Published OnlineFirst May 11, 2017; DOI: 10.1158/1078-0432.CCR-16-2900

Clinical Cancer Research

Intratumoral Injection of HSV1716, an Oncolytic Herpes Virus, Is Safe and Shows Evidence of Immune Response and Viral Replication in Young Cancer Patients

Keri A. Streby[1,2], James I. Geller[3], Mark A. Currier[2], Patrick S. Warren[4], John M. Racadio[5], Alexander J. Towbin[5], Michele R. Vaughan[1], Melinda Triplet[1], Kristy Ott-Napier[1], Devon J. Dishman[1], Lori R. Backus[3], Beth Stockman[3], Marianne Brunner[6], Kathleen Simpson[7], Robert Spavin[7], Joe Conner[7], and Timothy P. Cripe[1,2]

Abstract

Purpose: HSV1716 is an oncolytic herpes simplex virus-1 (HSV-1) studied in adults via injection into the brain and superficial tumors. To determine the safety of administering HSV1716 to pediatric patients with cancer, we conducted a phase I trial of image-guided injection in young patients with relapsed or refractory extracranial cancers.

Experimental Design: We delivered a single dose of 10^5 to 10^7 infectious units of HSV1716 via computed tomography–guided intratumoral injection and measured tumor responses by imaging. Patients were eligible for up to three more doses if they achieved stable disease. We monitored HSV-1 serum titers and shedding by PCR and culture.

Results: We administered a single dose of HSV1716 to eight patients and two doses to one patient. We did not observe any dose-limiting toxicities. Adverse events attributed to virus included low-grade fever, chills, and mild cytopenias. Six of eight HSV-1 seronegative patients at baseline showed seroconversion on day 28. Six of nine patients had detectable HSV-1 genomes by PCR in peripheral blood appearing on day +4 consistent with *de novo* virus replication. Two patients had transient focal increases in metabolic activity on ^{18}fluorine-deoxyglucose PET, consistent with inflammatory reactions. In one case, the same geographic region that flared later appeared necrotic on imaging. No patient had an objective response to HSV1716.

Conclusions: Intratumoral HSV1716 is safe and well-tolerated without shedding in children and young adults with late-stage, aggressive cancer. Viremia consistent with virus replication and transient inflammatory reactions hold promise for future HSV1716 studies. *Clin Cancer Res; 1–9. ©2017 AACR.*

Introduction

With the recent FDA approval of the herpes simplex type-1 (HSV-1) virus talimogene laherparepvec for melanoma by intralesional injection, oncolytic virotherapy is gaining recognition as an efficacious and safe cancer therapy. Oncolytic viruses have a large therapeutic index with limited toxic effects due to their tumor selectivity. Indeed, talimogene laherparepvec induced a 16% durable response rate as monotherapy in patients with advanced melanoma (1).

HSV-1 is an attractive platform for virotherapy, as it is one of the best characterized human viruses (2, 3) and its disease pathogenesis is well described (4). Diagnostic assays are standardized, and practitioners have ample clinical experience dealing with HSV-1 infections. In particular, HSV is one of the few human viral pathogens for which safe and clinically proven antiviral therapies are available.

We studied a similar virus to talimogene laherparepvec, HSV1716, an oncolytic virus derived from HSV-1 strain 17. Both viruses are attenuated from their wild-type counterparts by mutation in the *RL1* genes encoding ICP34.5, which confers neurovirulence (5, 6). Talimogene laherparepvec is also deleted for the gene encoding ICP47, which blocks antigen presentation to major histocompatibility complex class 1 and 2 molecules, and has the coding sequence for human granulocyte-macrophage colony-stimulating factor (GM-CSF) inserted in the place of ICP34.5. HSV1716 is incapable of replicating in the central nervous system (6–8) and has been extensively

[1]Division of Hematology/Oncology/BMT, Nationwide Children's Hospital, The Ohio State University, Columbus, Ohio. [2]Center for Childhood Cancer and Blood Diseases, Nationwide Children's Hospital Research Institute, Columbus, Ohio. [3]Division of Oncology, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio. [4]Department of Radiology, Nationwide Children's Hospital, Columbus, Ohio. [5]Department of Radiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio. [6]Translational Research Trials Office, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio. [7]Virttu Biologics Ltd, Biocity, Scotland, United Kingdom.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

This work is dedicated to the memory of Dr. Arceci and all his life's work on the behalf of children with cancer and serious blood diseases.

Corresponding Author: Timothy P. Cripe, Division of Hematology/Oncology/BMT, Nationwide Children's Hospital, 700 Children's Dr., Columbus, OH 43205. Phone: 614-722-3552; Fax: 614-722-3699; E-mail: Timothy.Cripe@nationwidechildrens.org

doi: 10.1158/1078-0432.CCR-16-2900

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Published OnlineFirst May 11, 2017; DOI: 10.1158/1078-0432.CCR-16-2900

Translational Relevance

Oncolytic variants of herpes simplex virus-1 have shown antitumor efficacy in adults with melanoma, glioma, and other cancers. One such oncolytic HSV, HSV1716, is genetically modified to target cancer cells for viral replication and cancer cell lysis. We and others have shown that HSV1716 delays tumor growth and is cytotoxic to various pediatric cancers in preclinical models. In this first evaluation of an oncolytic HSV-1 in children and young adults with cancer, we evaluated the safety and tolerability of HSV1716 administered directly by injection into tumors. HSV1716 was safe in the pediatric population with minimal toxicities noted. We also found evidence of virus replication in blood and acute inflammation on PET/CT imaging. Although no clinical responses were observed in this phase I trial, these findings prompt further investigation into optimal virus dosing, method of virus delivery, and combination therapies with other cancer treatments such as chemotherapy and/or immunomodulators.

characterized both *in vitro* and *in vivo*. It maintains expression of thymidine kinase, targetable by administration of acyclovir, thereby providing a "therapeutic safety net" in the unusual circumstance of viral replication escape and toxicity. Preclinically, human sarcoma and neuroblastoma cancers demonstrate replication of HSV mutants in cultured cells and human xenograft models in mice with notable antitumor effects (9–12). Phase I trials in more than 80 adult patients with cancer with CNS tumors, melanoma, and head and neck squamous cell carcinomas demonstrated the safety of HSV1716 with minimal toxicities (no attributable grade 3 or higher toxicities; refs. 13–16). HSV1716 demonstrated efficacy in a phase I trial of adults with glioblastoma multiforme (GBM) by showing sustained responses and increased survival without additional medical intervention in 3 of 12 patients (15). One patient with GBM remained alive at last follow-up with no tumor progression 10 years after HSV1716 injection without additional medical intervention (unpublished).

Herein, we report the first clinical trial of HSV1716 in pediatric patients with cancer. We sought to determine the safety of intratumoral injection of HSV1716 in children and young adults with non-CNS solid tumors and to determine the dose-limiting toxicities (DLT) of intratumoral HSV1716. Our secondary aims were to assess the antiviral immune response, systemic viremia, and viral shedding after intratumoral HSV1716 injection. We also measured the antitumor activity of HSV1716 within the confines of a phase I trial.

Patients and Methods

This trial received a waiver regarding the need for public discussion from the NIH Recombinant DNA Advisory Committee. Each participating institution's local Institutional Review Board approved the trial. It was conducted under FDA Investigational New Drug BB-13196 and registered on clinical trials.gov (NCT00931931). We obtained informed consent from patients 18 years or older and/or from parents or legal guardians of patients younger than 18 years. Child assent was obtained in accordance with local institutional policies.

Eligibility – inclusion criteria

The trial population included patients with recurrent or refractory incurable non-CNS solid tumors and patients were aged $\geq$7 to $\leq$30 years at the time of virus injection. Patients were required to have a Karnofsky (age $\geq$ 16) or Lansky (age < 16) performance score of >50%. Organ function requirements included adequate bone marrow function (absolute neutrophil count $\geq$ 750/mL in absence of G-CSF for 72 hours or PEG-GCSF for 14 days, platelet count > 100,000/mL, and hemoglobin $\geq$ 9 g/dL), adequate renal function [serum creatinine $\leq$ 1.5 $\times$ upper limit of normal (ULN) for age or creatinine clearance or radioisotope GFR $\geq$ 70 mL/min/1.73 m^2), adequate hepatic function [total bilirubin $\leq$ 2 times ULN for age, alanine transaminase (ALT) $\leq$ 2.5 $\times$ ULN for age, and albumin $\geq$ 2 g/dL], adequate hemostatic function [prothrombin time (PT)/international normalized ration (INR) and activated partial thromboplastin time (aPTT) < 1.5 $\times$ ULN for age], adequate central nervous system function (baseline CNS conditions $\leq$ grade 2 per CTCAE v3.0), and adequate cardiac function (shortening fraction > 25% by echocardiogram, no focal wall motion abnormalities, and no evidence of ischemia or significant arrhythmia on electrocardiogram). Patients with primary brain malignancies were excluded from the trial but asymptomatic patients with treated brain metastases were eligible for enrollment. We required patients to test negative for Hepatitis B surface antigen, Hepatitis C antibody, and HIV-1 and HIV-2 antibodies at or within 3 months prior to trial entry. Patients also must have fully recovered from the acute toxicities of previous therapies prior to trial enrollment. Patients could not have received myelosuppressive chemotherapy within 28 days prior to study entry or non-myelosuppressive therapy within 14 days; could not have received biologic agents within 7 days prior to trial entry; no local palliative radiation therapy within 14 days and no myeloablative radiation therapy within 42 days prior to trial entry; no immunoablative or myeloablative stem cell transplant within 6 months prior to trial entry; and no investigational agent within 28 days prior to trial entry.

In addition, patients needed to have at least one cancer lesion amenable to HSV1716 administration by needle via imaging guidance without undue risk. The lesion(s) had to be at least 3 times greater than the volume of HSV1716 to be injected (based on available lots, the volumes were 1 mL of HSV1716 injected for dose levels 1 and 2, 5 mL for dose level 3). One lesion had to meet criteria in the first 2 dose levels and the sum total of up to 3 lesions could meet criteria in the third dose level. We recorded the longest diameter for the injected target lesion(s) as the baseline longest diameter, which we used as reference to further characterize the objective tumor response. The response of the injected target lesion(s) determined whether the patient was eligible for part 2 of the trial in which patients could consent to receive up to 3 additional monthly doses of HSV1716. To be eligible, all injected tumors were required to be characterized as stable disease or better using a modified version of the RECIST. All measurable uninjected tumors were also identified and followed on imaging and were classified as localized or distant metastases from the site of the primary tumor.

Eligibility – exclusion criteria

Exclusion criteria included a history of allogeneic stem cell transplant, currently pregnant or breast feeding, unable or

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Published OnlineFirst May 11, 2017; DOI: 10.1158/1078-0432.CCR-16-2900

unwilling to give voluntary informed consent/assent, significant infection or other severe systemic disease or medical/surgical condition deemed significant by the PI, PEG-GCSF within 14 days or G-CSF within 72 hours of trial entry, and planned use of antiviral therapy between 2 days prior to HSV1716 administration up to 28 days after HSV1716 administration.

Clinical trial design and treatment

NCT00931931 opened as a single-center phase I trial at Cincinnati Children's Hospital Medical Center (Cincinnati, OH) and was subsequently expanded to include enrollment at Nationwide Children's Hospital (Columbus, OH). The dose-escalation portion of the trial enrolled patients in a 3 + 3 fashion. Baseline assessments included organ function, HSV serologies, and relevant imaging studies such as computed tomography (CT) and/or MRI and ^{18}fluorine-deoxyglucose PET/CT imaging. All patients underwent general anesthesia to ensure safety and proper needle placement with imaging guidance. Patients received a single dose of HSV1716. Patients then recovered and were monitored in the hospital overnight for any adverse events. Peripheral blood was collected for bacterial culture, HSV PCR, and culture prior to injection on day 0 and at 1, 7, 14, 21, and 28 days after HSV1716 injection. The HSV PCR assay was our standard hospital clinical laboratory assay, which utilizes a primer for a 148-basepair fragment for the gene encoding glycoprotein B that is present on both wild-type HSV and HSV1716. Patients were discharged after the 24-hour laboratory draw, and/or it was medically appropriate to discharge the patient home. They returned on days 4, 7, 14, 21, and 28 for laboratories and physical examinations to monitor adverse events and organ function and immune response and virus studies. Patients were eligible for part 2 of the trial, in which patients could receive up to 3 more doses after 28 days, each a minimum of 28 days apart, if they showed a tumor response in the injected lesion(s) of stable disease or better. Injection of subsequent doses required a second consent/assent. The requirement of the 28-day interval between virus doses and between patients was mandated by the FDA as a safety measure as this was the first study of an oncolytic herpes virus in children. The requirement of general anesthesia to safely administer the virus into these deep-seated tumors also limited the frequency of intratumoral virus delivery.

DLTs

Toxicity was graded according to the NCI Common Toxicity Criteria (CTCAE) v3.0. DLT was any grade 3 or grade 4 toxicity, grade 2–4 neurologic or allergic toxicity, that was possibly, probably, or definitely attributable to participation in the study (with the exclusion of grade 3 flu-like symptoms, grade 3 anorexia, and grade 3 pain or infection at the injection site). The highest tested and tolerated dose was predefined as the highest dose level of HSV1716 administered at which no more than 1 of 6 patients experienced a DLT.

Evaluation of clinical activity

Baseline imaging was obtained within 14 days prior to the first HSV1716 dose, then again at 14 days following injection (via amendment after patient HSV03) and at 28 days, then as clinically indicated until withdrawal from the trial. All measurable lesions were deemed target lesions and were followed for response as appropriate for cancer type and location. We evaluated response according to modified RECIST guidelines at days 14 and 28. The modification varied from RECIST v1.0 as we measured the longest diameter instead of the sum of the longest diameters.

Virus production, handling, and administration

Vials of HSV1716 were manufactured according to Good Manufacturing Practice (GMP) standards by BioReliance at either 1.0×10^5 (used in dose level 1) or 2.0×10^6 infectious units (i.u.) used in dose levels 2 (1 vial) and 3 (5 vials). Infectious units are defined as the equivalent of plaque-forming units (PFU) per mL. Quality assessment HSV1716 control vials were obtained from Virttu Biologics. HSV1716 was stored in an ultralow freezer (−80°C) until patient arrival.

Frozen vial(s) were transported on dry ice to the interventional radiology suite, draped with a lead shield during fluoroscopy/CT scanning for needle placement, and hand-thawed prior to injection through a straight needle followed by a 1-mL flush of normal saline. Thawing of HSV1716 vials required 13 minutes on average (range, 5–25). Vials were checked immediately for clarity and particulate matter, sprayed, and wiped down with 70% ethanol. The time elapsed from completion of the thaw to injection was 7 minutes on average.

All vials contained an additional 0.1 mL of HSV1716 for quality assurance testing. Immediately following injection, vials containing residual HSV1716 were transported on ice to the laboratory for post-procedure virus titer assessment using the standard plaque assay procedure as previously described (17). In addition, control HSV1716 vials were thawed and assayed for quality assurance. We followed standard biosafety level 2 precautions. The acceptable range established for 10 control vials at 2×10^6 i.u. was 6.3×10^5 to 6.3×10^6 i.u. (2 SDs). All postinjection titers were within the expected range (Supplementary Table S1).

Results

Patient characteristics

A total of 9 patients aged 8 to 30 years were enrolled and fully evaluable for safety and toxicity. Three patients were accrued to each of 3 dose levels (1×10^5, 2×10^6, and 1×10^7 i.u.). Patient diagnoses included a variety of sarcomas, clival chordoma, malignant peripheral nerve sheath tumor (MPNST), and renal cell carcinoma (see Table 1). Most patients received at least 2 lines of therapy for relapsed or refractory disease prior to enrollment on this trial (one exception being the patient with renal cell carcinoma who was only previously treated with sunitinib). All 3 of the dose level 3 patients had their doses split into different needles (2 of the patients had 2 needles placed within the same tumor; HSV09 had 3 separate tumors injected).

Serologic responses and toxicities

Eight of the 9 patients were serologically negative for anti-HSV1 antibodies at baseline, and most patients converted following injection by day 28 (Table 2). Only HSV02 was serologically positive prior to HSV1716. No DLTs were noted in any of the patients. Two patients had grade 3 back pain (later resolved to grade 1) related to HSV1716 and/or the intratumoral injection procedure. Grade 1 and 2 adverse events possibly or probably attributable to HSV1716 included fever, chills, and mild laboratory abnormalities such as anemia and leukopenia (Table 3). HSV09, whose dose was split into 3 different parenchymal lung lesions, remained hospitalized for an additional

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Published OnlineFirst May 11, 2017; DOI: 10.1158/1078-0432.CCR-16-2900

Table 1. Patient demographics

Patient	Diagnosis	Age, y	Prior chemotherapy regimens (no.)	Previous radiation Tx	Time from Dx to Tx, mo	Disease at trial entry	HSV1716 dose, i.u.	Location of injected tumor
HSV01	Parameningeal rhabdomyosarcoma	13	VCR, Irino, Doxo, CTX, Etop; navelbine/CTX; vorinostat/bortezomib (3)	IMRT 50.4 Gy	23	Large local recurrence; lung metastases	1×10^5	Skull base
HSV02	Extremity Ewing sarcoma	21	CTX/Topo/VCR/Ifos/Carbo/Etop; CTX/Topo; Irino; Gem/Tox (4)	Yes	75	Multiple lung metastases	1×10^5	Left lung metastasis
HSV03	Spinal/Paraspinal MPNST	19	Ifos/Doxo; Carbo/Etop x2; sirolimus; vorinostat/bortezomib (4)	IMRT 59.4 Gy to abd; IMRT 45 Gy to pelvis	43	L3 paraspinal mass; spinal canal lesions lower thoracic to sacrum	1×10^5	Paraspinal mass (L3)
HSV04	Thoracic osteosarcoma (in context of Li Fraumeni)	19	Doxo/Cisplat/MTX; Gem/Dox; IMC-A12/Tem; Etop (4)	None	27	2 paraspinal masses, lung metastases, humeral lesion, L2 vertebral body lesion	2×10^6	Paraspinal mass at T8/9 (recurrent primary tumor)
HSV05	Clival chordoma	10	VCR/Doxo/CTX/Ifos/Etop; erlotinib; sirolimus (3)	74 Gy	53	Clival recurrence, lesions in orbit, posterior to cerebellum and hard palate	2×10^6	Left orbital metastasis
HSV06	Retroperitoneal rhabdomyosarcoma	8	VCR/dactino/CTX; Irino/Doxo; Ifos/Etop; vinorelbine/Bevaz; Tem; Topo (4)	41.4 Gy primary; 3 Gy spinal	50, 51	Localized large tumor recurrence at primary tumor site; no metastatic disease	2×10^6	Recurrent retroperitoneal mass
HSV07	Renal cell carcinoma of the left kidney	16	Sunitinib (1)	None	7	Localized recurrence retroperitoneum, supraclavicular node metastases; localized node metastases	1×10^7	Recurrent retroperitoneal mass
HSV08	Osteosarcoma of the left tibia	16	Ifos/Etop; Gem/Dox; Zometa x2; Bev; Oxali/Irino; Doxo/Cisplat; sorafenib (7)	None	45	Lung metastases (multiple)	1×10^7	Right pleural metastasis
HSV09	Chondrosarcoma of the left distal femur	30	IDH-1 inhibitor (1)	60 Gy to leg; 54 Gy leg	48	Innumerable bilateral pulmonary metastases; possible local recurrence in the distal left femoral prosthesis and left calf regions	1×10^7	2 right and 1 left upper lobe lung metastases

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Table 2. Patient serologic responses to single dose of intratumoral HSV1716

Patient	WBC	ALC	ANC	HSV-1 PCR						HSV-1 IgG	
Day	0	0	0	0 and 1	4	7	14	21	28	Baseline	28
HSV01	4.9	686	3,626	–	–	–	–	–	–	–	+
HSV02	4.7	1,739	1,692	–	–	–	–	–	–	+	+
HSV03	6	1,140	4,140	–	+	–	–	–	–	–	+
HSV04	3	600	2,040	–	+	+	+	+	+	–	+
HSV05	4.4	1,408	2,640	–	–	–	–	–	–	–	–
HSV06	8	1,040	5,600	–	+	–	–	–	–	–	–
HSV07	6	1,260	4,080	–	+	–	–	–	–	–	ND
HSV08	4.3	1,419	2,451	–	+	–	–	–	–	–	+
HSV09	6.1	1,769	3,721	–	+	+	–	–	–	–	+

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ND, not done; PCR, polymerase chain reaction; WBC, white blood count.

24 hours due to monitoring of a pneumothorax, an expected complication of inserting a needle into the intrapleural space and/or pleural cavity.

Three of 4 patients eligible for part 2 of the trial (more HSV1716 doses) based on stable disease of the injected lesion(s) at days +14 or +28 declined further injections because of the treating oncologist preference or concern for disease progression elsewhere. Patient HSV06 elected to receive an additional injection (denoted as "II" in Table 4), with no significant adverse events noted with either dose.

Viremia and virus shedding

No viral shedding was observed in any patient on this trial as all HSV-1 cultures including blood, buccal swab, and urine at all study visits through day 28 were negative. PCR for HSV-1 genomes were also negative in all buccal swab and urine samples. Blood PCR for HSV-1 genomes were negative at baseline, day 0, and day +1 following virus injection. In contrast, blood PCR for HSV-1 genomes at day +4 turned positive in 1 patient at dose level 1, 2 patients at dose level 2, and all 3 patients at dose level 3 (6 of 9 patients total). In 2 patients, PCR remained positive at day +7, and in one of those patients (HSV04), it remained positive through day 28. Unfortunately, this patient's disease rapidly progressed leading to hospice care so we were unable to confirm viral clearance at a later time point.

Disease responses

No patients had tumor shrinkage in directly injected (Table 4) or uninjected (Table 5) lesions. Four of 5 patients evaluated at day +14 had stable disease by cross-sectional imaging. Three of 7 patients evaluated at day +28 had stable disease, and one of these patients had a decrease in PET standardized uptake values (SUV; HSV09).

Interestingly, in 2 of 3 patients who had multiple PET/CTs, we observed an increase in SUV either at day +14 or +28, which we initially interpreted as disease progression, followed by a spontaneous decrease back to or near baseline on subsequent images (Fig. 1). In one case, the exact geometric configuration of the increased PET signal became completely negative on subsequent scans (Fig. 1A). In another patient, we also observed a parallel flare in an uninjected metastatic tumor (Fig. 1B).

As shown in Table 4, patients treated at the first 2 dose levels had a median survival of 2.25 months, whereas the 3 patients treated at the highest dose level had a median survival of 7 months. These 3 patients also went on to other forms of therapy after discontinuing HSV1716 treatment (HSV07 received cabozantinib, HSV08 received cryoablation to the remaining tumors, and HSV09 received everolimus and pazopanib). As this is a very small number of patients all treated with different therapies after HSV1716, we are unable to draw any conclusions about the role HSV1716 may have played in their prolonged survival.

Table 3. Adverse events possibly, probably, or definitely attributable to intratumoral HSV1716 administration

Adverse events	Grade 1	Grade 2	Grade 3
Anemia	1		
Leukopenia	1	1	
Lymphopenia	1		
Neutropenia	1	1	
Chills	1		
Fever	2		
Bruising	2		
Constipation	1	1	
Nausea	1		
Anxiety		1	
Back pain	2		2
Headache	2	1	
Chest pain	1	1	
Pleurisy	1		
Atelectasis	1		
Pneumothorax	1	1	

Discussion

Children with relapsed/refractory solid tumors continue to have very poor outcomes and significant toxicities from their various cancer therapies. Novel strategies and treatment modalities are urgently needed. The field of oncolytic virotherapy continues to gain momentum and offers the potential of improved outcomes with fewer toxicities for cancer patients. On the basis of our results, we conclude that intratumoral administration of a single dose of HSV1716 in children with relapsed/refractory non-CNS solid tumors is safe and well-tolerated. All observed adverse events that were likely attributed to virus were low grade and transient. The majority of patients enrolled in this trial were HSV-1 seronegative, suggesting that pediatric patients may benefit the most from HSV virotherapy if pre-existing anti-HSV-1 immunity is ultimately found to diminish antitumor efficacy.

Intratumoral HSV1716 resulted in systemic viremia as evidenced by initially negative and subsequent appearance of HSV-1 by PCR in the peripheral blood in most patients. The lack of a PCR signal in the peripheral blood of patients HSV01 and HSV02 may reflect that the dose used was insufficient, the location was not prone to generating viremia, or their particular tumor did not support robust virus replication. Preclinically, MPNST models show robust herpes virus replication (18), which may account for the PCR signal even with the lower dose of HSV1716 in patient

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Table 4. Disease response and PET SUV changes relative to baseline in each injected tumor after each dose of intratumoral HSV1716

Patient	Location of injected tumor	Day 14 CT/MRI	Day 14 PET	Day 28 CT/MRI	Day 28 PET	Time from Tx to death, mo
HSV01	Skull base	N/A	N/A	ND	ND	1
HSV02	Left Lung metastasis	N/A	N/A	PD (2.6 to 3.3 cm)	SUV ↑ (8.3 to 9.9)	2
HSV03	Paraspinal mass at L3	N/A	N/A	PD (11.8 to 12.5 cm)	SUV ↑ (3.8 to 7.5)	3
HSV04	Recurrent paraspinal mass at T8/9	PD (2.5 to 4.7 cm)	SUV ↑ (13.6 to 19.6)	ND	ND	1
HSV05	Left orbital metastasis	SD (3.4 to 3.4 cm)	SUV ↓ (5 to 3.8)	PD (4.8 cm)	ND	2.5
HSV06-I[a]	Recurrent retroperitoneal mass (posterior)	SD (11.6 to 11.1 cm)	SUV ↑ (minimal to 2.4)	SD (11 cm)	SUV ↑ (4.7)	8
HSV06-II	Retroperitoneal mass (anterior)	SD (11 to 10.5 cm)	SUV stable (4.7 to 4.8)	SD (10.3 cm)	SUV ↓ (2.52)	7
HSV07	Recurrent retroperitoneal mass	SD (10.4 to 9.7 cm)	SUV stable (4.96 to 4.9)	SD (10.6 cm)	ND	25
HSV08	Right pleural metastasis	PD (5 to 6.3 cm)	SUV ↑↑ (3.2 to 10.1)	PD (7 cm)	SUV ↑ (4)	7
HSV09-A[b]	Right upper lobe metastasis	SD (3.3 to 3.4 cm)	SUV ↑ (4.1 to 4.9)	SD (3.3 cm)	SUV ↑ (4.6)	6.5
HSV09-B	Left upper lobe metastasis	SD (2.8 to 2.9 cm)	SUV stable (2.6 to 2.6)	SD (2.8 cm)	SUV stable (2.7)	
HSV09-C	Right upper lobe metastasis	SD	SUV ↑	SD	SUV stable	

Abbreviations: N/A, not applicable; ND, not done; PD, progressive disease; SD, stable disease.
[a]Patient HSV06 had 2 cycles of an injection of HSV1716 (HSV06-I and HSV06-II).
[b]Patient HSV09 had 3 injected target lesions (A, B, C).

HSV03. The lack of an HSV PCR signal in patient HSV05 may suggest that chordoma cells do not support virus replication and/or that certain anatomic locations may not be favorable to producing viremia (i.e., a tumor in the skill base protruding into the nasal cavity and orbit). In contrast, HSV04 had a persistent PCR signal suggesting robust replication within this patient's osteosarcoma. Interestingly, HSV04 had a low absolute lymphocyte count (ALC; 600) at the time of virus injection, but in this small study, it is difficult to draw any conclusions on how a low ALC may impact the ability of HSV1716 to replicate. We hypothesize the prolonged persistence of HSV detection could be due to the inhibition of immunosuppressor cells within the tumor such as regulatory T cells, but further research is required to determine any relationship between virus persistence and the immune microenvironment.

Most but not all patients converted their HSV-1 immune serology following virus injection. We did not observe any differences in toxicities between seronegative and seropositive patients. The reasons 2 of 8 patients tested in this trial failed to convert to seropositive are unclear, but it is possible they had ineffective or delayed antiviral immunity, as both were heavily pretreated with chemotherapy. Although both patients had relatively normal WBC, ALC, and absolute neutrophil count (ANC) levels, the capacity of their immune systems is unknown. Further research into the functionality of the immune system at various time points in cancer treatment may be warranted to guide immunotherapy trials. As implied above regarding viremia, location of the tumor and virus injection may also play a role in seroconversion if there is limited access of immune cells to virus antigens.

Two patients notably had a transient increase in PET uptake that resolved spontaneously. The possible causes of increased glucose utilization are tumor progression or pseudoprogression, the latter from inflammation due to virus infection or stimulation of antitumor immunity. In patient HSV06, we administered a second dose at the site of uptake and, following persistence of signal 12 days later, observed complete disappearance of signal by day 27, suggesting that area of tumor was necrotic. Unfortunately, the rest of this child's large tumor mass continued to progress and the child ultimately succumbed to disease. In patient HSV08, we also observed an immediate swelling and transient increase in PET signal. The fact that the PET signal spontaneously faded suggests that it was most likely consistent with an inflammatory response to virus. We do not know if the swelling, which may have been due to edema or tumor progression, would have also eventually diminished, as the patient subsequently underwent cryotherapy ablation at the choice of the treating physician. The fact that an uninjected lesion also transiently flared on PET may indicate that localized HSV1716 infection had a systemic antitumor immune effect.

Table 5. Disease response and PET SUV change relative to baseline after single dose of intratumoral HSV1716 in noninjected target lesions

Patient	Localized or distant metastases	Day 14 CT/MRI	Day 14 PET	Day 28 CT/MRI	Day 28 PET
HSV01	Bilateral upper lung lobes	N/A	N/A	ND	ND
HSV02	Lung	N/A	N/A	PD	Not measured
HSV04	Local (T3/4 paraspinal mass)	PD (10.5 to 14.5 cm)	SUV ↑ (22.5 to 24.6)	ND	ND
HSV05-A	Clival recurrence (local)	SD (3.9 to 3.8 cm)	SUV stable (3.9 to 4)	PD (4.7 cm)	ND
HSV05-B	Mets posterior to cerebellum (local)	SD (1.3 to 1.5 cm)	Not measured	SD (1.4 cm)	ND
HSV07	Subcarinal node (distant)	SD (2.9 to 3.2 cm)	SUV stable (4.3 to 4.5)	SD (3.4 cm)	ND
HSV08-A	Left anterior perihilar metastatic lesion (distant)	PD (1.9 to 3 cm)	SUV ↑↑ (2.5 to 6.6)	PD (5.7 cm)	SUV stable (2.5)
HSV08-B	Metastatic lesion in left upper lobe of lung (distant)	PD (1 to 2.5 cm)	SUV ↑ (1.2 to 2.8)	PD (4.2 cm)	SUV ↑ (3.1)
HSV09	Pretracheal lymph node (distant)	SD	SUV stable (4.1 to 4.2)	SD	SUV stable (4.1)

NOTE: Letters A and B indicate different measurable but uninjected lesions in the same patient. HSV03 and HSV06 did not have any noninjected lesions.
Abbreviations: N/A, not applicable; ND, not done; PD, progressive disease; SD, stable disease.

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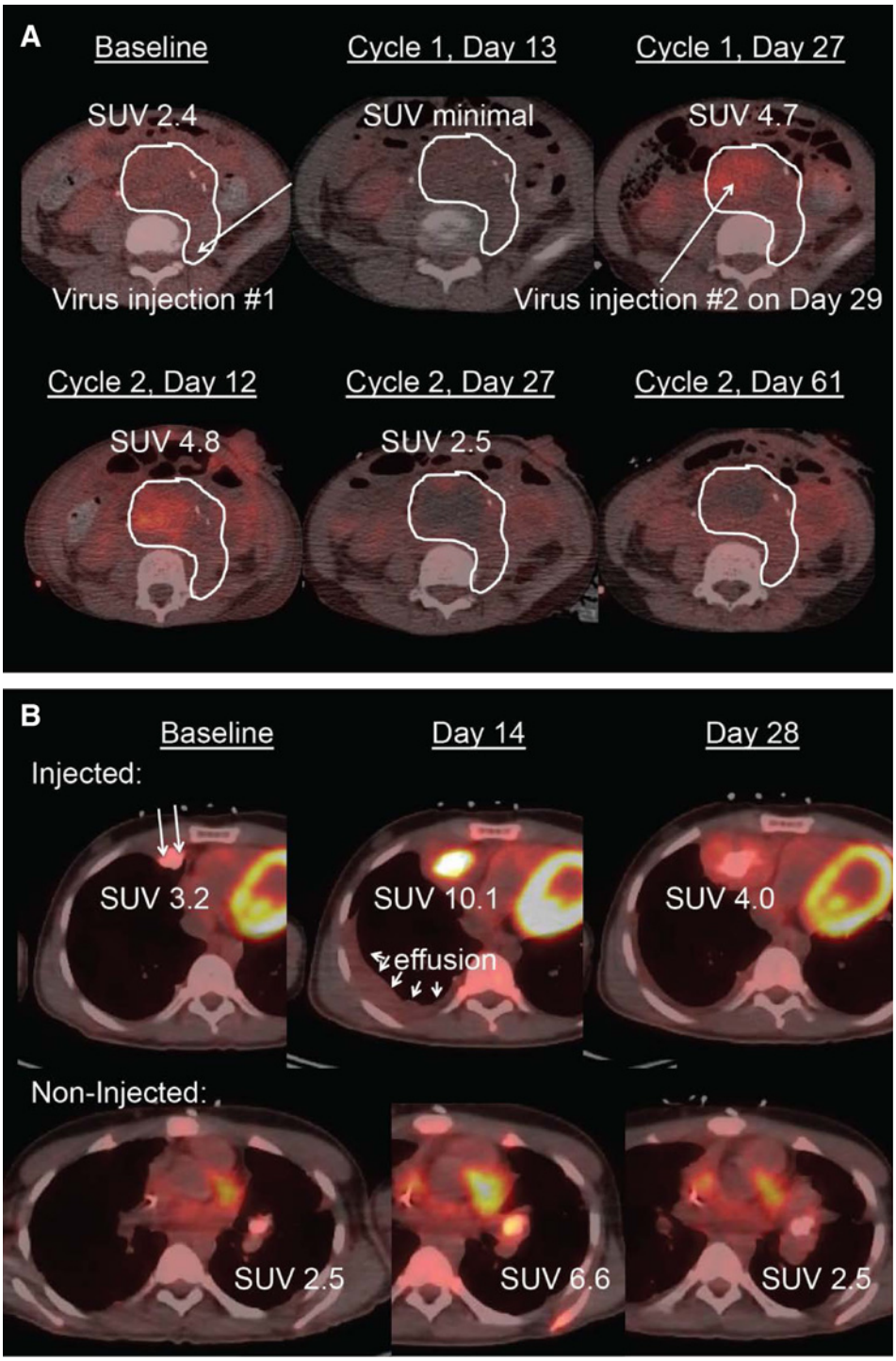

Figure 1.

Inflammatory reactions following virus injection as detected by PET/CT. Baseline images, needle tracks and injection sites (arrows), and follow-up scans are shown for 2 patients who experienced a transient increase in SUV uptake following virus injection that ultimately returned near baseline. Although initially interpreted as tumor progression, in retrospect, the spontaneous decrease suggests the uptake was due to a transient inflammatory reaction to virus (pseudoprogression). **A,** Patient HSV06. The tumor mass is outlined in white. C, Cycle; D, Day. Notice the area of uptake drops to zero, suggesting tumor necrosis in the exact geographic distribution of the uptake. **B,** Patient HSV08. Notice the pleural effusion (white arrows) that developed coincident with the increased PET signal, both of which spontaneously resolved. In addition to the injected right chest wall lesion, the uninjected left hilar lesion also showed a transient increase in PET signal suggesting a systemic effect.

Two non-pathogenic wild-type oncolytic viruses (seneca valley virus and reovirus), and one attenuated pathogenic virus (vaccinia virus), have also been studied in children and showed few toxicities but little evidence of disease response (19–21). Of these and the current pediatric trials, this trial using HSV1716 and the trial using vaccinia virus utilized intratumoral virus administration, whereas the other 2 trials used intravenous or systemic administration. The best method of virus delivery remains unclear. Thus, we are also conducting a parallel portion of this clinical trial with HSV1716 administered intravenously in pediatric patients with relapsed/refractory solid tumors. Certainly, intravenous dosing is significantly less complicated because of the lack of need for sedation, nor imaging guidance. A potential concern for systemic dosing is the development of antiviral antibodies that might limit systemic delivery to tumor sites, so its use in a pediatric

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setting where most patients are seronegative may prove to be advantageous.

Pediatric patients with cancer typically enter phase I trials at a later stage in their disease, mostly with high tumor burdens and aggressive cancers. In contrast, patients in the Amgen trial of talimogene laherparepvec in adults had slowly growing, albeit advanced stage, melanoma. In the melanoma trial, the average time to disease response was 4 months and patients were injected with 10^8 infectious units of virus every 2 weeks for a minimum of 24 weeks, despite disease progression during that time (1, 22). Rather than from a direct lytic effect, the implication is that the majority of response resulted from antitumor immunity, which may take weeks to months to become robust. Thus, one rational approach to achieve enhanced benefit for pediatric patients with cancer is to deliver higher and more doses of oncolytic virus than given in our trial. We plan to investigate more frequent dosing in subsequent studies, now that we have more evidence of safety with oncolytic herpes viruses as shown in this trial. The talimogene laherparepvec trial also demonstrated that higher doses of oncolytic HSV are safe in adults by intralesional injection; however, these data were not available until near the end of our clinical trial. Thus, we only included dose-escalation to 1e7 i.u., as this was the highest dose studied in adults with HSV1716.

Unlike for melanoma, prolonged virotherapy as a single agent may not be feasible given the rapid growth of most pediatric solid tumors. Thus, the effective use of virus may require combination therapy with targeted therapies, chemotherapy, or low-dose radiotherapy to slow tumor growth while allowing time for virolytic or viroimmunotherapeutic effects to develop. Preclinical studies support these approaches (23–25), although concurrent therapies should be chosen and perhaps timed carefully to not interfere with virus replication (26) or the development of virus-induced antitumor immunity. In addition, giving oncolytic virotherapy earlier in the disease course may also allow time to develop an antitumor immune response. Finally, herpes virotherapy may be enhanced by combination with other immune adjuncts such as T-cell checkpoint inhibitors (27, 28).

In conclusion, although none of the patients had objective responses, the evidence of virus replication and inflammatory reactions we observed in pediatric patients with cancer following intratumoral injection of HSV1716 is promising. We propose that using more doses of HSV1716 in addition to combination studies with other cytotoxic or cytostatic agents, radiation and/or other immunomodulators warrant further investigation. We also propose further research regarding the relationship of virus replication and the development of antitumor immunity in pediatric cancer to maximize the efficacy of oncolytic herpes virotherapy.

Disclosure of Potential Conflicts of Interest

K.A. Streby is a consultant/advisory board member for Amgen Inc. A.J. Towbin reports receiving commercial research grants from Guerbet and Siemens, royalties from Elsevier, and is a consultant/advisory board member for Applied Radiology. R. Spavin is a corporate counsel for Virttu Biologics Limited. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J.I. Geller, M.A. Currier, A.J. Towbin, R. Spavin, J. Conner, T.P. Cripe

Development of methodology: J.I. Geller, M.A. Currier, A.J. Towbin, R. Spavin, T.P. Cripe

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.A. Streby, J.I. Geller, M.A. Currier, J.M. Racadio, A.J. Towbin, B. Stockman, T.P. Cripe, P.S. Warren

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.A. Streby, J.I. Geller, M.A. Currier, A.J. Towbin, R. Spavin, J. Conner, T.P. Cripe

Writing, review, and/or revision of the manuscript: K.A. Streby, J.I. Geller, M.A. Currier, J.M. Racadio, A.J. Towbin, M.R. Vaughan, M. Triplet, M. Brunner, K. Simpson, R. Spavin, J. Conner, T.P. Cripe

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.A. Streby, M.A. Currier, M. Triplet, K. Ott-Napier, D.J. Dishman, L.R. Backus, K. Simpson, R. Spavin, J. Conner, T.P. Cripe

Study supervision: J.I. Geller, R. Spavin, T.P. Cripe

Other (Product Manager responsible for the supply of investigational product): K. Simpson

Acknowledgments

We thank members of the Office for Clinical and Translational Research at Cincinnati Children's Hospital Medical Center (CCHMC) including Sheri Selk, Susan Radtke, Rubina Dosani, Chris Canter, and Jeanie Bailey, as well as the Division of Oncology clinical research team including Rebecca Turner, Kristina Kruskamp, Stacey Crane, and Renee Richardson. We thank members of the Nationwide Children's Hospital Division of Hematology/Oncology/BMT Clinical Research Office including Myeshia Harmon, Melissa Jaskiewicz, Karen Krajcovic, Katie Suchy, and Amy Yekisa and members of the H12 nursing staff including Deborah Hockett, Sam Howorka, and Sarah Vogel. We thank nurse navigators Paula Fisher (CCHMC) and Paula Sanborn (NCH). We thank the CCHMC infectious disease and infection control teams for oversight including David Bernstein, Beverly Connelly, and Michael Cloughessy. We thank Denise Lagory in the CCHMC investigational pharmacy and Jill Blind in the NCH investigative pharmacy. We thank members of the CCHMC interventional radiology department including Kamlesh Krukreja, Manish Patel, and Neil Johnson and members of the Nationwide Children's Hospital interventional radiology department including William Shiels (in memorial) and Mark Hogan. We thank the referring oncologists as well as the patients and their families who participated in this trial. Finally, we thank Robert Arceci (in memorial), Paul Sondel, E. Anders Kolb, and Eileen King for serving on the data safety monitoring board and reviewing the safety/toxicity data.

Grant Support

This trial was funded by R01 FD003717-03 to T.P. Cripe from the FDA Orphan Drug Grant Program and by Solving Kids Cancer, Virttu Biologics Ltd, Alex's Lemonade Stand Foundation, Cancer Free Kids, teeoffagainstcancer.org, and The Katie Linz Foundation.

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Received November 17, 2016; revised December 22, 2016; accepted January 13, 2017; published online August 1, 2017.

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Intratumoral Injection of HSV1716, an Oncolytic Herpes Virus, Is Safe and Shows Evidence of Immune Response and Viral Replication in Young Cancer Patients

Keri A. Streby, James I. Geller, Mark A. Currier, et al.

Clin Cancer Res Published OnlineFirst May 11, 2017.

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