

Rationale and Design of a Phase 1 Clinical Trial to Evaluate HSV G207 Alone or with a Single Radiation Dose in Children with Progressive or Recurrent Malignant Supratentorial Brain Tumors

Alicia M. Waters,¹ James M. Johnston,² Alyssa T. Reddy,³ John Fiveash,⁴ Avi Madan-Swain,³ Kara Kachurak,⁵ Asim K. Bag,⁶ G. Yancey Gillespie,² James M. Markert,^{2,3} and Gregory K. Friedman^{3,*}

Departments of ¹Surgery, Division of Pediatric Surgery, ²Neurosurgery, ³Pediatrics, Division of Hematology/Oncology, ⁴Radiation Oncology, and ⁶Radiology, University of Alabama at Birmingham, Birmingham, Alabama; ⁵Division of Hematology/Oncology, Children's of Alabama, Birmingham, Alabama.

Primary central nervous system tumors are the most common solid neoplasm of childhood and the leading cause of cancer-related death in pediatric patients. Survival rates for children with malignant supratentorial brain tumors are poor despite aggressive treatment with combinations of surgery, radiation, and chemotherapy, and survivors often suffer from damaging lifelong sequelae from current therapies. Novel innovative treatments are greatly needed. One promising new approach is the use of a genetically engineered, conditionally replicating herpes simplex virus (HSV) that has shown tumor-specific tropism and potential efficacy in the treatment of malignant brain tumors. G207 is a genetically engineered HSV-1 lacking genes essential for replication in normal brain cells. Safety has been established in preclinical investigations involving intracranial inoculation in the highly HSV-sensitive owl monkey (*Aotus nancymai*), and in three adult phase 1 trials in recurrent/progressive high-grade gliomas. No dose-limiting toxicities were seen in the adult studies and a maximum tolerated dose was not reached. Approximately half of the 35 treated adults had radiographic or neuropathologic evidence of response at a minimum of one time point. Preclinical studies in pediatric brain tumor models indicate that a variety of pediatric tumor types are highly sensitive to killing by G207. This clinical protocol outlines a first in human children study of intratumoral inoculation of an oncolytic virus via catheters placed directly into recurrent or progressive supratentorial malignant tumors.

Keywords: HSV, oncolytic, virotherapy, pediatric, brain tumors, G207

INTRODUCTION

Malignant childhood brain tumors

CENTRAL NERVOUS SYSTEM TUMORS are the most common solid neoplasm of childhood accounting for approximately 25% of all childhood malignancies and the leading cause of cancer-related morbidity and mortality.^{1,2} While outcomes for children with low-grade, localized tumors are excellent, there remains a significant subset of patients with high-grade malignancies that have very poor outcomes despite conventional therapies including surgery, chemotherapy and radiation. For patients with high-grade recurrent tumors, traditional treatment usually only provides a brief interval of disease control.^{3,4}

Furthermore, patients who survive their disease after traditional therapy often have damaging lifelong sequelae such as hormone dysfunction, neurosensory impairment, and neurocognitive changes that are attributed to the treatment.⁵⁻⁷ Novel innovative treatments that improve outcomes and lessen harmful side effects are urgently needed.

Neoplastic therapy using genetically engineered herpes simplex virus

Brain tumors are suitable targets for intervention using conditionally replicating viruses that replicate and kill tumor cells while sparing the postmitotic, nondividing cell population of the brain.

*Correspondence: Dr. Gregory K. Friedman, Department of Pediatrics, Division of Hematology/Oncology, University of Alabama at Birmingham, 1600 7th Avenue South, Lowder 512, Birmingham, AL 35233. E-mail: gfriedman@peds.uab.edu

Attenuated herpes simplex virus (HSV) has a number of potential advantages as a neoplastic therapy for pediatric brain tumors. HSV-1 has been extensively studied; it is a large, enveloped, double-stranded DNA virus with a genome of approximately 152 kb, encoding at least 80 genes.⁸ The genome is divided into long and short unique regions, each flanked by characteristic repeated sequences. Essential and nonessential genes for HSV-1 replication in the brain and in brain tumors have been identified, making it possible to genetically engineer a virus capable of selective replication in pediatric brain tumor cells.^{9–12} HSV is a neurotropic virus, making nervous system tumors ideal targets for the virus. The attenuated virus remains immunogenic and promotes danger signals that can reverse tumor immune evasion, increase cross-presentation of tumor antigens, and promote an antitumor immune response.¹³ This antitumor immune response may occur even in the absence of virus permissivity.¹⁴ Lastly, because these viral constructs retain the thymidine kinase gene, they are highly susceptible to readily available, effective antiviral agents in the unlikely event that a mutant virus produces toxicity in normal brain tissue.

Engineered HSV-1 G207 (Fig. 1) contains deletions in both loci of the γ_1 34.5 gene and a disabling *lacZ* insertion in U_L39 , that encodes the large subunit of viral ribonucleotide reductase.¹² The γ_1 34.5 “neurovirulence” gene encodes a critical multifunctional protein (ICP34.5) that enables the virus to replicate in normal cells by blocking host cell shutoff of protein synthesis, inhibiting the induction of antiviral host cell genes, and preventing autophagy that can limit virus replication.^{15,16} In the absence of this protein, the virus fails to induce HSV encephalitis, even when injected in high amounts directly into the central nervous system (CNS) of susceptible animal models.^{17,18} Additionally, a portion of the latency associated transcripts is encoded on the DNA strand opposite the γ_1 34.5 genes. Thus, the capacity to express genes necessary for the virus to establish and escape latency is lost when the γ_1 34.5 gene is altered.¹⁹ The *lacZ* insertion provides added protection for normal cells because ribonucleotide reductase is important for nucleotide synthesis needed for viral replication.^{20,21} In the postmitotic brain, dividing tumor cells provide cellular ribonucleotide reductase, which is utilized by the mutant virus to facilitate replication in cancer cells.

Preclinical and clinical data to support the safety and efficacy of the proposed study

G207 has been inoculated intracerebrally without deleterious side effects in BALB/c mice and in

Aotus nancymai owl monkeys at single doses up to 10^9 plaque-forming units (pfu).^{17,18} Owl monkeys are exquisitely sensitive to wild-type HSV-1, similar to human neonates, with 10^3 pfu of wild-type HSV-1(F) causing death.¹⁸ Magnetic resonance imaging (MRI) analysis of G207-treated nonhuman primates showed no CNS abnormalities, and neuropathologic evaluation demonstrated local inflammation at the site of injection, but no other brain abnormalities. Histopathological evaluation of multiple organs revealed no evidence of altered cellular architecture or HSV immunoreactive cells.

A phase 1 clinical trial of G207 in adults with high-grade glioma was conducted at the University of Alabama at Birmingham (UAB) and Georgetown University, followed by two additional phase 1 studies conducted at UAB.^{22–24} Twenty-one adult patients were treated in seven cohorts of three each by stereotactic intratumoral injection of HSV G207 in a standard dose escalation fashion from 1×10^6 pfu as a single injection in 0.1–0.3 mL up to 3×10^9 pfu given at 0.2 mL each in five sites.²² While some patients developed complications frequently seen with malignant glioma, no serious adverse events were ascribed to G207, and therefore a maximum tolerated dose was not reached. Eight of twenty MRI-evaluable patients had a decrease in their enhancement volume at 1 month postinoculation. Two patients had an extended disease-free survival of >5.5 years, and another patient who died of an unrelated stroke 9 months after G207 treatment had no evidence of recurrent disease at autopsy.^{22,25}

In a phase 1b study, six patients with recurrent glioblastoma multiforme received two doses of G207 totaling 1.15×10^9 pfu, with 13% of this total dose injected prior to tumor resection via a catheter placed stereotactically into enhancing portion of the tumor.²³ Two to five days later, the tumor was resected *en bloc* with the catheter in place, and the remainder of G207 dose was injected into the brain surrounding the resection cavity. Radiographic and neuropathologic evidence suggestive of viral replication and antitumor activity was seen in three patients. Furthermore, increases in tumor-infiltrating T lymphocytes and macrophages were seen in three of six and five of six patients respectively. This study suggested that multiple administrations of G207 was safe and that injection into brain bordering the tumor bed was safe.

A third phase 1 study examined the safety of stereotactic intratumoral administration of G207 at 1×10^9 pfu followed within 24 h with a single 5 Gy radiation dose in nine adults with recurrent high-grade gliomas.²⁴ Preclinical studies demonstrated

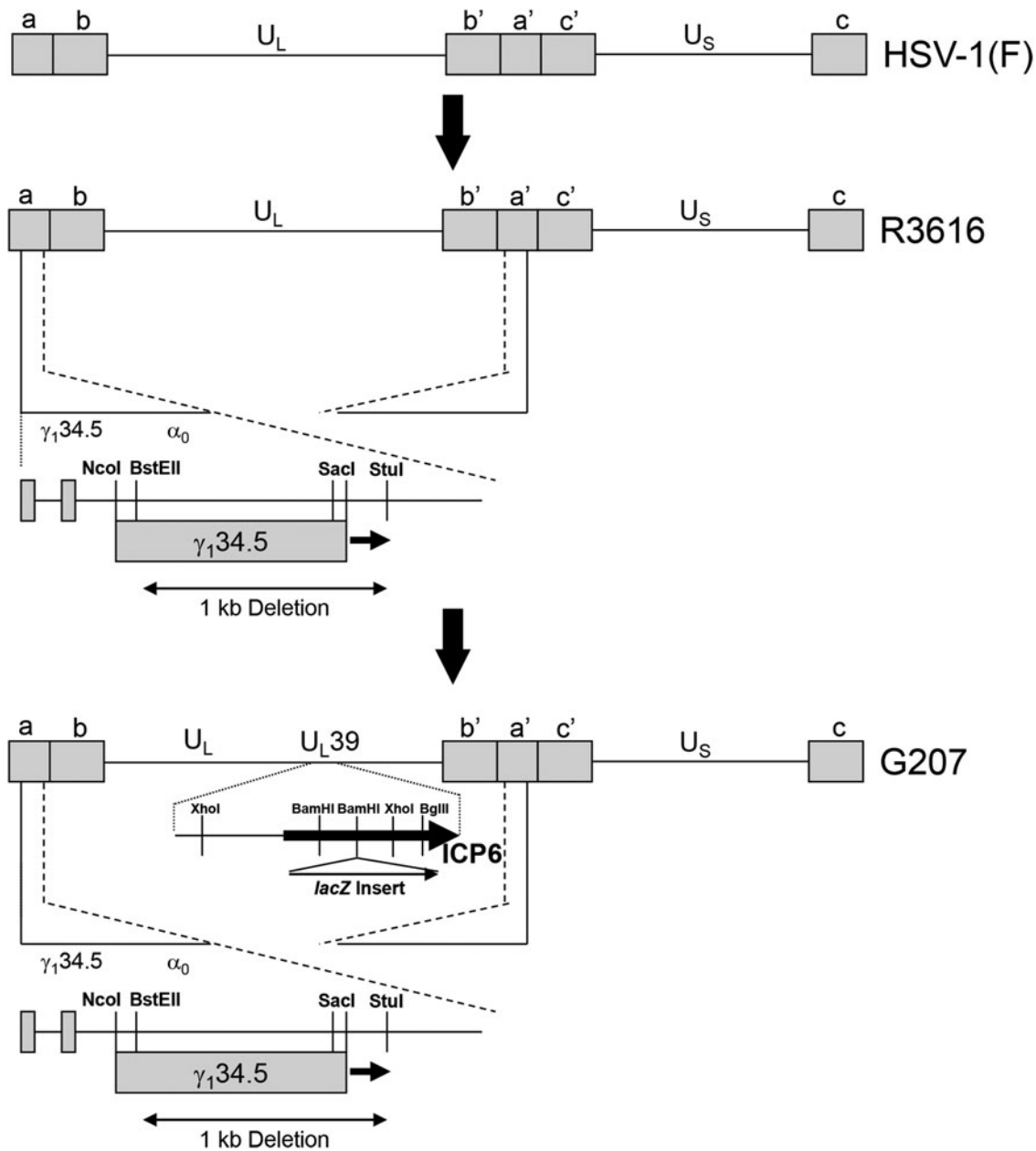


Figure 1. Schematic representation of the derivation of G207 HSV. Chou *et al.* deleted both copies of the $\gamma_134.5$ gene from herpes simplex virus strain F (HSV-1(F)), a temperature-sensitive clinical isolate, to create recombinant virus R3616.⁹ Only one of the two deletions is shown for simplicity. This deletion ablated the ability of the virus to overcome interferon resistance in normal cells and made the virus aneurovirulent. Mineta *et al.* modified R3616 by inserting the *Escherichia coli* β -galactosidase gene (*lacZ*) to produce a functional deletion of HSV U_L39, which encodes the heavy chain or ribonucleotide reductase (infected cell protein 6 [ICP6]), as described by Goldstein and Weller.^{12, 20} This second disabling deletion was performed to ensure added safety for intracerebral human trials. (Copyright [2009] The American Society of Gene Therapy. Reproduced with permission from *Molecular Therapy* [Friedman *et al.*³⁸]).

in vivo synergism of engineered HSV-1 and radiation through several potential mechanisms.^{26–30} Ionizing radiation increases transcription of cellular genes that complement viral gene deletions such as ribonucleotide reductase and potentially activates late HSV-1 promoters through activation of the p38 mitogen-activated protein kinase pathway thereby enhancing virus replication.^{28,29} Also, increased cellular activity due to the DNA damage response after radiation may improve viral replication.

Lastly, radiation may synergize with oncolytic HSV-1 by facilitating enhancement of antitumor innate and adaptive immune responses. In the phase 1 trial, treatment was well tolerated and no dose limiting toxicities occurred. Six of nine patients had a stable or partial response for at least one time point, and three had marked radiographic responses to treatment (see Fig. 2 for example of response). Two patients had an initial response, then demonstrated recurrences and were able to be

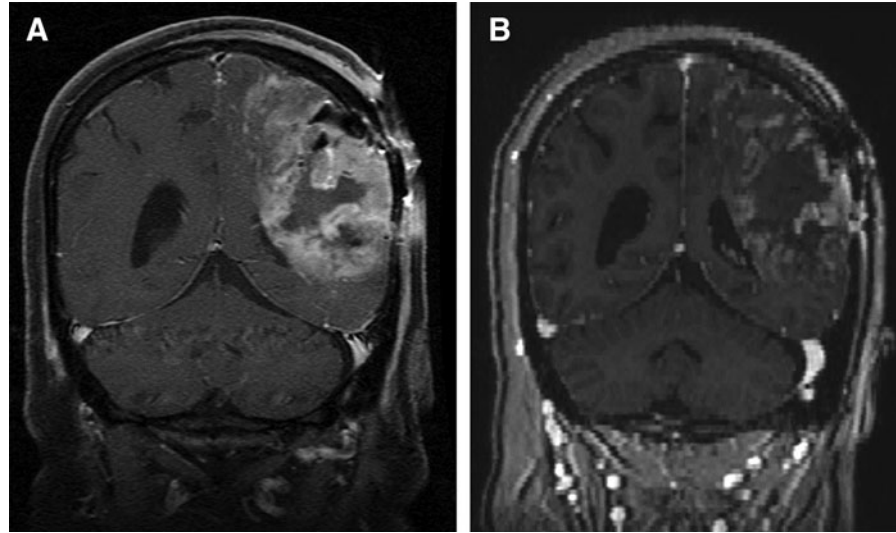


Figure 2. Response to G207 in an adult with recurrent glioblastoma multiforme. T1-weighted gadolinium-enhanced coronal magnetic resonance imaging images. **(A)** Pre-inoculation of G207. **(B)** One month post-G207 inoculation demonstrating clear response to G207.

retreated under patient-specific protocols; both patients exhibited antitumor effects in response to the second dose of virus as well. Taken together, approximately half of the 35 adults treated in the phase 1 adult high-grade glioma G207 trials had radiographic or neuropathologic evidence of response at a minimum of one time point.

Preclinical evidence supports advancing G207 to clinical testing in children with malignant brain tumors; preclinical studies demonstrated that a variety of pediatric tumor types, including neural and glial tumors, express the primary HSV entry receptor nectin-1 (CD111), an adhesion molecule, and are highly sensitive to engineered HSV both *in vitro* and *in vivo*.^{31–34} Comparing the sensitivity of eight pediatric patient-derived high-grade brain tumor xenografts (4 medulloblastoma, 2 glioblastoma, 1 supratentorial primitive neuroectodermal tumor [PNET], 1 anaplastic ependymoma) with six adult glioblastoma xenografts to G207, Ring *et al.* determined that on average, pediatric tumor cells expressed CD111 in significantly greater amounts and were 22-fold more sensitive than the adult tumors *in vitro*, requiring a substantially lower dose to kill 50% of the cells in 72 h.³⁵ These data suggest that malignant pediatric brain tumors may be more sensitive to G207 than adult glioblastoma, and pediatric patients may be the ideal candidates for this therapy. Importantly, CD133+ glioma and medulloblastoma cancer stem cells—which play a significant role in tumor aggressiveness, chemotherapy resistance, radioresistance, recurrence, and metastasis—were highly sensitive to G207 and showed no inherent resistance to oncolytic HSV-1 compared

with CD133- tumor cells.^{32,33,36} Furthermore, three pediatric patient-derived xenograft models of molecular group 3 medulloblastoma, which is the most difficult to treat subgroup of medulloblastoma and portends a poor prognosis, were very sensitive to killing by G207 *in vitro*, and G207 significantly prolonged survival in athymic nude mice bearing intracranial tumors compared with saline.³³

The safety and efficacy established in the three phase 1 adult studies and the promising preclinical data in pediatric patient-derived xenograft models form the bases to extend HSV G207 alone or combined with a single dose of radiation to the pediatric population. Certainly, pediatric patients have the potential to respond differently than adults to G207. They are more likely to be seronegative for HSV at the time of injection and therefore will require close monitoring for seroconversion and/or any evidence of HSV-related sequelae.

STUDY OBJECTIVES

The primary objective of this study is to assess the safety and tolerability of G207 administered intratumorally via stereotactic infusion alone or followed by a single dose of radiation within 24 h of G207 administration in children three years of age or older with recurrent or progressive malignant supratentorial brain tumors. Safety and tolerability will be assessed by adverse events and laboratory tests.

The secondary objectives are to obtain preliminary information concerning the potential efficacy of and biological response to G207 alone or combined with a single dose of radiation in pediatric

patients with recurrent or progressive malignant brain tumors. This will be assessed by radiographic response, patient performance scale, progression-free and overall survival, measuring antiviral immune response and presence of G207 in blood, saliva, and conjunctiva. Additionally, the impact of this new therapy on patients' quality of life and caregiver stress will also be assessed.

STUDY DESIGN

This study is a phase 1, open-label clinical trial of HSV G207 to assess its safety and tolerability in pediatric patients (3–18 years old) with recurrent or progressive supratentorial malignant brain tumors who have failed standard of care therapy: surgery, radiotherapy, and who may or may not have had chemotherapy or another form of treatment. A traditional 3+3 design is used (Table 1). The initial dose is 1×10^7 pfu of G207 in a volume of 2.4 mL for the first three patients (dose level 1). If two or more patients have toxicity at the starting dose level, a cohort will be added at 1×10^6 pfu (dose level –1). If two or more patients have toxicity at this dose, the study will be terminated. Three additional patients may be added at any dose level if toxicity in one patient is observed. If toxicity in a second patient is observed at any dose level, no further patients will receive that dose level, and the dose immediately below will be declared the maximum tolerated dose. There will be a minimum 28-day observation period between the first and second patient of each cohort, a 7-day observation period between patient 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.

Any grade 3 or 4 nonhematologic toxicity, as defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0, that is possibly, probably, or likely related to G207 will be considered a dose-limiting toxicity. Based on previous clinical trial experience with G207, worsening neurologic deficits will not be considered dose-limiting if it resolves back to the patient's baseline within one week of completing treatment. 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 dose-limiting toxicity if symptoms improve to baseline within 7 days of completing treatment. Patients will be followed closely for potential HSV toxicity. 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 preoperatively and at 1, 3, 5, 7, 9, and 12 months (see Table 2 for study schedule). PCR and cultures will also be performed at the same time points to check for HSV in saliva, conjunctival secretions, and blood. MRI will be performed on day 3 or 4 after treatment and at months 1, 3, 5, 7, 9, and 12. Because the virus is immunogenic and may stimulate an immune cell-related inflammatory response in the tumor and cause pseudoprogression, which has been seen in engineered HSV-1 studies in adults, the Immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria will be used for assessing tumor response.³⁷ The appearance of enlarging or new lesions 6 months or less from the initiation of an immunotherapy alone does not define progressive disease. Confirmation of disease progression on follow-up imaging after initial radiographic progression will be determined if there is no new or substantially worsened neurological deficits that are not due to comorbid events or concurrent medication, and it is 6 months or less from receiving G207. If follow-up imaging confirms disease progression, the date of actual progression will be back-dated to the date of initial radiographic progression. 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.

Dosing will start at an initial dose level of 1×10^7 pfu of G207 and will then escalate by a one log increment to a dose of 1×10^8 pfu. The rationale to start at 1×10^7 pfu, which is higher than the initial starting dose of G207 in adults but lower than the highest safe dose used (3×10^9), was based on our primary concern for safety. Children may respond differently to the virus than adults, both in terms of the oncolytic effect, based on our preclinical data suggesting that pediatric tumors may be more sensitive than adult tumors, and the immune response generated against the virus and/or tumor.³⁶ Furthermore, responses to G207 in adults were not dose dependent, with re-

Table 1. Dose escalation

Dose Level	Patients	Dose (pfu)	Volume	No. of Loci
–1	3	1×10^6	2.4 mL	1–4
1	3 (+3)	1×10^7	2.4 mL	1–4
2	3 (+3)	1×10^8	2.4 mL	1–4
3	3 (+3)	1×10^7 + 5 Gy radiation	2.4 mL	1–4
4	3 (+3)	1×10^8 + 5 Gy radiation	2.4 mL	1–4

Dose level –1 will be utilized only if needed due to toxicity at dose level 1. pfu, plaque-forming units.

Table 2. Patient study schedule

	Pre	Day 0	Day 1	Day 2	Day 3 or 4	Day 7	Day 14	Day 28	Month 3	Month 5	Month 7	Month 9	Month 12 [‡]
History and physical	X					X		X	X	X	X	X	X
Complete metabolic panel, magnesium and phosphorous	X			X				X					
Complete blood count with differential and platelets including lymphocyte markers	X			X		X	X	X					
Cystatin C	X												
HIV serology	X												
HSV antibody titer	X							X	X	X	X	X	X
HSV detection (Saliva, conjunctival secretions, and blood)**	X		X		X	X	X	X	X	X	X	X	X
Serum pregnancy	X												
Head CT scan		X	X [#]										
G207 administration		X											
MRI	X*				X			X	X	X	X	X	X [†]
UA	X												
PT/PTT	X												
Neurological status on exam	X	X [§]	X	X	X	X	X	X	X	X	X	X	X
Performance score	X		X	X	X	X	X	X	X	X	X	X	X
Quality of life evaluation***	X							X	X	X			X
Vital signs	X	X [§]	X	X	X	X	X	X	X	X	X	X	X

* Must be within 14 days of scheduled treatment

** Additional samples for shedding analysis should be taken when clinical signs warrant (i.e. if patients show signs of herpes virus infection due to reactivation)

*** Optional consent

Only for patients who receive radiation therapy

§ Frequency post-operatively defined by institutional standards and medical need

† Will also be done at 18 and 24 months, and yearly thereafter, as patient's health permits

‡ 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 for up to 15 years after treatment with G207. Particular attention will be paid to any symptoms (neurological or otherwise) consistent with HSV infection

CT, computed tomography; HSV, herpes simplex virus; MRI, magnetic resonance imaging; PT/PTT, Prothrombin Time/Partial Thromboplastin Time; UA, urinalysis.

sponses seen at this starting dose and below this dose (1×10^6). If there are no unacceptable (dose-limiting) toxicities in the initial cohorts, the dose levels will be repeated in combination with a single 5 Gy dose of radiation. The decision to repeat the same two doses of G207 with a single dose of radiation was based on the previously discussed preclinical and clinical rationale to combine radiation with G207 and to monitor closely for any additional toxicity caused by the combination therapy. Subjects will be enrolled sequentially. Treatment will be escalated as outlined in Table 1.

SUBJECT SELECTION AND WITHDRAWAL

Inclusion criteria

All study subjects must meet the following inclusion criteria to be included in the study.

1. Age ≥ 36 months and < 19 years
2. Pathologically proven malignant supratentorial brain tumor (including glioblastoma multiforme, giant cell glioblastoma, anaplastic astrocytoma, PNET, atypical teratoid/rhabdoid tumor, germ cell tumor, or other high-grade malignant tumor) which is progressive or recurrent despite

standard of care treatment including surgery, radiotherapy, and/or chemotherapy and have a life expectancy of ≥ 8 weeks

3. Lesion must be ≥ 1.0 cm in diameter and surgically accessible as determined by MRI
4. Patients must have fully recovered from acute treatment related toxicities of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study
5. Myelosuppressive chemotherapy: patients must have received their last dose at least 3 weeks prior (or at least 6 weeks if nitrosurea)
6. Investigational/biologic agents: patients must have recovered from any acute toxicities potentially related to the agent and received last dose ≥ 7 days prior to entering this study (this period must be extended beyond the time during which adverse events are known to occur for agents with known adverse events ≥ 7 days)
7. Monoclonal antibodies: At least three half-lives must have elapsed prior to study entry
8. Radiation: Patients must have received their last fraction of craniospinal radiation (> 24 Gy) or total body irradiation ≥ 3 months prior to

study entry. Patients must have received focal radiation to symptomatic metastatic sites or local palliative radiation >4 weeks prior to study entry

9. Autologous bone marrow transplant: Patients must be ≥ 3 months posttransplant prior to study entry
10. Normal hematological, renal, and liver function (absolute neutrophil count $\geq 1000/\text{mm}^3$, platelets $\geq 100,000/\text{mm}^3$, PT Prothrombin Time or PTT (Partial Thromboplastin Time) $\leq 1.3 \times$ control, creatinine $\geq 70 \text{ mL/min/1.73 m}^2$, total bilirubin $\leq 1.5 \text{ mg/dL}$, transaminases ≤ 3 times above the upper limits of the institutional norm)
11. Patients <10 years, modified Lansky score ≥ 60 ; patients ≥ 10 years, Karnofsky score ≥ 60
12. Written informed consent in accordance with institutional and U.S. Food and Drug Administration (FDA) guidelines must be obtained from patient or legal guardian

Exclusion criteria

All patients with the following conditions will be excluded from participation in the study:

1. Any treatment outside the allowable guidelines outlined in inclusion criteria
2. Acute infection, granulocytopenia, or medical condition precluding surgery
3. Pregnant or lactating females
4. Prior history of encephalitis, multiple sclerosis, or other CNS infections
5. Tumor involvement which would require ventricular, cerebellar, or brainstem inoculation
6. Prior participant in experimental viral therapy (e.g., adenovirus, retrovirus, or herpes virus protocol)
7. Required steroid increase within 1 week prior to injection
8. Known HIV seropositivity
9. Concurrent therapy with any drug active against HSV (acyclovir, valaciclovir, penciclovir, famciclovir, ganciclovir, foscarnet, cidofovir) or any immunosuppressive drug therapy (except dexamethasone or prednisone)

Subject recruitment and screening

Up to 24 pediatric patients, ages 3 to 18 years, with recurrent/progressive malignant supratentorial brain tumors will be enrolled in this study.

Informed consent

The general principles underlying the therapeutic strategy of this trial will be explained in non-medical, lay language to the patient and/or the patient's caretakers. Potential risks and benefits will be discussed, as well as the time involvement necessary for appropriate follow-up care. Alternative therapies for pediatric patients with recurrent/progressive malignant brain tumors will be discussed, including standard (re-resection, repeat fractionated radiotherapy, and/or chemotherapy) and experimental interventions (biological agents, gene therapy, and novel combinations and administration routes of existing agents). The patient's caretaker(s) will be provided with a summary of this information as contained in the informed consent form (see Supplementary Data; Supplementary Data are available online at www.liebertpub.com/humc) and will be given as much time as needed to make a deliberate decision concerning the involvement of their child in this trial. Additionally, patients will be provided an assent form (see Supplementary Data) asked if they assent to the therapy except in cases where assent is waived due to the child's age, maturity, or psychological state. Those patients and families who do not decide to enroll in this trial will be provided with the best standard of care available.

STUDY DRUG

G207 is a genetically engineered altered herpes simplex virus that has been demonstrated to be aneurovirulent secondary to deletions of both copies of the $\gamma_134.5$ gene and a disabling *LacZ* insertion at U_L39. G207 is supplied by Aettis, Inc., in sterile, labeled, 1.0 mL cryovials containing 0.12 mL of G207 suspended in the storage buffer Dulbecco's phosphate-buffered saline and 10% glycerol. The vials should remain frozen at -60°C or below until use. G207 will be stored in the pediatric hematology/oncology pharmacy at Children's of Alabama prior to being injected.

STUDY PROCEDURES

After informed consent has been obtained and all screening procedures completed according to the protocol (Table 2), treatment may commence. Utilizing the frameless stereotactic system, children under general anesthesia will undergo a stereotactic guided biopsy. First, a frozen section biopsy for histopathological confirmation of neoplastic cells will be taken. If tumor is not present on frozen section, patients will not undergo G207 inoculation. Following frozen section demonstration of recurrent

tumor, up to four silastic catheters (PIC-030 Neuro-Infusion Catheter (Sophysa; 30 cm × 2.0 mm outer diameter, 1.00 mm inner diameter), equipped with a stainless steel stylet and a 6 French compression hub) will be passed to stereotactically predefined coordinates of enhancing tumor. The catheters will be exteriorized, primed with sterile vehicle (Dulbecco's phosphate-buffered saline +10% glycerol; Alanza, Inc.) for injection, the scalp wound(s) closed and the patient allowed to recover in the Pediatric Intensive Care Unit. The following morning, a post-operative head computed tomography (CT) scan will be obtained to confirm the location of each catheter within the tumor. If needed, and it is possible to do so at the bedside, the surgeon will adjust the position of the catheter tip by slightly withdrawing it to a more desirable location. Otherwise, malplaced catheters will not be utilized for the infusion of virus.

The total amount of G207, as defined by each patient's dose level, will be delivered in a total volume of 2.4 mL administered in up to four catheters, each attached to a separate syringe mounted in a microprocessor-controlled infusion pump for a total infusion rate of 0.4 mL/h (Table 3). The study drug syringe will be connected to the infusion tubing that has been flushed with sterile vehicle. The infusion tubing (PIT-400; Sophysa) will then be primed with 3.4 mL of the diluted G207 virus preparation in the syringe and connected to the catheter. The infusion period will begin with a 35 min flush of the sterile vehicle solution from the catheter at a rate of 0.4 mL/h/catheter. After completion of the flush the pump will be reprogrammed to the appropriate rate for the study drug infusion, and the infusion will occur over 6 h. This interval was chosen based on previous stability testing to ensure no detectable loss of virus activity. If delivery at the desired rate is not possible through an individual catheter, the rate of delivery through that catheter may be slowed and the rate of delivery through the remaining catheters increased as possible to attempt to maintain total delivery rate at 0.4 mL/h. Infusion may extend up to 8 h, if needed due to catheter malfunction. Vitals signs (temperature, blood pressure, pulse, and respiratory rate) and neurologic function (Glasgow Coma Scale and limited neurological exam) will be mon-

itored every hour during the infusion. Approximately 1 h after the completion of the infusion, the catheters will be removed at the bedside. Stable patients may be transferred to be a general inpatient room at that time and will have vital signs and neurologic function monitored every 2 h for the first 24 h. Subsequently, the frequency of monitoring will be determined by the physician(s) based on the medical condition of the patient.

For patients receiving radiation, a 100 SAD linear accelerator with at least 6 MV beam energy will be utilized for radiation treatment. On day 1 after receiving G207, all patients will undergo simulation and radiation treatment. A custom aquaplast immobilization device will be fabricated, and axial CT images will be collected at a slice thickness of no less than 3 mm. The preoperative MRI will be utilized for treatment planning. This study will be registered/fused to the simulation CT scan. The target volumes and prescription doses for radiation planning are shown in the Table 4 and follow The International Commission on Radiation Units and Measurements (ICRU)-62 conventions. The goal of treatment planning is coverage of the planning target volume 1 (PTV1) and 2 (PTV2) by the prescription 100% isodose line with none of the PTV1 to receive greater than 120% (6 Gy). The dose to brainstem and optic apparatus (nerves and chiasm) will be limited to 3 Gy or less unless coverage of the gross tumor volume would be compromised. The 2 mm margin for setup and registration error may be dosimetrically compromised in cases where gross tumor is very close to critical structures such as the brainstem or optic apparatus. In no case will gross disease receive less than the prescription dose. Generally, it is expected that multiple axial or nonaxial beams (5–9) will be required to generate an acceptable plan.

STATISTICAL PLAN

This study is a phase 1 standard dose-escalation study of safety and toxicity. It is anticipated that patients with several different histologic tumor types will be enrolled in this study, since the primary endpoint is safety and not efficacy. Data from the study will be presented in a descriptive manner with careful analysis of any effects that G207 may have on survival, cognitive function,

Table 3. Plan for loading rate and infusion rate for HSV G207

Number of Catheters	Initial Loading Rate per Catheter	Final Infusion Rate per Catheter
1	0.4 cc/hour	0.4 cc/hour
2	0.4 cc/hour	0.2 cc/hour
3	0.4 cc/hour	0.13 cc/hour
4	0.4 cc/hour	0.1 cc/hour

Table 4. Target volume for radiation planning

Volume	Definition	Dose
Gross tumor volume	Enhancing residual plus resection cavity	–
Clinical target volume	T2 abnormality	–
Planning target volume 1	Gross tumor volume plus 2 mm	5 Gy
Planning target volume 2	Clinical target volume plus 2 mm	3 Gy

or development of herpes infection. In general, descriptive statistics will be used to summarize the safety and efficacy variables collected and the baseline demographic data. Continuous variables will be summarized using mean, standard deviation, minimum, medium, and maximum. Categorical variables will be summarized using frequency counts and percentages. Additional exploratory analyses of the data on some of the secondary endpoints will be conducted as deemed appropriate.

SAFETY AND ADVERSE EVENTS ANALYZED

Any serious, life-threatening, suspected or unexpected adverse event that occurs during the conduct of this study, regardless of relationship to test article or procedures, will be reported within 24 h and a written report filed within 7 days after the event to the medical monitors, the investigator's Institutional Review Board, the UAB Clinical Trials Monitoring Committee, and the FDA. Also, grade 2–3 unexpected events will be filed as soon as possible, but in no case later than 15 calendar days after determination that the event qualifies for reporting with the Institutional Review Board and FDA.

RISK–BENEFIT ASSESSMENT

The primary objective of the study is to provide safety and tolerability data on intratumoral injection of G207 in small cohorts of pediatric patients with malignant supratentorial brain tumors. The children's risk level for this study is 2, because the research involves greater than minimal risk but presents the prospect of direct benefit to the individual child (45 CFR 46.405). G207 has been tested on very few brain tumor patients (<40), and therefore, little is known about side effects in humans. Side effects that have been seen in patients shortly after experimental treatment with G207 include headache, nausea, drowsiness, weakness, anemia, and leukopenia. Although not reported, side effects could include more severe reactions such as viral infections with flu-like symptoms and allergic reactions to G207 ranging from itching and hives to difficulty breathing or the development of brain irritation or edema including swelling due to HSV encephalitis and/or hepatitis. If there is concern for encephalitis, a CT scan or MRI scan will be performed and the antiviral drug acyclovir started. Surgical biopsy or lumbar puncture may be necessary. There may also be unpredictable side effects, and participation in this study may not be of any direct medical benefit to

the patient. If the patient responds to this therapy as seen in some adult patients, the tumor may shrink and the symptoms caused by the tumor may improve. We expect that there may be an improvement in the patient's quality of life and decrease in caregiver stress from baseline to subsequent administrations if there is a response to the treatment. Furthermore, there may be indirect benefits; information learned from this study may help patients with brain tumors in the future.

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AUTHOR DISCLOSURE

Drs. Markert and Gillespie are founders of and own stock and stock options (<8% interest) in Aettis, Inc., a biotech company that has licensed M032 HSV from The Board of Trustees of the University of Alabama for the University of Alabama at Birmingham and is developing other oncolytic HSVs that are not the subject of this current investigation. Dr. Gillespie currently serves as one of five unpaid members of the Board of Directors for Aettis, Inc. Dr. Gillespie is a founder of and owns stock and stock options (<10%) in Maji Therapeutics, which is developing other HSVs that are not the subject of the current investigation. Drs. Markert and Gillespie 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 oncolytic HSV. 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. 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 oncolytic HSVs to treat localized and metastatic cancers. This is generally, but not specifically, related to the subject matter of this investigation.

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