHSV ever reaches the tumor.\(^{28,29}\) Despite these impediments, several preclinical and proof-of-concept studies have demonstrated tumor shrinkages and even cures following systemic delivery of oHSVs.\(^{23,30-33}\) Although similar levels of success have yet to materialize in human clinical trials, it should be noted that the majority of studies conducted thus far were not designed to gauge objective responses, but rather to assess the safety of delivering oHSVs系统ically. To date, no dose-limiting toxicities have been reported and side-effects have generally been limited to mild flulike symptoms.\(^{34-36}\)

**MECHANISMS OF ANTITUMOR EFFICACY**

Originally the antitumor efficacy of oncolytic viruses was thought to be caused solely by the direct oncolytic effects of the virus’ natural lytic replication cycle; however, data have shown oHSV stimulation of the host immune response is partially responsible for antitumor efficacy. Intratumoral injection of G207, which contains deletions in both copies of the \(\gamma_134.5\) gene and an ICP6 disabling insertion of the \(lacZ\) gene encoding \(\beta\)-galactosidase into the \(UL39\) locus from the wild-type isolate HSV-1(F) strain, in only one of two bilateral tumors implanted in immunocompetent mice caused regression of both the injected and uninjected tumors in syngeneic mouse melanoma, colon cancer, and pediatric neuroblastoma models (see Table 1 for summary of viruses and Figure 1 for structural schematics of first-generation oHSVs discussed in the text).\(^{37-41}\) It was later elucidated that this phenomenon was caused in part by activation of CD8\(^+\) cytotoxic T-cells specific for tumor antigen and cured mice rechallenged with tumor cell injection were immune to a second tumor development.\(^{38}\) Fraser also demonstrated a critical role of the immune response following intratumoral injection of HSV strain 1716.\(^{42}\) Virus injected into melanoma lesions previously implanted in the brain-induced B-cell, CD4\(^+\) T-cell, and CD8\(^+\) T-cell responses specific to HSV.\(^{43}\) Virus injection of tumors in RAG2\(^{-/-}\) mice failed to impact tumor growth in these studies, while treatment of immunocompetent mice resulted in significant tumor regression. In several model systems, the addition of immunomodulatory genes into the virus such as interleukin-12 (IL-12) and granulocyte macrophage colony stimulating factor (GM-CSF) enhanced the antitumor effect of oHSVs.\(^{23,44-47}\) In fact, such a virus expressing GM-CSF (talimogene laherparepvec, T-VEC, formerly known as OncoVex\(^{GM-CSF}\)), a JS-1 strain HSV-1 with genetic deletions in ICP34.5 and ICP47, has recently undergone phase 3 testing for melanoma and will likely be the first FDA-approved
oncolytic virus (see Figure 2 for structural schematics of second generation, armed HSV mutants discussed in the text). Similar activation of cytotoxic T-cells has also been seen in pediatric rhabdomyosarcoma bearing immunocompetent mice treated with rRp450 oncolytic HSV. These studies suggest that oHSV not only causes direct oncolysis during tumor infection, but also activates the host antitumor immune response, potentially preventing tumor recurrence via the adaptive immune response. As a practical consideration for future oHSV trials in children, current therapies to treat advanced pediatric solid tumors are highly immunosuppressive and this may act to lessen the host antitumor immune response.

EFFECT OF HSV IMMUNITY ON ANTITUMOR EFFICACY

One of the most common questions arising about the use of oncolytic viruses is the effect of immunity, either pre-existing immunity that would presumably thwart any therapeutic effects or immunity triggered following initial exposure that would presumably hamper repeated dosing. This is a particularly relevant when considering the application of oHSV in children since most children are seronegative for HSV-1 throughout childhood and into adolescence. Indeed, following infection with most strains of wild-type HSV, mice mount vigorous innate and adaptive immune responses. HSV has evolved mechanisms such as Fc receptor binding to subvert adaptive antiviral immunity, but innate immune factors such as complement have been shown in some cases to limit viral delivery to tumor sites.

Although the HSV-encoded ICP47 gene inhibits class I MHC antigen presentation, mice still mount antigen-specific CD8+ cytotoxic T lymphocyte responses when infected with HSV in vivo. To increase the efficacy of mutant HSV, Todo et al. deleted the ICP47-encoding gene (α47, U512) in G207 (renamed GΔ47) and observed significantly better antitumor effects, in both immune competent and immunocompromised mice. It had been shown years earlier that deletion of U512 placed U511, an archaic gene that HSV adapted to defeat host antiviral responses, under the control of the U512 promoter, changing its expression pattern from late to immediate-early and enhancing virus replication. This essentially overcomes some of the attenuation of Δγ134.5 deletion.

HSV infection induces an early NK-cell response that can be detected in the spleen, which is followed by viral-specific CD4+ and CD8+ T-cell responses. In C57BL/6 mice, over 90% of the CD8+ T-cell response is focused on a single immunodominant epitope from the glycoprotein B gene. Nevertheless, preimmunization of mice with wild-type HSV reduced but did not eliminate HSV-mediated gene transfer after direct intratumoral injection in a brain tumor model. In other models, preimmunization had little effect on efficacy following intratumoral or intravascular virus injection. In one setting, mice immunized to HSV actually exhibited enhanced survival following oHSV therapy. Thus, pre-existing or subsequent immunity to HSV does not appear to be a hard-stop barrier for the use of HSV as an oncolytic virus, either using single or even multiple dosing.

CHALLENGES AND ARMING STRATEGIES

A number of challenges exist to maximizing the benefit of oHSV in children, though strategies have been developed to overcome many of these obstacles. Tumor genotype and phenotype heterogeneity is common even among tumors of the same classification. For
example, molecular characterization of pediatric medulloblastoma has identified four distinct molecular subgroups with unique genetic alterations and distinct clinical outcomes. Initial studies suggest that oHSV is capable of targeting the highest risk subgroups. While HSV can target many tumor types, not all tumors have a favorable environment for HSV replication. Expression of the primary entry molecule nectin-1 (CD111; poliovirus receptor-related protein 1), a cell surface adhesion molecule widely expressed in cell lines of different lineages, is variable and has been shown to at least partially predict HSV sensitivity in many tumor types. Low CD111 expression (<20%) has been associated with tumor resistance to oHSV in some but not all model systems. Overexpression of CD111 in resistant malignant peripheral nerve sheath tumor cell lines improved cell-to-cell spread of virus but did not make resistant tumors sensitive suggesting that other mechanisms such as the tumor antiviral response may prevent a productive infection even in tumors with high CD111 expression. One approach to improve virus entry involves retargeting the virus to a different receptor that is highly expressed in pediatric tumor cells but has low expression in normal tissue. A variety of receptors have been used effectively in preclinical studies including HER-2, EGFR, Musashi1, IL-13 receptor α2, and urokinase plasminogen activator. More research is needed to examine pediatric tumors for expression of these receptors and to develop new viruses based on receptors unique to pediatric tumors.

The tumor microenvironment can pose several obstacles for oHSV. The extracellular matrix and areas of tissue necrosis, which can be seen in high-grade tumors, may impair the spread of oHSV. Of note, many pediatric high-grade tumors, such as Ewing sarcoma, neuroblastoma, rhabdomyosarcoma, and medulloblastoma to name a few, are classified as small round blue cell tumors. Histologically these tumors are highly cellular and composed of tight sheets of small, round cells with scant cytoplasm. The increasedcellularity and less interstitial matrix of the tumors may facilitate infection of nearby cells. To potentially target the microenvironment of pediatric tumors, certain transgenes can be inserted; molecules can be produced during oHSV replication to degrade the extracellular matrix and inhibit angiogenesis. Enzymes such as chondroitinase, a bacterial enzyme that removes chondroitin sulfate from proteoglycans, and tissue inhibitor of metalloproteinases 3 (TIMP3), a potent inhibitor of metalloproteinases that control remodeling of the extracellular matrix, improved survival in preclinical in vivo tumor models including pediatric neuroblastoma. Antiangiogenesis proteins platelet factor 4 (CXCL4), angioatin, endostatin, and vasculostatin have augmented oHSV targeting of gliomas in animal models. Hypoxia, a key regulator of the tumor microenvironment, has been shown to decrease infectivity and cytotoxicity of first-generation γ134.5-deleted oHSV in part due to decreased virus activation of p38 MAPK. Activation of p38 MAPK has been shown to enhance expression of late viral genes in wild-type HSV-1. Approaches which may improve virus replication in hypoxia include using a tumor-specific hypoxia-inducible factor-responsive promoter or augmenting late viral protein synthesis through expression of the human cytolomegalovirus PKR evasion gene, IRS1 (HSV C134). Importantly, cancer stem cells, which proliferate in response to hypoxia and are resistant to traditional chemotherapy
and radiation, do not have any inherent resistance to oHSV in multiple pediatric tumor types and may be effectively targeted by oHSV.\textsuperscript{68,71,91,92}

In addition to targeting the primary tumor site, metastatic disease must be targeted by oHSV. Human clinical trials have mainly focused on intratumoral delivery, although novel routes of delivery such as intraarterial or intravenous injection are being studied. Both the oncolytic effect of the virus and the antitumor response engendered by the virus that may facilitate removal of the virus can result in tumor killing. Determining the right balance and timing of these effects is critical to maximize oHSV. Approaches to inhibit the immune response and to induce an immune response and produce a vaccine-like effect are being tested.

Transgenes that stimulate an antitumor immune response, modify apoptosis, convert prodrugs to cytotoxic agents, and increase susceptibility to radiotherapy have been utilized to improve the efficacy of oHSV and may be applicable to pediatric cancers. A variety of immunostimulatory cytokines have been shown to enhance oHSV including IL-12 and GM-CSF.\textsuperscript{48,93} A cytochrome P450 (CYP2B1) transgene which converts cyclophosphamide to phosphoramid mustard resulted in decreased tumor growth in animal models when given with cyclophosphamide including in models of pediatric rhabdomyosarcoma in which cyclophosphamide is a standard chemotherapeutic.\textsuperscript{94,95} Virus expressing the noradrenaline transporter (NAT) gene can result in the accumulation of the noradrenaline analog metaiodobenzylguanidine (MIBG) in infected cells, resulting in cellular susceptibility to targeted radiotherapy using radiolabeled (131)I-MIBG.\textsuperscript{91} Because this latter agent is primarily in clinical use for pediatric neuroblastoma patients, initial safety trials will likely need to be in that population.

**SAFETY**

While the majority of adults are seropositive for HSV-1, most children are seronegative with the incidence increasing throughout childhood from under 20\% at age 1–4 to 39\% by adolescence.\textsuperscript{50} While seronegativity may provide a treatment advantage, it may also make patients more sensitive to infection. Nevertheless, extensive preclinical and clinical testing of a variety of oHSVs have confirmed safety and suggest that oHSV will be safe in children. Early studies focused on central nervous system inoculation of G207. Mice injected intracerebrally or intracerebroventricularly with G207 survived with no symptoms of disease, and no infectious virus particles were found by PCR in the brains of mice after 8 weeks.\textsuperscript{96} Similarly, nonhuman primates (Aotus nancymaeae), which are exquisitely susceptible to wild type HSV-1 analogous to human neonates, were safely inoculated with G207 intracerebrally without evidence of HSV-induced histopathology or dissemination.\textsuperscript{97}

In an attempt to determine the effect of G207 on the developing mammalian brain, Radbill \textit{et al.}\textsuperscript{98} injected 4-day-old murine pups with G207 or saline intracerebrally and tested long-term physical development, exploratory behaviors, and cognitive performance of the mice. There were no significant adverse neurodevelopmental outcomes in the oHSV treated group; however ventriculomegaly was seen in five of seven G207-treated mice compared to one saline treated mouse. This side-effect was thought to be due to the freehand injection of the virus, which resulted in varying amounts of virus delivered to the parenchyma and the
ventricles and due to local trauma produced by the 30-gauge needle and the 2 µl volume inoculated. The authors acknowledged that the model may be more comparable to a third trimester human fetus than a young child; however, based on the findings, they recommended that an initial study of G207 in children should exclude patients with tumors in the ventricles and patients less than 2 years of age.

Three phase 1 clinical trials of G207 in adults with recurrent malignant glioma demonstrated safety.\textsuperscript{19,99,100} No dose-limiting toxicities or HSV encephalitis occurred when up to $3 \times 10^9$ plaque-forming units (pfu) of G207 were inoculated stereotactically into five sites of enhancing tumor. G207 was also safely inoculated in two separate doses several days apart and into surrounding brain tissue of the resection cavity after tumor removal. When given in combination with a single 5 gray dose of radiation administered within 24 hours of virus inoculation, G207 was well tolerated without significant toxicity. In all three trials, radiographic and neuropathologic evidence of antitumor activity were seen in nearly half of all patients including six of nine patients with stable disease or a partial response when G207 was combined with radiation. Another $\gamma$134.5-deleted virus, HSV1716, which was derived from wild-type isolate, strain 17, has been safely used in adult patients with high-grade gliomas in the United Kingdom.\textsuperscript{20,21,101} The virus caused no adverse clinical symptoms when injected intratumorally or into brain adjacent to excised tumor. Furthermore, there was no toxicity in both HSV seropositive and seronegative patients, and clinical, radiographic, and histologic evidence of antitumor activity was seen. A second-generation oncolytic HSV that produces interleukin-12 (IL-12) (M002, expresses murine IL-12 under the transcriptional control of the murine EGR-1 promoter; M032, expresses human IL-12) was safe with intracerebral injection in mice and Aotus nonhuman primates, and a clinical phase 1 study in adults with recurrent high-grade glioma is ongoing at the University of Alabama at Birmingham (ClinicalTrials.gov Identifier: NCT02062827).\textsuperscript{93,102}

Several first- and second-generation viruses have proven safe when given alone or in combination with chemoradiation in adult clinical trials in various tumor types outside the central nervous system. HF-10, a spontaneously mutated virus that was derived from the parent virus strain HF, was piloted in patients with recurrent head and neck squamous cell carcinoma or with recurrent metastatic breast cancer. Cutaneous or subcutaneous metastases were injected with virus safely and subsequently excised.\textsuperscript{103,104} Histopathological examination revealed significant cell death in both tumor types. A phase 1 dose escalation clinical trial of HF10 in patients with pancreatic cancer was likewise safe with no adverse side-effects, and evidence of response with three of six patients having stable disease and one patient having a partial response.\textsuperscript{105}

Safety of GM-CSF producing T-VEC has been established in several adult clinical trials. Multidose intratumoral injections of the virus were safe in patients with cutaneous or subcutaneous deposits of melanoma or breast, head and neck or gastrointestinal tumors.\textsuperscript{106} These results led to a phase 2 trial in unresectable metastatic melanoma.\textsuperscript{107} Patients received intratumoral injections of up to 4 ml of virus at $10^8$ pfu/ml as frequently as every 2 weeks for up to 24 months. Transient flu-like symptoms were the primary adverse effects. Overall response rate by Response Evaluation Criteria in Solid Tumors was 26% and some durable responses in both injected and uninjected lesions including visceral sites were seen. A phase
In addition to being safe when administered alone, T-VEC injected intratumorally every 3 weeks, combined with cisplatin-based chemoradiotherapy was well tolerated in patients with untreated stage III/IV squamous cell cancer of the head and neck. Viral replication was confirmed in injected and adjacent uninjected tumors with virus detected at levels higher than the input dose.

While oHSV has been primarily tested via the intratumoral route, NV1020 has been administered safely by hepatic arterial infusion in patients with metastatic colorectal carcinoma to the liver. This virus was initially developed as a vaccine against HSV-2; it was constructed from HSV-1 (strain F) by deleting a portion of the thymidine kinase (tk) gene and by replacing the sequences representing the internal inverted repeat and adjacent genes (U1.56) in the L component with a fragment of the HSV-2 genome encoding the glycoproteins G, D, I, and a portion of E. Interestingly, while it contains one intact copy of \( \gamma_1 \)34.5 gene in the terminal repeat region, it is neuro-attenuated 10,000-fold in comparison to wild-type HSV. In the initial dose-escalating phase 1 trial in subjects with colorectal carcinoma liver metastases, patients received a single 10-minute infusion of the virus at up to \( 10^8 \) pfu. Escalations in dose were stopped when virus was detected emerging through the hepatic venous circulation. Serious adverse events possibly or probably related to the virus were limited to a transient rise in \( \gamma \)-glutamyltransferase, diarrhea, or leukocytosis, and no significant effect on liver function was seen. A follow-up multicenter phase 1/2 study explored the safety of four weekly doses of hepatic arterial infused NV1020 followed by two or more cycles of conventional chemotherapy. Toxicities related to the virus included mild to moderate febrile reactions and two patients developed grade 3/4 lymphopenia. Half of the patients had stable disease after NV1020 administration. A similar study is underway using the virus rRp450 (NCT01071941).

**SUMMARY**

From the preceding, it is clear that oHSVs are safe in adults as agents for the treatment of cancer by a variety of delivery routes and offer several advantages. This is a large DNA virus that does not integrate into the host genome and a substantial amount of its DNA is not essential for the infection of and replication in tumor cells. This offers the potential for foreign therapeutic gene delivery to tumor cells to facilitate its efficacy by improving its percolation through tumor tissue and by engendering a strong immune response to the virus and to the infected tumor cells. To the extent that pre-existing immunity may hasten clearance of the virus, the pediatric population may be ideal for study of oHSVs as most young patients are seronegative. In Part II, we evaluate the potential efficacy of oHSV virotherapy in children with malignant diseases, highlight preclinical and clinical advances in children, and discuss future prospects for utilizing oHSVs in pediatric patients.

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CONFLICT OF INTEREST

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Figure 1.
Structural schematics of first-generation herpes simplex virus (HSV) recombinants. This depiction of a linearized DNA molecule of HSV-1 shows the relevant features of each of several mutant viruses described in the text. Most of these constructs have demonstrated to be safe and efficacious in animal models and some have been advanced to clinical trials. Neuroattenuation has been achieved primarily by deletion of one or both copies of the neurovirulence gene, $\gamma_134.5$, or by other deletions. Attempts to enhance virus replication without increasing toxicity are shown by using tumor-specific transcriptional targeting where $\gamma_134.5$ gene expression is driven by a gene promoter expressed primarily in tumor cells. HCMV, human cytomegalovirus.
Second-generation herpes simplex virus mutants are defined as those first-generation constructs that now have been armed with an antitumor therapeutic gene, as indicated in this schematic for several of the viruses described in the text. CMV, cytomegalovirus; GM-CSF, granulocyte macrophage colony stimulating factor.
# Table 1

Summary of oncolytic HSVs discussed in the text

| Virus         | Deletions                                                                 | Foreign gene/promoter insertion                                      | References         |
|---------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------|---------------------|
| C134          | Deletions in both copies of γ134.5 gene                                    | IRS1 gene under control of an HCMV immediate early promoter           | 88                  |
| G207          | Deletions in both copies of γ134.5 gene and disabling lacZ insertion within ICP6 gene | None                                                                  | 19, 37–41, 68, 71, 73, 93, 96–100 |
| HF10          | Deletions resulting in UL43, 49.5, 55, 56 and latency-associated transcript inactivation | None                                                                  | 103–105            |
| HSV1716       | Deletions in both copies of γ134.5 gene                                    | None                                                                  | 20, 21, 42, 101    |
| M002          | Deletions in both copies of γ134.5 gene                                    | Murine IL-12 under the transcriptional control of the murine early-growth response-1 promoter (Egr-1) | 68, 71, 92, 93     |
| M032          | Deletions in both copies of γ134.5 gene                                    | Human IL-12 under the transcriptional control of the murine early-growth response-1 promoter (Egr-1) | 102                |
| NV1020        | Deletion in thymidine kinase (tk) locus and across the joining region of the long and short components of the HSV-1 genome | HSV-1 DNA fragment encoding the tk gene fused to the α gene promoter | 34–36              |
| rRp450        | Deletions of ICP6                                                          | Rat CYP2B1                                                            | 28, 49, 94         |
| Talimogene Laherparepvec (T-VEC) | Complete deletions of the genes encoding ICP34.5 and ICP47 | GM-CSF, CMV promoter                                                  | 48, 106–108        |

CMV, cytomegalovirus; GM-CSF, granulocyte macrophage colony stimulating factor; HCMV, human cytomegalovirus.