PROTOCOL—CLINICAL DEVELOPMENT

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Design and Rationale for First-in-Human Phase 1 Immunovirotherapy Clinical Trial of Oncolytic HSV G207 to Treat Malignant Pediatric Cerebellar Brain Tumors

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Brain tumors represent the most common pediatric solid neoplasms and leading cause of childhood cancer-related morbidity and mortality. Although most adult brain tumors are supratentorial and arise in the cerebrum, the majority of pediatric brain tumors are infratentorial and arise in the posterior fossa, specifically the cerebellum. Outcomes from malignant cerebellar tumors are unacceptable despite aggressive treatments (surgery, radiation, and/or chemotherapy) that are harmful to the developing brain. Novel treatments/approaches such as oncolytic virotherapy are urgently needed. Preclinical and prior clinical studies suggest that genetically engineered oncolytic herpes simplex virus (HSV-1) G207 can safely target cerebellar malignancies and has potential to induce an antitumor immune response at local and distant sites of disease, including spinal metastases and leptomeningeal disease. Herein, we outline the rationale, design, and significance of a first-in-human immunotherapy Phase 1 clinical trial targeting recurrent cerebellar malignancies with HSV G207 combined with a single low-dose of radiation (5 Gy), designed to enhance virus replication and innate and adaptive immune responses. We discuss the unique challenges of inoculating virus through intratumoral catheters into cerebellar tumors. The trial utilizes a single arm open-label traditional 3 + 3 design with four dose cohorts. The primary objective is to assess safety and tolerability of G207 with radiation in recurrent/progressive malignant pediatric cerebellar tumors. After biopsy to prove recurrence/progression, one to four intratumoral catheters will be placed followed by a controlled-rate infusion of G207 for 6 h followed by the removal of catheters at the bedside. Radiation will be given within 24 h of virus inoculation. Patients will be monitored closely for toxicity and virus shedding. Efficacy will be assessed by measuring radiographic response, performance score, progression-free and overall survival, and quality of life. The data obtained will be invaluable in our efforts to produce more effective and less toxic therapies for children with high-grade brain tumors.

Keywords: pediatric brain tumors, cerebellum, clinical trials, HSV, immunotherapy, virotherapy

INTRODUCTION

Tumors of the central nervous system (CNS) represent the most common pediatric solid tumors and the leading cause of morbidity and mortality in children with cancer.[1,2] Survival rates of children with malignant CNS tumors are generally dismal despite aggressive treatments, including surgery, chemotherapy, and/or radiation.[1,2] Moreover, for children who are cured, conventional therapies produce debilitating, irreversible long-term effects on the developing brain, including neurocognitive and neuroendocrine dysfunction, psychomotor delays, and hearing loss for many survivors.[3] With this in mind, innovative treatments are greatly needed, and oncolytic immunovirotherapy with genetically engineered herpes simplex virus (HSV-1) has emerged as a promising targeted approach for treating pediatric malignant brain tumors.[4,5]

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Oncolytic HSV-1 (oHSV) G207 has been the most comprehensively investigated virus in human brain tumors.[6–9] G207 has both copies of the ''neurovirulence'' $\gamma_1$34.5 gene deleted. This gene encodes infected cell protein 34.5, which is essential for a productive infection of quiescent normal cells by preventing the normal antiviral response.[10] For additional safety, the U_L39 gene, encoding ICP6, the heavy chain of viral ribonucleotide reductase, has also been inactivated through the insertion of the bacterial gene encoding beta-galactosidase (*LacZ*).[11] Beyond providing additional protection, this enables G207 to be easily identified from wild-type HSV-1. The safety of intracranial inoculation of G207 was demonstrated in both mice and highly HSV-sensitive owl monkeys (*Aotus nancymai*).[12,13] The proven safety and efficacy of G207 in preclinical studies led to three Phase I studies in adults.[11] A Phase I adult study in recurrent glioblastoma (GBM) demonstrated the safety of intratumoral injections of G207 with dosages up to 3×10^9 plaque forming units (PFU) at five sites.[6] In a Phase Ib adult recurrent GBM study, G207 was safely injected in two doses (totaling 1.15×10^9 PFU) pre- and post-tumor resection, including the tumor and normal brain in the adjoining cavity of the tumor resection.[7] A subsequent Phase I recurrent GBM trial confirmed the safety of intratumoral infusion of G207 (1×10^9 PFU) combined with a single 5 Gy dose of focal radiation within 24 h.[8] Radiation results in a DNA repair response that increases virus replication and intratumoral spread and may enhance anti-tumor innate and adaptive immune response.[14–17] A maximum tolerated dose was not reached in all three trials, and radiographic and neuropathological evidence of responses and viral replication were seen in approximately half of the patients.

Based on the safety and efficacy seen in the adult trials, and preclinical evidence indicating that pediatric brain tumors, including the cancer stem cell fraction, are highly sensitive to G207 and significantly more sensitive than adult GBM,[18–20] a pediatric trial (3–18 years old) in recurrent/progressive supratentorial malignant brain tumors was initiated (clinical trials.gov Identifier: NCT02457845).[9] Although most adult brain tumors are supratentorial, pediatric brain tumors are more commonly infratentorial and arise in the cerebellum.[1,2] Morbidity and mortality remain high for children with malignant infratentorial brain tumors, highlighting a need for an infratentorial G207 trial in children.[1–3] Thus, based on the infratentorial propensity of pediatric brains tumors, a recent preclinical study demonstrating heightened efficacy of G207 in targeting pediatric malignant embryonal brain tumors that arise in the cerebellum such as medulloblastoma,[20–22] and promising preliminary data from the pediatric supratentorial trial,[23,24] a G207 protocol to target cerebellar tumors was developed. Accordingly, herein we outline the recently approved and actively recruiting protocol for this Phase 1 open-label single-arm clinical trial, entitled, ''Phase 1 Trial of Engineered HSV G207 in Children with Recurrent or Refractory Cerebellar Brain Tumors'' (ClinicalTrials.gov Identifier: NCT03911388). We highlight the significance of this first-in-human trial and discuss the unique challenges of inoculating the virus through intratumoral catheters in this area of the brain.

STUDY OBJECTIVES

The primary objective is the safety and tolerability of oHSV G207, administered through controlled rate infusion, with/without a 5 Gy dose of radiation in progressive and/or recurrent malignant pediatric cerebellar brain tumors. Secondary objectives include assessment of efficacy and biological response to G207 by examining radiographic response, performance score, quality of life (optional consent), virologic shedding, and changes in HSV-1 antibody titers.

STUDY DESIGN

The University of Alabama at Birmingham (UAB) Institutional Review Board (IRB) has approved this Phase 1 study (IRB-300003130). The trial will initially be performed at a single tertiary care pediatric university hospital, Children's of Alabama, in partnership with UAB. We anticipate enrolling six patients per year, and we will open the study at additional sites as needed. The open-label single-arm trial will employ a 3 + 3 design with four dose-escalation cohorts (Table 1). The initial dose level of 1×10^6 was selected based on previous experience from the pediatric supratentorial study. Up to 24 children 3–18 years old with recurrent/progressive malignant cerebellar tumors will be enrolled in the study for an estimated 24–36 months.

SUBJECT SELECTION AND WITHDRAWAL

Inclusion criteria

Children 3–18 years old with a pathologically proven malignant cerebellar brain tumor that is progressive or recurrent despite standard of care (*e.g.*, surgery, chemotherapy, and/or radiation) will be eligible for the study. Children with secondary malignant cerebellar tumors without curative treatment options are also eligible. Lesions must be between 1.0 and 3.0 cm in diameter and surgically accessible. Larger tumors may be debulked and

Table 1. *Dose-escalation plan*

Dose Level	*Patients*	*Dose (PFU)*
–1	3	1×10^5
1	3 (+3)	1×10^6
2	3 (+3)	1×10^6 + 5 Gy radiation
3	3 (+3)	1×10^7 + 5 Gy radiation
4	3 (+3)	1×10^8 + 5 Gy radiation

PFU, plaque forming units.

treated if ≤3.0 cm after surgery. Patients must have fully recovered from acute treatment-related toxicities from all prior chemotherapy, immunotherapy, and/or radiotherapy before entering the study. They must have normal hematological, renal, and liver function, a performance score of ≥60%, and a life expectancy of ≥8 weeks.

Exclusion criteria

Subjects not on a stable dose of steroids for ≥1 week or on any concurrent therapy with a drug active against HSV are excluded. Likewise, patients with an acute infection, a medical condition preventing surgery, a history of multiple sclerosis, HIV, encephalitis, or CNS infection, or who are pregnant or lactating are excluded. Lastly, tumor involvement that would require brainstem or ventricular injection or access through a ventricle to administer the therapy is likewise excluded.

Subject recruitment and screening

Patients treated locally and at distant sites referred by their primary neuro-oncologist or neurosurgery or their family will be prescreened for potential eligibility. Informed consent (and assent when appropriate) will be obtained on all patients before screening. For those who consent, patients will undergo a complete history and physical examination, neurologic evaluation, and routine preoperative clinical laboratory testing before treatment. They will also have a baseline HSV-1 antibody titer and HSV quantitative polymerase chain reaction (PCR) of saliva, conjunctival secretions, and blood to confirm no evidence of HSV-1 shedding pretreatment. Patients will undergo a study-specific contrast-enhanced magnetic resonance imaging (MRI) scan. Tumor size will be determined using the maximal two-dimensional cross-sectional tumor measurements, transverse × width, using either T1- or T2-weighted images. For patients/families that consent (optional), a neuropsychologist will perform a baseline quality-of-life assessment.

Informed consent

Before undergoing screening for the study and treatment, informed consent and assent (when appropriate) will be obtained in accordance with the U.S. Food and Drug Administration (FDA) and UAB IRB guidelines.

STUDY DRUG

G207 was derived from genetic engineering of the wild-type isolate HSV-1(F); it has both copies of the neurovirulence $\gamma_1$34.5 gene deleted and a *lacZ* gene insertion at the U_L39 gene encoding ICP6, which inactivates ribonucleotide reductase for added protection.[11] These genetic changes make G207 aneurovirulent, replication competent, and selectively oncolytic. After infecting tumor cells, the virus replicates and lyses the tumor cells allowing progeny to infect adjacent tumor cells, augmenting the therapeutic effect throughout the tumor. Importantly, the immunogenic virus promotes danger signals that can increase tumor antigen cross-presentation and stimulate an antitumor immune response. This response may occur even without tumor cell permissivity to the virus and at distant sites of disease not infected with G207.[25–27] Thus, the oncolytic effect of the virus, which is limited to tumor cells, and the secondary antitumor immune response stimulated by the virus provide a dual attack on directly infected and distant uninfected cancer cells.

STUDY PROCEDURES

Under general anesthesia, patients will undergo stereotactic image-guided craniotomy, biopsy, and intraoperative histopathological analysis to document the presence of tumor cells, followed by placement of up to four catheters (PIC-030 Neuro-Infusion Catheter; Sophysa) for G207 infusion as previously described.[23] Catheter placement and treatment with G207 will only proceed if viable recurrent tumor is present on the frozen section. At the discretion of the neurosurgeon, debulking of the tumor may be performed before catheter placement based on the size of the tumor and/or concern for mass effect. After placement, the catheters will be exteriorized and primed with Dulbecco's phosphate-buffered saline (PBS) +10% glycerol (Alanza, Inc.), and the scalp wound(s) will be closed. Patients will recover in the intensive care unit, which is standard of care after any neurosurgical procedure. The following day, the location of each catheter will be confirmed with a CT scan; catheter tips must be ≥0.5 cm from the ventricle and subarachnoid space and ≥1 cm away from the brainstem. Catheters that are malplaced can be adjusted by the neurosurgeon at the bedside when possible; otherwise, malplaced catheters will not be used for G207 infusion.

G207 infusion

G207 will be infused based on the patient's dose level (Table 1) in a total volume of 2.4 mL divided by the number of usable catheters. Each catheter will be separately connected to infusion tubing (PIT-400; Sophysa) after the tubing is first flushed with sterile PBS +10% glycerol (Alanza) and then primed with 0.4 mL of the diluted G207 virus preparation in a syringe. Each syringe will be mounted to a microprocessor-controlled infusion pump. The sterile vehicle from when each catheter was primed during the surgery will be flushed out with an initial infusion rate of 0.4 mL/h for 36 min (0.24 mL). Subsequently, the controlled rate of infusion of G207 will be initiated at a rate based on the number of catheters so that 2.4 mL of total volume is infused for the 6-h infusion (Table 2). This slow infusion is designed to facilitate the spread of G207 throughout the tumor and increase tumor cell killing. At the end of the infusion, the catheters will be

Table 2. *Controlled rate infusion of G207 per hour for a total of 6 h based on the number of catheters placed*

No. of Catheters	*Infusion Rate for Each Catheter*
1	0.4 mL/h
2	0.2 mL/h
3	0.13 mL/h for two catheters 0.14 mL/h for one catheter
4	0.1 mL/h

clamped. To allow any virus at the tip of the catheters to permeate the surrounding tissue and to minimize efflux up the catheter tracts, the catheters will be removed at the bedside by the neurosurgical team at least 1 h after completion of the infusion. Because the catheters are placed centrally within areas of the tumor to maximize coverage, any small amount of efflux as the catheters are removed likely remains within the tumor. After removal of catheters, stable patients may be moved to a general inpatient room for continued monitoring.

Radiation therapy

For dose level 2–4, patients will receive radiation within 24 h of virus infusion. Simulation may occur at the screening visit or on the day of radiation administration. Axial CT images will be collected, and in cases wherein the tumor is debulked, a postoperative contrast-enhanced brain MRI will be utilized for treatment planning. Otherwise, the screening brain MRI will be used for treatment planning, and the study will be registered to the CT scan from the simulation. The planning target volume for 5 Gy will be 2 mm outside areas of T1 gross residual disease, the resection cavity at the primary site, and any site of gross tumor progression including progressive leptomeningeal disease (LMD). For leptomeningeal tumors in the spine, the planning target volume for 5 Gy will be further expanded 3–5 mm. In cases of no prior radiation to the spine, 5 Gy will be prescribed. If prior radiation has been administered at the level, then the radiation dose will be reduced to 3 Gy. The planning target volume for 3 Gy will be 2 mm outside areas of T2 or FLAIR abnormality. However, for tumors that do not have T1 abnormality, high-risk portions of the T2 or FLAIR abnormality will receive 5 Gy.

Follow-up and response assessment

Patients will be followed closely and potential effects from HSV will be monitored. For the patient study schedule, see Table 3. Medical monitors with expertise in diagnosis and treatment of herpes encephalitis will review all patient data and will be available for immediate consultation should any suspicion or concerns for infection arise. HSV antibody titers will be measured pre- and post-treatment to look for evidence of seroconversion. PCR of the saliva, conjunctival secretions, and blood will also be performed to look for virus shedding.

Response assessment will be performed by comparing MRI at baseline with subsequent MRIs starting 28 days after treatment. This period for the initial response assessment was chosen based on evidence of MRI changes from prior G207 studies.[6–8] Two-dimensional measurements obtained at the baseline scan will be compared with follow-up scans for assessment of response as described in the immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria and summarized as complete response, partial response, stable disease, or progressive disease. Owing to the possibility of pseudoprogression, an initial radiographic progression requires verification on follow-up MRIs if it is ≤6 months from receiving G207 and there are not new or deteriorating neurological deficits unrelated to comorbidities or other medications. Furthermore, new lesions alone will not define progression when ≤6 months from receiving G207. During this interval, the patient may be watched or treated with bevacizumab and/or steroids at the discretion of the investigators. If disease progression is confirmed on follow-up MRIs, the date of initial radiographic progression will be considered the date of progressive disease. Patients whose tumor shows radiographic and clinical evidence of progression will be declared a treatment failure and may be considered as candidates for any other available therapy. Nonetheless, patients who have received G207 will continue long-term follow-up even after receiving other cancer therapies.

STATISTICAL PLAN

As a Phase 1 dose-escalation study, data will be presented through descriptive statistics with a focus on G207 and its safety. Because our preclinical data indicate that multiple pediatric brain tumor types are sensitive to G207 and this is a safety and tolerability study,[20] various tumor types will be assessed in this trial and the analyses will be focused as a main effects model considering varied tumor types and within tumor type. Continuous variables associated with adverse events/toxicities, progression-free survival, and overall survival will be presented as mean, median, mode, and standard deviation; categorical variables will be presented as percentages and/or counts. Univariate and multivariate analyses will be performed as deemed appropriate for analysis of our endpoints.

SAFETY AND ADVERSE EVENTS

Definitions

Toxicity is defined by the National Cancer Institute Common Terminology Criteria for Adverse Events 5.0 (NCI CTCAE v.5.0). A dose limiting toxicity (DLT) is defined as any grade 3 or 4 toxicity that is considered as being possibly, probably, or likely related to G207 within 30 days of G207 inoculation with the following excep-

Table 3. *Patient study schedule*

	Pre	*Day −1*	*Day 0*	*Day 1*	*Day 2*	*Day 3/4*	*Day 7*	*Day 14*	*Day 28*	*Month 3*	*Month 5*	*Month 7*	*Month 9*	*Months 12, 18, and 24*[a,b]
History and physical	X						X	X	X	X	X	X	X	X
Vital signs	X		X[c]	X	X	X	X	X	X	X	X	X	X	X
Neurological status on examination	X		X[c]	X	X	X	X	X	X	X	X	X	X	X
CMP, Mg and phosphorous	X				X		X		X					
CBC with lymphocyte markers	X				X		X	X	X	X	X	X	X	X
Cystatin C	X[d]													
Urinalysis	X													
PT/PTT	X													
HIV serology	X													
HSV antibody titer	X								X	X	X	X	X	X
HSV detection (PCR on saliva and conjunctiva, and blood)[e]	X			X		X	X	X	X	X	X	X	X	X
Serum pregnancy-adolescents	X													
CT scan	X[f]		X											
Biopsy/placement of catheter(s)		X												
Dosing			X											
Radiation				X[g]										
MRI	X[h]	X[i]				X			X[j]	X[j]	X[j]	X[j]	X[j]	X[j]
Performance score	X			X	X	X	X	X	X	X	X	X	X	X
Quality of life evaluation[k]	X								X	X	X			X
Blood sample[k]	X				X		X	X	X	X	X	X	X	X

[a]After the 24-month visit, studies will be performed yearly thereafter, as patient's health permits.
[b]All subjects will continue to receive yearly follow-up by clinic visits (or telephone call for those who cannot be seen in person) for assessment of adverse events/serious adverse events that might be related to G207 for up to 15 years after treatment with G207.
[c]Frequency postoperatively defined by institutional standards and medical need.
[d]Only for patients with elevated creatinine level on complete metabolic panel.
[e]Additional samples for shedding analysis should be taken when clinical signs warrant.
[f]Only for patients who receive radiation therapy. Patients may get simulation CT scan at screening or day 1.
[g]Only for patients in dose levels 2, 3, and 4.
[h]Must be within 14 days of treatment. A complete spine MRI is recommended to evaluate for disease for radiation planning.
[i]For patients who have tumor debulking, they will have a postoperative MRI to evaluate tumor after debulking and to be used for radiation planning.
[j]For patients with known leptomeningeal and/or spinal disease, a standard-of-care spinal MRI is recommended to evaluate disease.
[k]Optional consent. Quality of life evaluation will be performed at screening, 1, 3, 5, and 12 months.
CBC, complete blood count; CMP, complete metabolic panel; Mg, magnesium; PCR, polymerase chain reaction.

tions: (1) neurological deterioration will not be considered a DLT if it returns to baseline within 2 weeks of receiving G207. For example, increased weakness or aphasia that occurs during the infusion will be treated with increasing steroid doses and/or slowing of infusion rates, but not treated as a DLT; (2) any grade 3 or 4 neurological toxicity that improves with supportive care agents (bevacizumab and/or steroids) or that is determined to be tumor progression by the treating physician. Owing to the possibility of pseudoprogression, a well-known occurrence in oncolytic virotherapy and with the treatment of malignant brain tumors in adults and children, neurological changes may be treated with supportive care agents, bevacizumab and/or steroids[28–31], and (3) any grade 4 seizure that improves within 48 h of initiation of supportive care measures.

If one patient has a DLT at the starting dose, up to three additional patients will be treated at that dose. If two or more patients have toxicity at the starting dose, a cohort will be added at 1×10^5. If no significant (grade 3 or 4) toxicities are encountered within 28 days of completing this starting dose treatment cohort, a subsequent dose level of 1×10^6 PFU with a single 5 Gy of radiation directed to the tumor within 24 h of G207 inoculation will be used. If no DLTs are seen at either dose level, then the subsequent dose levels of 1×10^7 PFU plus 5 Gy of radiation and 1×10^8 PFU plus 5 Gy of radiation will be used. If there are no DLTs in the highest cohort, up to three additional patients may be added to ensure safety at that dose. If G207 plus radiation causes toxicity, the escalation dose scheme for G207 may continue without radiation. The maximally tolerated dose will be defined as the dose immediately below any dose that causes grade 3 or 4 toxicities in two patients. There will be a minimum 28-day observation period between the first and second patient of each cohort, a 7-day observation period between patients 2 and 3 enrolled in a cohort, and a 28-day observation period from one cohort to the next to allow for evaluation of potential toxicity.

Adverse events and management

Focal neurologic deficits will be investigated with imaging and managed appropriately. Cerebral edema is common postoperatively with oHSV administration and is managed with standard measures such as dexametha-

sone, whereas more significant symptomatic cases will undergo consideration for further tumor resection and/or craniectomy. In case of cerebral edema and the need for bevacizumab, it may be given ≥14 days after initial surgery for biopsy and catheter placement to allow adequate time for healing of the surgical incision. Bevacizumab will be given every 2 weeks at a dose of 5–10 mg/kg intravenously. Dosage adjustments or cessation of drug may be made should adverse events related to bevacizumab occur (uncontrolled hypertension, proteinuria, bleeding, thrombosis, etc.) and will be made by the prescribing neuro-oncologist. Previous tumor progression on bevacizumab does not preclude its use herein. An increase in steroid dose will be kept to a minimum as patient neurologic condition permits.

Small hematomas may undergo serial observation with imaging, yet large hematomas will undergo consideration for evacuation. In case of seizure, it is permitted to use antiepileptic medicines for control of partial or generalized seizures. Concern for encephalitis due to fever or altered mental status will be managed with imaging, and if it confirms hemorrhagic necrosis beyond the postoperative cavity, a needle core biopsy and cerebrospinal fluid sample will be obtained to evaluate for G207/HSV-1 virus. Intravenous acyclovir will be given while the pediatric neuropathologist evaluates the histology. The medical monitor will be consulted immediately for any suspected cases of encephalitis and be integrally involved in evaluation and treatment decisions. These will be reported per NCI CTCAE 5.0.

Reporting of serious adverse events or unanticipated problems

Any serious and unexpected suspected adverse reaction that occurs during the conduct of this study, regardless of relationship to test article or procedures, will be reported within 24 h to the external Data and Safety Monitoring Board (DSMB), medical monitors, and UAB Comprehensive Cancer Center Clinical Trials Monitoring Committee (CTMC), and a written report filed within 7 days. The study will be paused for the following events: death (except due to motor vehicle accident or clear progressive disease), two instances of grade 4 toxicity, disseminated HSV infection, or any severe life-threatening neurologic complications including uncontrolled seizures of 48 h, HSV encephalitis, or mental status changes requiring intubation. The pause will be in effect to allow assessment by the DSMB, medical monitors, principal investigator, UAB CTMC, and the FDA to review the events to decide whether the study should be terminated or can be resumed.

Risk/benefit assessment

The risk/benefit assessment is Children's Risk Level #2: research involving greater than minimal risk but presenting the prospect of direct benefit to the individual child subjects involved in the research.

DISCUSSION

This Phase 1 trial represents the first oncolytic immunovirotherapy study (in any age patient) that delivers an oncolytic virus by controlled rate infusion through catheters into the cerebellum and the first ever infratentorial oHSV virotherapy trial. G207 has been utilized in four Phase 1 supratentorial brain tumor trials (three adult and one pediatric) with an excellent safety profile and evidence of radiographic, neuropathological, and/or clinical responses in many patients.[6–9,23, 24,32] The three adult G207 trials targeted malignant gliomas arising in the cerebrum where >90% of adult gliomas occur.[33] These studies excluded a small number of patients with tumors that would require basal ganglia, brainstem, posterior fossa, or ventricular inoculation of G207 to minimize potential risks. As the first oHSV brain tumor trial in children, the pediatric G207 supratentorial trial (NCT02457845) used similar exclusion criteria although any malignant tumor histology was eligible because the primary objective of the study was safety and tolerability; in addition, preclinical data indicate that a diverse group of malignant pediatric brain tumors is sensitive to G207, and pediatric supratentorial malignant tumors are rare tumors.[1,9,20]

Unlike adult brain tumors, the majority of pediatric brain tumors are infratentorial with the cerebellum being the most common location.[34] Several highly aggressive malignant brain tumors may originate in or proximate to the cerebellum including medulloblastoma, the most common malignant brain tumor of childhood, ependymoma, high-grade glioma, and atypical teratoid/rhabdoid tumors. These tumors result in high morbidity and mortality even with aggressive treatments including surgery, radiation, and/or chemotherapy, which result in long-term disabilities in survivors. Furthermore, LMD and metastases occur in up to 30–50% of newly diagnosed and recurrent malignant cerebellar tumors.[35–37] Outcomes are poor for patients with LMD, which is typically minimally responsive to conventional therapies. Thus, there is a great need to develop novel targeted immunotherapies like G207 to treat aggressive malignant pediatric cerebellar tumors.

Whether G207 alone or combined with radiation can safely and effectively target malignant cerebellar tumors and disseminated, LMD is unknown; this Phase 1 clinical trial will begin to resolve these important questions. Preclinical studies support the safety of injecting G207 in the cerebellum; in the immunocompetent HSV-1–sensitive mouse strain CBA/J, G207 was systemically nontoxic with normal brain and organ pathology after the virus was inoculated into the normal cerebellum.[22] Safety will be the highest priority as cerebellar tumors provide unique challenges for oHSV delivery and treatment: (1) frameless stereotactic placement of intratumoral catheters in the cerebellum is technically more difficult than in supratentorial locations, (2) cerebellar tumors are often in

proximity to the ventricle that requires precise placement of the catheters to avoid direct ventricular inoculation, and (3) the posterior fossa is a smaller area in proximity to the brainstem and, therefore, patients are more likely to become symptomatic if there is swelling or hemorrhage in the area. Thus, the starting dose in this cerebellum trial is a log lower than the starting dose in the supratentorial study. Additional safety measures were added to the inclusion/exclusion criteria of the cerebellum study; the maximum tumor size allowed is 3.0 cm and the neurosurgeon has discretion to debulk a tumor before catheter placement based on the size of the tumor or concern for potential mass effect.

In addition to demonstrating safety of the approach, preclinical studies indicate that G207 can effectively target malignant cerebellar tumors. G207 increased survival in mice bearing a highly aggressive *MYC*-overexpressed group 3 murine medulloblastoma.[22] Studies in patient-derived xenograft models indicate that pediatric embryonal tumors such as medulloblastoma are highly sensitive to G207 and appear to be even more sensitive than pediatric glial tumors.[20,21] In addition, preclinical studies using G207 and $\gamma_1$34.5-deleted oHSV HSV1716 in immunocompetent murine tumor models demonstrated that in addition to local cytotoxic viral replication in tumor cells, the viruses induce systemic antitumor immune responses in uninoculated tumors and metastases.[27,38] Similarly, responses were seen in uninjected sites of disease in melanoma patients receiving another $\gamma_1$34.5-deleted oHSV talimogene laherparepvec (T-VEC), which is the first oncolytic virotherapy agent that is FDA approved for treating melanoma.[39,40] These studies indicate that G207 may be able to target malignant cerebellar tumors and induce antitumor immune responses in areas distant from G207 inoculation such as spinal metastases and LMD.

Lastly, combining low-dose radiation with G207 therapy may provide additional synergy to target pediatric malignant cerebellar tumors, including metastatic and LMD. When combined with oHSV, radiation has been shown to increase oHSV viral replication and intratumoral spread, reduce tumor volumes, and prolong survival in mice bearing intracranial high-grade glioma.[14–16] Furthermore, radiation may enhance the antitumor innate and adaptive immune response to target local and distant disease.[17] Radiation releases tumor antigens leading to increased tumor antigen presentation and induces chemokines that recruit effector T cells, thereby priming tumor-specific cytotoxic T cells and enhancing T cell homing and function in tumors.[17] Together, these preclinical and clinical studies provide significant rationale for the proposed Phase 1 clinical trial and suggest that G207 can safely target cerebellar malignancies and has potential to generate an antitumor immune response at all sites of disease. The knowledge gained from this study will be invaluable in our efforts to synthesize more effective and less toxic therapeutic strategies for children with high-grade brain tumors.

DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official views of the U.S. FDA or the National Institutes of Health.

AUTHOR DISCLOSURE

Treovir, LLC, has licensed G207 and all of the surrounding intellectual property from Aettis, Inc. Drs. Markert, Gillespie, and Whitley are cofounders and each owns ~25% of the corporation. Dr. Gillespie became the chief scientific officer effective July 1, 2020. Dr. Bernstock has been granted equity interests in Treovir, LLC, in compensation for his role as a key advisor and consultant. Drs. Gillespie and Whitley are founders of and own stock and stock options (<10%) in Maji Therapeutics, which is developing other HSVs that are not the subject of the current investigation. Drs. Markert, Gillespie, and Whitley were also founders of and owned stock and stock options (<8%) in Catherex Inc., a biotechnology company that had licensed additional intellectual property related to oHSV. Catherex, Inc., was sold to Amgen, Inc., on December 18, 2015, and they no longer participate in any decision making or have any control of any aspect of Catherex or Amgen, although they did receive proceeds from the sale of the company. Drs. Markert, Gillespie, and Whitley hold equity in Aettis, Inc., a company that holds oncolytic HSVs in a storage facility. Dr. Gillespie has served as a paid advisor to the program project at the Ohio State University that seeks to find improved methods for application of distinct oHSV to treat localized and metastatic cancers. This is generally, but not specifically, related to the subject matter of this investigation. All of these potential conflicts of interest have been reported to and are being managed by the UAB Office of Conflicts of Interest. Dr. Bernstock has positions/equity in CITC Ltd. and Avidea Technologies and is member of the POCKiT Diagnostics Board of Scientific Advisors.

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