Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Friedman GK, Johnston JM, Bag AK, et al. Oncolytic HSV-1 G207 immunovirotherapy for pediatric high-grade gliomas. N Engl J Med 2021;384:1613-22. DOI: 10.1056/NEJMoa2024947

This supplement contains the following items:

1. Original protocol (pages 2-44; statistical analysis plan, pages 29-31)
2. Final protocol (pages 45-90; statistical analysis plan, pages 73-76)
3. Summary of changes to the protocol (page 91)
4. Summary of changes to the statistical analysis plan (page 92)

Phase I Clinical Trial of HSV G207 Alone or with a Single Radiation Dose in Children with Recurrent Supratentorial Brain Tumors

IND No.: IND 16294, approved January 14, 2015

Site: **University of Alabama at Birmingham**
Principal Investigator: Gregory K. Friedman, M.D., Dept. of Pediatrics, Hematology/Oncology

Co-Investigators: Alyssa T. Reddy, M.D., Dept. of Pediatrics, Hematology/Oncology
James M. Johnston Jr, M.D., Dept. of Surgery, Div. of Neurosurgery
James M. Markert, M.D., Dept. of Surgery, Div. of Neurosurgery
John Fiveash, M.D., Dept. of Radiation Oncology
Asim K. Bag, M.D., Dept. of Radiology
Avi Madam-Swain, Ph.D., Dept. of Pediatrics, Hem./Oncology

Medical Monitors: Andres Forero, M.D., Director, Clinical Trials Review Committee
Tony McGrath, M.D., Dept. of Pediatrics
Shannon Ross, M.D., Dept. of Pediatrics

Table of Contents

Appendices

Study Synopsis

Title of Study:	Phase I Clinical Trial of HSV G207 Alone or With a Single Radiation Dose in Children with Recurrent Supratentorial Brain Tumors
Principal Investigator:	Gregory K. Friedman, M.D., Dept. of Pediatrics, Hematology/Oncology
Co-Investigators:	Alyssa T. Reddy, M.D., Dept. of Pediatrics, Hematology/Oncology James M. Johnston Jr, M.D., Dept. Neurosurgery James M. Markert, M.D., Dept. of Neurosurgery John Fiveash, M.D., Dept. of Radiation Oncology Asim K. Bag, M.D., Dept. of Radiology Avi Madam-Swain, Ph.D., Dept. of Pediatrics, Hem./Oncology
Medical Monitors:	Andres Forero, M.D., Director, Clinical Trials Review Commitee Tony McGrath, M.D., Dept. of Pediatrics Shannon Ross, M.D., Dept. of Pediatrics
Study Center:	University of Alabama at Birmingham
Clinical Phase:	Phase I (open-label)
Study Rationale:	Outcomes for children with recurrent supratentorial brain tumors are extremely poor
Study Objectives:	Primary: To assess the safety and tolerability of G207 administered intratumorally via stereotactic infusion alone or followed by a single dose of radiation within 24 hours of G207 administration in children with recurrent or progressive malignant supratentorial brain tumors. To establish a maximum tolerated dose (MTD) or maximal planned dose if no dose-limiting toxicity is observed. Secondary: 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 by assessing radiographic response, performance scale, progression-free and overall survival, immune response, and presence of G207 in blood, saliva, and conjunctiva.
Investigational Drug:	G207 is a genetically altered herpes simplex virus that has been demonstrated to be aneurovirulent secondary to deletions of both copies of the $\gamma_1$34.5 gene. After stereotactic biopsy to confirm tumor recurrence, up to 4 silastic catheters will be passed to predefined coordinates of enhancing tumor. Subsequently, patients

will be inoculated with 2.4 ml of G207 at one of two doses over 6 hours alone or followed by a single 5 Gy fraction of radiation within 24 hours of virus inoculation.

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

Treatment Indication: Progressive or recurrent growth of malignant supratentorial brain tumor after initial surgery, radiotherapy, and/or chemotherapy. Patients with brainstem, cerebellar or intraventricular tumors will not be eligible.

Study Design: This study will be a Phase 1, open-label study of HSV G207 to assess its safety and tolerability. A traditional 3 + 3 design will be used. The following dose escalation scheme will be used:

Dose Level	Patients	Dose (pfu)	Volume	# Loci
-1	3	$1x10^6$	2.4 ml	1-4
1	3 (+3)	$1x10^7$	2.4 ml	1-4
2	3 (+3)	$1x10^8$	2.4 ml	1-4
3	3 (+3)	$1x10^7$ + 5 Gy radiation	2.4 ml	1-4
4	3 (+3)	$1x10^8$ + 5 Gy radiation	2.4 ml	1-4

The first three patients enrolled will be entered at the starting dose (dose level 1). If no significant toxicities are encountered, subsequent doses will be escalated as outlined above. If significant toxicity is encountered in one patient at the starting dose, 3 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 x 10^6 plaque-forming units (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 maximally tolerated dose (MTD). There will be a minimum 28 day observation period between the first and second patient in each cohort and a 28 day observation period from one cohort to the next to allow for evaluation of potential toxicity. It will take an estimated 24-30 months to complete patient accrual.

Safety Analysis: Patient monitoring will be appropriately tapered from the initial intensive monitoring in the Pediatric Intensive Care Unit immediately after biopsy and catheter placement, to an inpatient room at Children's of Alabama, and then to regular clinic follow-up visits. In addition to regularly scheduled screening tests looking specifically for G207 shedding and viremia, other routine evaluations will include vital sign measurements, neurological exams, neurological performance measurements, magnetic

resonance imaging studies (MRIs), chemistry and hematology laboratories, urinalysis, neuropsychological testing, and quality of life questionnaires (quality of life measures are optional consent). Two medical safety monitors, who have expertise in herpes encephalitis, will be integrally involved in the assessment of toxicity throughout the study. Safety/tolerability will be assessed by adverse events and laboratory tests. Descriptive statistics will be provided for selected demographic, safety data overall and by dose group. Additional exploratory analyses of the data will be conducted as deemed appropriate.

Study Duration: Patients will have close scheduled follow up for 24 months following treatment with G207. Annual follow up appointments will be made for patients who survive longer than 24 months. 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.

1.0 Introduction:

Primary CNS tumors are the most common solid neoplasm of childhood and the leading cause of cancer related death in pediatric patients. Approximately 25% of children with brain tumors will not be long-term survivors of their disease despite aggressive treatment with combinations of surgery, radiation, and chemotherapy. 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 lacking genes essential for replication in normal brain cells. G207 has been shown to be efficacious in the treatment of both human and rodent tumors in murine models. Safety has been established following pre-clinical investigation involving intracranial inoculation in the highly HSV-sensitive owl monkey (*Aotus nancymai*). A Phase I study in adults with recurrent glioblastoma multiforme (GBM) demonstrated safety of intratumoral injections of G207 (1). Patients in this study were treated with doses escalating from 1×10^6 pfu to 3×10^9 without serious adverse events or toxicity. In a Phase Ib study in adults with recurrent GBM, patients were safely injected with two doses of the G207 (totaling 1.15×10^9 pfu) pre- and post-tumor resection (2). A follow-up phase I trial of G207 given in combination with a single 5 Gy dose of radiation for recurrent GBM demonstrated safety and radiographic responses (3). A maximum tolerated dose was not reached in all 3 trials.

2.0 Background and Rationale:

2.1 Disease Processes Involved in 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 a leading cause of cancer related morbidity and mortality (4). Although survival rates for low-grade, localized tumors have improved, there remains a significant subset of patients with high-grade, unresectable and/or disseminated tumors that have very poor outcomes despite conventional treatment approaches including surgery, chemotherapy and radiation. For patients with high-grade recurrent tumors, traditional treatment usually only provides a brief interval of disease control (5,6). Furthermore, patients who survive their disease after traditional therapy often have long-term sequelae such as hormone dysfunction, neurosensory impairment, and neurocognitive changes that are attributed to the treatment (7-10).

2.2 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 post-mitotic, non-dividing cell population of the brain. Many vectors have been considered for neoplastic therapy, including naked DNA, liposomes, and viruses. Attenuated HSV has a number of potential advantages as a neoplastic therapy for pediatric brain tumors. While traditional chemotherapy requires cancer cells to be dividing, HSV can enter a non-dividing cancer cell, replicate, and lyse the cell.

HSV-1 is an enveloped, double-stranded DNA virus (11). It has a genome of approximately 150 kb encoding for at least 80 genes. The genome is divided into long and short unique regions, each flanked by characteristic repeated sequences. Genetically altered, conditionally replicating HSV has been generated containing a variety of gene deletions and their effectiveness as therapeutic agents has been examined *in vitro* and *in vivo* in numerous models of

metastatic and primary tumors. These experiments provide valuable information on the efficacy and safety in the animal models. HSV-1 vectors have the advantage of being neurotropic. Genes essential for HSV-1 replication in the brain have been identified (12,13). Studies for brain tumor therapy have included alterations in one or more of the viral genes thymidine kinase, DNA polymerase, uracil DNA glycosylase, ribonucleotide reductase and $\gamma_1$34.5 (14-20). Thymidine kinase, DNA polymerase, uracil DNA glycosylase and ribonucleotide reductase are all viral enzymes necessary for nucleotide synthesis and replication. It has been clearly demonstrated experimentally that by altering these genes, either by deletion, insertion, or point mutation in the coding region, viruses will not be able to replicate in post-mitotic cells such as neurons. The viruses, however, are able to utilize cellular enzymes and factors present in dividing tumor cells to infect and kill them.

There are two copies of $\gamma_1$34.5 gene; one on each side of the two inverted repeat regions of the HSV genome. This gene encodes a critical bifunctional protein that enables the virus to replicate in neurons and also blocks a normal host response to infection. This gene is felt to be responsible for the neurovirulence of HSV. In the absence of this protein, the virus fails to induce HSV encephalitis even when injected in high amounts into the CNS of susceptible animal models. Additionally, a portion of the latency activated transcripts is encoded on the DNA strand opposite the $\gamma_1$34.5 genes. Thus the capacity to express genes necessary for the virus to establish latency is lost when the $\gamma_1$34.5 gene is altered. The deletion of the neurovirulence gene produced a greater survival of nude mice with human gliomas and retained the tk gene, making it susceptible to acyclovir which is considered standard of care treatment for HSV infections (21). Thus, in the unlikely event that a mutant virus produces toxicity in normal brain tissue, effective antiviral agents are readily available.

2.3 G207 Development and Preclinical Studies

G207 is a modified HSV that contains 1) deletions of both copies of $\gamma_1$34.5 and 2) a viral ribonucleotide reductase disabled secondary to disruption of the U_L39 gene by insertion of the *E. coli LacZ* coding region (16). Both mutations had previously been independently investigated in animal models. The second modification was based on the observation that a virus deficient in ribonucleotide reductase (22), a non-essential enzyme for HSV replication in dividing cells, showed severe attenuation in non-dividing cells while remaining proficient in killing proliferating Vero cells and human tumor cells in culture (21). These features were incorporated into G207 in order to maximally decrease the neurovirulence and yet maintain the ability of the virus to replicate in tumor cells. Other favorable properties of G207 are its easy detection due to the *lacZ* reporter gene, temperature sensitivity, and hypersensitivity to the antiviral drug acyclovir due to the retained tk gene, and interruption of the UL39 gene (19). Genetically altered viruses such as G207 with multiple mutations offer the safest approach for any proposed clinical trial.

A variety of pediatric tumors, including neural and glial tumors, express the primary HSV entry receptor CD111 and are sensitive to engineered HSV both *in vitro* and *in vivo* (23-27). When comparing the sensitivities of adult and pediatric glioma xenograft lines (xenolines) to G207, Friedman et al. demonstrated that the pediatric xenoline was more sensitive than 6 adult xenolines tested (28). Both pediatric glioma cells and primitive neuroectodermal tumor (PNET)/medulloblastoma cells were sensitive to G207 with a low dose of virus required to kill 50% of cells *in vitro*. Furthermore, brain tumor stem cells (also known as brain tumor-initiating

cells) from pediatric brain tumors were sensitive to $\gamma_1$34.5-deleted HSV suggesting that engineered HSV may be able to target this chemotherapy and radiotherapy resistant population (26,27) In athymic nude mice bearing intracranial tumors, oHSV prolonged survival significantly with several long-term survivors, including malignant pediatric brain tumors (26,30-31). G207 significantly prolonged survival of nude mice bearing human tumors either subcutaneously or intracranially when injected at titers of 10^6-10^7 plaque-forming units (pfu).

G207 has also been inoculated in young BALB/c mice via four different routes: intracerebrally, intracerebroventricularly, intravenously, and intrahepatically with no adverse effects or symptoms of disease (32). In addition, 10^9 pfu of G207 injected intracerebrally into the HSV sensitive owl monkey, *Aotus nancymai*, did not produce any deleterious side effects, whereas 10^3 pfu of HSV-1(F) virus was lethal (33). MRI analysis of G207 treated monkeys showed no CNS abnormalities. Neuropathologic evaluation showed local inflammation at the site of injection, but no other abnormalities. HSV DNA was detected throughout the brain by PCR analysis, but the unremarkable clinical course of the monkeys has not provided any evidence supporting the clinical significance of this finding. Histopathological evaluation of multiple organs revealed no evidence of altered cellular architecture or HSV immunoreactive cells.

The fundamental difference between G207 and other vector systems is that G207 is a conditionally replicating vector capable of replication in tumor cells, but not in the surrounding brain tissue. Conditionally replicating HSV is desirable when considering that, following resection, more than 10^9 tumor cells remain *in situ*. Other methods of treatment, e.g., a retroviral vector with the HSV tk, will not likely be able to target this many cells, especially considering the ongoing cellular proliferation of the remaining glioma cells. The ability of G207 to selectively replicate may allow for the delivery of sufficient vector into the tumor bed to potentially destroy most of the cells that remain after conventional therapy.

As G207 represents an excellent vector for evaluation in clinical studies for the treatment of malignant brain tumors, a G207 Master Viral Bank and clinical lot of virus have been produced by MediGene, Inc. (Now Aettis, Inc.) in accordance with Good Manufacturing Practice (GMP) regulations and tested according to the Points to Consider for the Testing of Cell Lines Used in the Manufacture of Biological Products.

2.4 Genetically-engineered HSV-1 and Radiation

A number of pre- clinical studies have combined the selective oncolytic activity of genetically modified viruses with radiotherapy. Advani et al. first reported the interaction between the modified HSV-1 R3616 (the parent molecule of G207) and radiation (34). In nude mice with subcutaneous U87 malignant glioma xenografts implanted in the flank, R3616 injection followed by fractionated radiotherapy not only in improved viral replication and distribution, but significantly increased tumor regression (observed in 22 of 33 mice receiving combined therapy versus 4 of 33 mice receiving R3616 alone). Similarly, a later study used immunohistochemistry staining of intracranial glioma xenografts in nude mice to demonstrate a 2- to 5-fold enhancement of viral replication when inoculation of R3616 was followed with fractionated radiotherapy (35). Longer survival times were observed for the mice in this study receiving the combined therapy, and the interaction of radiation and R3616 was significantly shown to be synergistic by statistical analysis based on a proportional hazards model.

Markert et al. observed that although there was not a statistically significant difference in

the median survival of nude mice with implanted human malignant gliomas that received only G207 versus G207 combined with radiation (p=0.09), the latter group was the only group with a majority of long- term survivors (70% in the group receiving combination therapy versus 20-30% in the groups receiving either G207 or radiation alone) (20). Of note, this study used two 5 Gy fractionated doses of radiotherapy given at 4 and then 24 hours, doses lower than the prior studies and more feasible for delivery in a clinical setting. Further preliminary work has compared the survival in mice receiving G207 followed by a single 5 Gy dose of irradiation given at varying time intervals (4 hours, 24 hours, and 120 hours). The group receiving radiation at 24 hours has demonstrated markedly improved survival over the groups receiving radiation at 4 or 120 hours (60% versus 30-40% at 91 days) (G.Y. Gillespie, personal communication). Importantly, none of the pre-clinical *in vivo* studies observed any mortality that could be attributed to a potential adverse synergism of these two modalities.

Several mechanisms have been proposed to explain the observed *in vivo* synergism of G207 and radiation. Because viruses with varying mutations have shown enhancement in response to radiation (Advani et al. observed a similar response in a second HSV-1 mutant), it appears unlikely that the irradiated cells are simply increasing production of a single protein which substitutes for a missing viral gene function, although Stanziale, et al, showed that radiation potentiated the antitumor efficacy of G207 by upregulating ribonucleotide reductase (34, 36). Since the HSV mutants replicate more efficiently in dividing tumor cells, it has been hypothesized that the enhanced viral replication may be accountable to a higher proportion of tumor cells in S phase after radiation. Other theories suggest that tumor cells may modify cytokine expression in response to radiation, or that the local inflammatory response to radiation may somehow facilitate increased viral replication within the tumor cells (20). Although the mechanisms underlying this synergy have not been fully elucidated, the results of the pre-clinical trials nonetheless provide strong rationale to begin investigating the safety of these combined modalities in humans.

2.5 Previous Clinical Experience

A Phase I clinical trial in adults was completed in March 2000 under BB-IND-7393 with HSV G207 (1). The genetically altered virus was supplied as clinical grade virus by the study sponsor, MediGene, Inc. Twenty-one adult patients with recurrent malignant glioma (GBM or anaplastic astrocytoma) were treated in seven cohorts of three each by stereotactic intratumoral injection of HSV G207 in a standard dose escalation fashion. Doses ranged from 1 $x10^6$ pfu as a single injection in 100 to 300 µl up to 3 x 10^9 pfu given at 200 µl each in five sites. While some patients developed complications frequently seen in patients with high-grade gliomas, no toxicity or serious adverse events could unequivocally be ascribed to G207. In fact, a toxic dose level was not reached during this trial because available viral production techniques limited the total dose that could be administered. There was also radiographic and neuropathologic evidence suggestive of anti-tumor activity and long-term presence of viral DNA in some cases. In a phase Ib study, 6 patients with recurrent GBM received two doses (one administered pre-tumor resection and one post resection in the resection cavity) of G207 totaling 1.15 x 10^9 pfu (2). Viral replication was demonstrated and radiographic and neuropathologic evidence suggestive of antitumor response was reported. No patient developed HSV encephalitis or required treatment with acyclovir.

A third Phase I study examined the safety of stereotactic intratumoral administration of G207 followed within 24 hours with a single 5 Gy radiation dose in patients with recurrent

malignant gliomas. Treatment was well tolerated and no dose limiting toxicities were experienced. Importantly, 6 of 9 patients had a stable or partial response for at least one time point. The safety established in these three studies forms the basis to extend HSV G207 to the pediatric population. Pediatric patients may 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, especially encephalitis.

2.6 Safety Information

The safety of G207 has been addressed both by the deliberate molecular engineering of the virus (as discussed in Section 2.3), as well as by extensive *in vitro* and *in vivo* testing of precursors to the clinical material, as well as on the clinical material. *In vitro* testing has included the following:

SUMMARY OF G207 TESTING

G207 Master Virus Bank (MVB)	
Test	*Result*
Bulk Cell Harvest	
Sterility	Pass
Mycoplasma	Negative
Final Filled Vials	
Sterility (21 CFR 610.12)	Pass

Lot Release (7/3/97)	
Test	*Result*
Bulk Cell Harvest	
Sterility	Pass
Mycoplasma	Negative
Final Filled Vials: Sublot A	
Sterility (21 CFR 610.12)	Pass
General Safety (21 CFR 610.11)	Pass
LAL	9.8 EU/mL
Final Filled Vials: Sublot B	
Sterility (21 CFR 610.12)	Pass
General Safety (21 CFR 610.11)	Pass
LAL	< 1.0 EU/mL
Bacteria/Fungistasis USP XXII	Pass

HSV infection is widespread among healthy adults and children alike. Influenced by socioeconomic and geographic factors, HSV seropositivity in children five years of age is 20-30%; this increases to 40-80% by the second decade of life (37). In the Phase I study with G207 in adults, only 1 of 5 seronegative patients demonstrated seroconversion after G207 administration. Importantly, no specific clinical decline seemed to be attributable to this seroconversion, therefore suggesting that children may not be at an increased risk secondary to their lower incidence of seropositivity. Although neonates are more susceptible to the

development of HSV encephalitis (HSE) than adults (the sensitivity of a human neonate is generally equivalent to the sensitivity of *Aotus nancymai*), other pediatric patients beyond the neonatal period develop HSE at an incidence similar to that in adults (one in 200,000 individuals).

Although HSE most often presents in neonates with generalized symptoms, HSE in adults and older children typically produces specific disturbances of the normal functions of the involved brain. It is unknown, however, if pediatrics patients will be more susceptible than adults to developing HSE or other herpes related infection after treatment with G207. Patients will be closely monitored during the clinical studies for indications of HSE (see Section 7.4) as well as other possible side effects with the administration of G207. In the event of the development of HSE, the tk gene, which confers sensitivity to gancyclovir and acyclovir, remains intact, allowing for medical management with high-dose acyclovir according to established methods (14). In general, children tend to have more complete recovery from HSE than adults who are >30 years of age (38).

Unlike whole brain radiotherapy, inoculation of G207 is a focal intervention, and as such is not expected to produce any neurodevelopmental insult in the pediatric patient greater than that which would be expected for other standard focal interventions in patients with this disease (surgery or focal radiation). The single 5 Gy dose of radiation proposed is to enhance virus replication and cancer cell killing (see Section 2.3.2) and was well tolerated in the adult Phase I study when combined with G207 (3). Palliative fractionated reirradiation at total doses up to 18 to 35 Gy has been safely used in children and adults with a variety of recurrent brain tumors (39-41). The patient population to be studied will have been heavily pretreated most likely with radiation and chemotherapy. They are therefore likely to have neurocognitive deficits upon entry into this study. A baseline neuropsychological assessment will be done at the time of study entry and during the course of therapy to monitor for any adverse neurodevelopmental impact on the patients receiving G207.

3.0 Study Objectives:

3.1 Primary Objective:

To assess the safety and tolerability of G207 administered intratumorally via stereotactic infusion alone or followed by a single dose of radiation within 24 hours of G207 administration in children with recurrent or progressive malignant supratentorial brain tumors. Safety/tolerability will be assessed by adverse events and laboratory tests. The dose escalation scheme for this study was based on the adult phase I study in which the maximum planned dose of 3 $x10^9$ was administered without significant adverse effects. The MTD or maximal planned dose, if no dose-limiting toxicity is observed, will be determined.

3.2 Secondary objective:

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 measuring radiographic response, determining patient performance scale, progression-free and overall survival, measuring antiviral immune response and presence of G207 in blood, saliva, and conjunctiva. The impact of this new therapy on patients' quality of life will also be assessed (optional consent).

4.0 Study Design:

4.1 Overview

Pediatric patients with recurrent or progressive supratentorial malignant brain tumors who have failed surgery, radiotherapy and who may or may not have had chemotherapy or other form of treatment will be eligible for the study. An MRI must demonstrate progression of enhancing residual tumor at least eight weeks after radiation treatment. Last chemotherapy must be 4 or more weeks from entry and patients must have normal hematologic function. Patients must be on a stable dose of steroids for at least one week prior to injection. HSV immune status will be determined both pre- and post-treatment.

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 (biological agents, gene therapy, and novel combinations and administration routes of existing agents) interventions. The patient's caretaker(s) will be provided with a summary of this information as contained in the informed consent form, and will be given as much time as needed to make a deliberate decision concerning the involvement of their child in this trial. While those who do not decide to enroll in this trial will be provided with the best standard of care available, those consenting to enrollment will undergo the following procedures.

Under general anesthesia, patients will undergo stereotactic image-guided craniotomy, stereotactic biopsy and intraoperative histopathological analysis to document tumor cells, followed by G207 inoculation. Treatment with G207 will only proceed if viable, recurrent tumor is present on the frozen section. Three patients will be entered at each dose. The initial dose will be 1×10^7 pfu of G207 in a volume of 2.4 ml for the first three patients. If no significant (Grade 3 or 4) toxicities are encountered within 28 days of completing this treatment cohort, a subsequent dose level of 1×10^8 will be used. If no dose limiting toxicities are seen at either dose level, the initial dose level (1×10^7) will be repeated followed by a single 5 Gy fraction of radiation directed to the tumor within 24 hours of G207 inoculation. If deemed safe, a final dose level of 1×10^8 combined with a single dose of 5 Gy of radiation will be used. If two or more patients have toxicity at the starting dose, a cohort will be added at 1×10^6. If two or more patients have toxicity at this lower dose, the study will be terminated. The maximally tolerated dose (MTD) will be defined as the dose immediately below any dose that causes Grade 3/4 toxicities in 2 patients. There will be a minimum 28 day observation period between the first and second patient of each cohort, a seven-day observation period between patient two and three enrolled in a cohort, and a 28 day observation period from one cohort to the next to allow for evaluation of potential toxicity. Patients will be followed closely with potential effects of HSV being monitored closely. 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-operatively, and at 1, 3, 5, 7, 9 and 12 months. PCR and cultures will also be performed at the same time points to check for HSV in saliva and conjunctival secretions and blood. MRI will be done on day 4 after treatment and at months 1, 3, 5, 7, 9 and 12. 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 (as described above). Nonetheless, patients who have received G207 will continue long-term follow-up even after receiving other cancer therapies.

4.2 Sample Size Considerations

This Phase I pilot study is conducted using a traditional (3+3) dose escalation design with four dosing cohorts. Thus the sample size for this study is up to 24 patients. In each cohort, 3 patients will be initially enrolled. Once the cohort of three patients has completed the toxicity and safety evaluation, additional patients will be enrolled into the next cohort or current cohort expanded following the dose escalation rules. Sample size has been determined based on practical consideration of the traditional 3+3 Dose Escalation design. Although accrual is expected to take 18-24 months for 12 patients, the actual time necessary for accrual is difficult to estimate due to an inability to predict the degree of toxicity that will be observed.

4.3 Statistical Considerations

This study is a Phase I clinical trial, standard dose-escalation study of safety and toxicity. A standard 3 + 3 design will be used. 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, 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, median, 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.

4.4 Treatment Summary

Dosing will start at an initial dose level of 1×10^7 pfu of G207, and will then escalate by one log increment to a dose of 1×10^8 pfu. If there are no unacceptable dose-limiting toxicities, the dose levels will be repeated in combination with a single 5 Gy dose of radiation. Subjects will be enrolled sequentially. Treatment will be escalated as follows:

Dose Level	Patients	Dose (pfu)	Volume	# Loci
-1	3	$1x10^6$	2.4 ml	1-4
1	3 (+3)	$1x10^7$	2.4 ml	1-4
2	3 (+3)	$1x10^8$	2.4 ml	1-4
3	3 (+3)	$1x10^7$ + 5 Gy radiation	2.4 ml	1-4
4	3 (+3)	$1x10^8$ + 5 Gy radiation	2.4 ml	1-4

There will be a minimum 28 day observation period between the first and second patient of each cohort, a seven-day observation period between patient two and three 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 non-hematologic toxicity, as defined by NCI CTCAE v.4, that is considered as being possibly, probably or likely related to G207 will be considered a dose-limiting toxicity (DLT). Based on previous clinical trial experience with G207, neurologic deterioration 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 DLT if symptoms improve to baseline within seven days of completing treatment. If one patient has a DLT at the starting dose, up to 3 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 1×10^6. If two or more patients have toxicity at this lower dose, the study will be terminated. If significant toxicity is encountered in one patient at subsequent dose levels, patient numbers will be increased to six for that dose level. If no further toxicity is observed, the dose escalation will continue. If 2 patients in a cohort have any Grade 3 or 4 non-hematologic toxicity, as defined by NCI CTCAE v.4, that is considered as being possibly, probably or likely related to G207, the dose level immediately below will be defined as the MTD. If only 3 patients had previously received the newly determined MTD (because no Grade 3/4 toxicity was observed), a second cohort of 3 patients will receive that dose to ensure that it is indeed safe. If there are no DLTs in the highest cohort, 3 additional patients will be added to ensure safety at that dose.

4.5 Stopping Rules

The study will be stopped if the MTD is established or if the lowest proposed dose causes unacceptable toxicity. The medical monitors and/or principal investigator will apply the stopping rules. 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 hours, HSV encephalitis, or mental status changes requiring intubation. The pause will be in effect to allow assessment by the medical monitors, principal investigator, UAB Comprehensive Cancer Center Clinical Trials Monitoring Committee (CTMC), and the FDA to review the events to decide if the study should be terminated or can be resumed.

4.6 Data and Safety Monitoring

All patient data will be maintained on UAB computers with appropriate password protection. A nurse clinician with expertise in clinical trials and the principal investigator will be responsible for all data collection and maintenance. This project will be conducted under the oversight of the UAB Comprehensive Cancer Center for which a Data and Safety Monitoring Plan has been established. This study will be under the supervision of the UAB CTMC which acts as a Data Safety and Monitoring Committee for patients with brain tumors who are enrolled in clinical trials at UAB. There is no Data and Safety Monitoring Board for this Phase I study. The NCI-approved Data and Safety Monitoring Plan is appended as attachment #1.

5.0 Study Population:

5.1 Inclusion Criteria

Patients meeting the following inclusion criteria will be eligible for the study:

5.1.1 Age $\geq$ 36 months and < 19 years

5.1.2 Pathologically proven malignant supratentorial brain tumor (including glioblastoma multiforme, giant cell glioblastoma, anaplastic astrocytoma, primitive neuroectodermal tumor, ependymoma, atypical teratoid/rhabdoid tumor, germ cell tumor, or other high-grade malignant tumor) which is progressive or recurrent despite standard care including surgery, radiotherapy, and/or

chemotherapy

5.1.3 Lesion must be > 1.0 cm in diameter and surgically accessible as determined by MRI

5.1.4 Patients must have fully recovered from acute treatment related toxicities of all prior chemotherapy, immunotherapy or radiotherapy prior to entering this study.

5.1.5 Myelosuppressive chemotherapy: patients must have received their last dose at least 3 weeks prior (or at least 6 weeks if nitrosurea)

5.1.6 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)

5.1.7 Monoclonal antibodies: At least 3 half-lives must have elapsed prior to study entry

5.1.8 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.

5.1.9 Autologous bone marrow transplant: Patients must be ≥ 3 months since transplant prior to study entry.

5.1.10 Normal hematological, renal and liver function (Absolute neutrophil count ≥ 1000/mm^3, Platelets ≥ 100,000/mm^3, PT or PTT ≤ 1.3 x control, Creatinine ≥ 70 mL/min/1.73 m^2, Total Bilirubin ≤ 1.5 mg/dl, Transaminases ≤ 3 times above the upper limits of the institutional norm)

5.1.11 Patients < 10 years, Modified Lansky score ≥ 60; patients > 10 years, Karnofsky score ≥ 60

5.1.12 Patient life expectancy must be at least 8 weeks

5.1.13 Written informed consent in accordance with institutional and FDA guidelines must be obtained from patient or legal guardian

5.2 Exclusion Criteria

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

5.2.1 Any treatment within the allowable guidelines outlined in section 6.1.

5.2.2 Acute infection, granulocytopenia or medical condition precluding surgery

5.2.3 Pregnant or lactating females

5.2.4 Prior history of encephalitis, multiple sclerosis, or other CNS infection

5.2.5 Tumor involvement which would require ventricular, cerebellar or brainstem inoculation

5.2.6 Prior participant in experimental viral therapy (e.g., adenovirus, retrovirus or

herpes virus protocol)

5.2.7 Required steroid increase within 1 week prior to injection

5.2.8 Known HIV seropositivity

5.2.9 Concurrent therapy with any drug active against HSV (acyclovir, valaciclovir, penciclovir, famciclovir, gancyclovir, foscarnet, cidofovir) or any immunosuppressive drug therapy (except dexamethasone or prednisone).

6.0 Study Methods and Procedures:

6.1 Designated Laboratories/Central MRI Review

6.1.1 Routine chemistry and hematology will be performed by Children's of Alabama (COA) laboratory.

6.1.2 All viral culture and viral serology will be performed by the UAB Diagnostic Virology laboratory (Mark Prichard, Ph.D., Director).

6.1.3 MRI/CT will be reviewed by a designated pediatric neuroradiologist at COA.

6.1.4 All pathology will be reviewed by the pediatric neuropathologist at COA.

6.2 Screening Evaluations

Prior to treatment, patients will undergo physical examination, neurologic evaluation and routine preoperative clinical laboratory testing as well as collection of saliva and conjunctival secretions and blood for HSV culture and serology. After having undergone a preoperative evaluation consistent with inclusion in the study, the study participant will undergo a contrast-enhanced MRI scan. Sedation will be used during the MRIs as necessary for the patient's comfort. Review of these MRI scans will be necessary to determine if the tumor location and size is such that the patient may be included in the study. Tumor size will be determined using the maximal 2-dimensional cross-sectional tumor measurements, transverse x width, using either T1 or T2 weighted images. This imaging study will be obtained a maximum of 15 days prior to the patient’s stereotactic inoculation. Also obtained within this timeframe will be assessment of quality of life by the Pediatric Hematology/Oncology Neuropsychology team at COA.

6.3 Intratumoral Inoculation with G207

After informed consent has been obtained and all screening procedures completed according to the protocol, treatment may commence. As a part of pre-operative evaluation, patients will receive a neurological exam the morning of G207 delivery. The patient will undergo a contrast enhanced MRI study using a frameless stereotactic system protocol. Utilizing the frameless stereotactic system, children under general anesthesia will undergo a stereotactic guided craniotomy. 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 4 silastic catheters (PIC-030 or equivalent: (30cm x 2.0mm outer diameter, 1.00mm inner diameter) Neuro-Infusion Catheter, 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 day, a postoperative 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, mal-placed 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 400 microliters (0.4 ml) per hour. However, the infusion of the first syringe(s) will actually last 3 hours and 35 minutes to allow flushing of the saline solution from the catheter(s). The infusion rate for the flush will be 400 microliters (0.4 ml) per hour for 35 minutes per catheter. After the 35 minute flush, the infusion rate will be changed to the appropriate rate for the G207 infusion. Subsequent to this, the rate of administration through each individual catheter shall be determined by the equation:

400 microliters (0.4 ml) per hour/number of active catheters.

The total volume administered from each syringe will be determined by the number of catheters actively infusing, using a volume of 400 microliters (0.4 ml) per hour as a guide. Each initial loaded syringe will be replaced at 3 hours 35 minutes after the infusion is started with a new loaded syringe that has been maintained at 4^{o}C and new infusion tubing. Refer to table below.

Table : Plan for Loading rate and Infusion Rate for G207 HSV

Number of Catheters	Initial Loading Rate for First 35 mins per catheter	Final Infusion Rate per catheter
1	0.4 cc/hour	0.4cc/hour
2	0.4 cc/hour	0.2cc/hour
3	0.4 cc/hour	0.13 cc/hour
4	0.4 cc/hour	0.1cc/hour

For each of the study drug infusion periods [active catheter(s)/pump(s)], the study drug syringe will be connected to the infusion tubing, that has been flushed with sterile PBS + 10% glycerol (Alanza) . The infusion tubing (PIT-400, Sophysa) will then be primed with 4.6 ml of the diluted G207 virus preparation in the syringe and connected to the catheter. The first infusion period will begin with a 35 minute flush of the saline solution from the catheter at a rate of 0.4ml/hour/catheter. After completion of the flush the pump will be reprogramed to the appropriate rate for the study drug infusion and the infusion begun. After the first period of infusion is complete, the infusion of the study drug from a single syringe at 400 microliters/hour should be 1.2ml. The infusion will be stopped, and the above procedure (minus the flush) repeated for the second syringe that has been stored at 4^{o}C and new infusion tubing. After two infusion periods, the delivery of the total planned dose should be complete.

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 400 microliters (0.4 ml) per hour. Two 3-hour infusion intervals were chosen based on previous stability testing to ensure no detectable loss of virus activity. Infusion may extend up to 12 hours, if needed due to catheter malfunction.

6.4 Patient Management Following Injection

After surgery, patients will be transferred from the recovery room to the Pediatric Intensive Care Unit where vital signs (temperature, blood pressure, pulse, respiratory rate) and neurologic function (Glascow Coma Scale and limited neurological exam) will be monitored every hour x 6, then every two hours overnight for the first 24 hours. Patients that appear to be stable after 24 hours will be moved to a general inpatient room at COA, and the frequency of monitoring will be determined by the attending physician(s) based on the medical condition of the patient.

6.4.1 FOCAL NEUROLOGICAL DEFICITS: General neurologic worsening (decreased state of consciousness) including focal neurological deficits or specific neurological worsening (paresis, dysphasia, etc., depending upon location of tumor), could be due to edema, hematoma, hydrocephalus, or encephalitis. An MRI or CT will be done to help determine the cause.

6.4.2 CEREBRAL EDEMA: This is common in tumor patients and occurs post-operatively and usually responds to standard measures for the treatment of increased intracranial pressure. Although medical management with systemic steroids is often very effective, patients occasionally require a ventriculostomy or surgical shunting procedure.

6.4.3 HEMATOMA: PT, PTT, and platelet count will be obtained. A small hematoma noted on a scheduled MRI may simply be watched and the patient treated as above for edema and then rescanned to exclude an enlarging lesion. A large hematoma or one associated with progressive neurologic deterioration may require operative evacuation.

6.4.4 ENCEPHALITIS: Patients who develop fever >101.5F and/or alterations in mental status/waning Glascow Coma Scale, or seizures will immediately undergo general physical and neurologic examination. If the patient has fever without any neurologic signs/symptoms, source for systemic infection will be investigated. If no source for fever can be found and fever persists for greater than 12 hours, evaluation for encephalitis will take place. Patients who have neurologic changes as above will also undergo immediate evaluation for encephalitis. CT or MRI will be performed. If this study demonstrates an increase in the area of hemorrhagic necrosis extending beyond the borders of the tumor Post-operative bed, a stereotactic biopsy will be taken to assess for presence of G207 or HSV-1(F) virus. This will consist of a minimum of 2-3 needle core biopsies which will undergo standard hematoxylin and eosin (H&E) staining, as well as immunostaining for HSV-1, PCR and culture, CD68, leukocyte common antigen, glial fibrillary acidic protein (GFAP), and X-gal staining for the beta-gal product. This will be reviewed by the Pediatric Neuropathologist at COA. While waiting for biopsy results, the patient will be empirically started on intravenous acyclovir at a dose of 30 mg/kg divided every 8 hours. At the time of biopsy, a sample of

CSF will be obtained if safe and checked for HSV by PCR to rule out HSV meningitis. Patients whose clinical course is suggestive of HSV encephalitis but imaging studies do not show a change from baseline, will undergo lumbar puncture for CSF examination as well. Those patients will be treated with empiric acyclovir as well. Patients who have tissue, CSF, or strong clinical evidence for HSV encephalitis will be treated for a 14-day course of acyclovir therapy. The medical monitor will be consulted immediately for any suspected cases of encephalitis and be integrally involved in evaluation and treatment decisions.

6.4.5 DISSEMINATED HSV: Disseminated multi-organ disease attributed to HSV is exceedingly uncommon, even in the immunocompromised host. Such disease has been encountered in newborns with disease identified as 'disseminated multi-organ involvement' and occurs in about 25% of newborns with this disease (incidence 1:10,000 deliveries) (42). In all circumstances, disseminated disease is associated with a systemic host response of fever, and findings compatible with sepsis, including hepatic dysfunction. To monitor for these rare events, most importantly, serial physical examinations will be performed to assess for fever and symptoms of sepsis or hepatic dysfunction. In addition, serial blood specimens will be obtained and cultured for infectious HSV at days 1, 3 or 4, 7, 14, 28; months 3, 5, 7, 9, 12, 18, 24; and yearly thereafter. Any positive cultures will be analyzed by PCR to discriminate between wild type HSV-1 and mutant G207 HSV. Should a patient develop fever, rash, or unexplained elevations in liver function tests in conjunction with a concomitant increase of infectious HSV by plaque assay in blood specimens (exceeding prior values by 2 logs), patients will undergo a 21 day course of treatment with acyclovir at a dose of 30mg/kg divided every 8 hours for presumed disseminated HSV infection. The medical monitor will be consulted immediately for any suspected cases of disseminated HSV and be integrally involved in evaluation and treatment decisions. Parenthetically, it should be noted that oncolytic HSV therapy for metastatic colon carcinoma involving the liver led to detectable HSV DNA in the blood but in the ABSENCE of clinical symptomatology (43); thus, increasing levels as well as fever will both be required to therapeutically intervene. Should two Grade 3 or 4 toxicities attributable to disseminated HSV infection occur in the same dose level, the prior dose level shall be declared the MTD and enrollment will be halted.

6.4.6 VIRAL SHEDDING DUE TO CSF LEAK OR BODY FLUID VIRAL CONTAMINATION: Patients with CSF leak will receive the standard of care by neurosurgery to close the leak. HSV culture and PCR of the CSF will be performed prior to closure. Any abnormal fluid collection at the surgical site will be assessed by neurosurgery and HSV culture and PCR of the fluid will be performed. Any signs of encephalitis or HSV dissemination will be managed as outlined above. Patients will be assessed for HSV shedding from body fluids (saliva, conjunctival secretions, and blood) routinely throughout the study (see Appendix A). Any patients with body fluid viral contamination will be placed on contact precautions until there are two negative follow-up samples.

6.5 Radiation Planning and Treatment

For patients receiving radiation, a 100 SAD linear accelerator with at least 6 MV beam energy will be utilized for radiation treatment.

6.5.1 Simulation and Immobilization

On postoperative day 1, all patients will undergo simulation and radiation treatment. The patient will generally be placed in the supine position, but prone positioning is allowed. 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.

6.5.2 Target Volumes and Treatment Planning

The postoperative 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 RT planning are shown in the table below and follow ICRU-62 conventions. The goal of treatment planning is coverage of the PTV1 and 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 chiasms) should be limited to 3 Gy or less unless coverage of the GTV would be compromised. The 2 mm margin for setup and registration error may be dosimetrically compromised in cases which gross tumor is very close to critical structures such as the brainstem or optic apparatus. In no case should gross disease receive less than the prescription dose.

Volume	**Abbreviation**	**Definition**	**Dose**
Gross Tumor Volume	GTV	Enhancing residual plus resection	-
Clinical Target Volume	CTV	2 abnormality	-
Planning Target Volume 1	PTV1	TV plus 2 mm	5 Gy
Planning Target Volume 2	PTV2	TV plus 2 mm	3 Gy

Generally, it is expected that multiple axial or non-axial beams (5-9) will be required to generate an acceptable plan. Field segmentation will often allow differential dosing to the PTV1 and PTV2 without computer optimization. Computer optimization and intensity modulated radiation therapy are allowed but not required. Plans utilizing computer optimization will need ion chamber measurement before delivery.

The treating radiation oncologist must review all treatment and setup/localization films before treatment.

6.6 Supportive Care Guidelines

Appropriate supportive care during the duration of the study includes the following:

6.6.1 Steroid administration for neurologic symptoms arising from increased edema or intracranial pressure

6.6.2 Proton pump inhibitors or H2 antagonists for control of steroid-induced gastric irritation

6.6.3 Anti-epileptic medicines for control of partial or generalized seizures

6.6.4 Post-operative neurological intensive care that is routine for the neurosurgical interventions involved in the administration of G207

6.6.5 Other than a restriction on medications with anti-HSV activity (to be given only for the management of an adverse event), there are not any limitations on concomitant medications that patients may receive for other co-morbidities.

6.7 Study Evaluations by Time Point (Appendix A)

6.7.1 Day 0 Evaluations

Vital signs (temperature, blood pressure, pulse, respiratory rate)
General physical and complete neurologic exam
Neurologic function (Glascow Coma Scale and neurological function) will be monitored every hour x 6, then every 2 hours overnight for the first 24 hours.

6.7.2 Day 1 - 4 Evaluations

Vital signs
Complete neurological exam
Modified Lansky or Karnofsky status determination (Appendix B)
MRI with and without contrast 72 hours after surgery. This MRI will be the first in a series of MRIs, and will be used a baseline for evaluation of potential clinical response. Should there be medical indication prior to 72 hours, MRI will be performed as deemed necessary by the Principal Investigator or medical monitors.
Chemistry Profile/Hematology with platelets and differential analysis, Day 2
Saliva and conjunctival secretions and blood will be checked for the presence of HSV by PCR and culture to evaluate for viral shedding and/or viremia, Day 3 or 4

6.7.3 Day 7 Evaluations

Neurological exam
Modified Lansky or Karnofsky status determination
Vital Signs

6.7.4 Day 14 Evaluations

Neurological exam
Modified Lansky or Karnofsky status determination
Vital Signs

6.7.5 Day 28 (+/- 4 days) Evaluation

Vital Signs
HSV antibody titer by ELISA
HSV culture and PCR of saliva and conjunctival secretions, and blood to rule out viral shedding and/or viremia
MRI with/without contrast
Chemistry Profile/Hematology with platelets and differential analysis
Complete neurological exam
Modified Lansky or Karnofsky score

6.7.6 Month 3, 5, 7, 9 and 12 (+/- 8 days) Evaluations

MRI with/without contrast
Complete neurological exam

HSV culture and PCR of saliva, conjunctival secretions, and blood to rule out viral shedding and/or viremia
HSV antibody titer by ELISA

6.7.7 Months 18, 24, and yearly Follow-up

All surviving study patients will receive follow-up clinic visits at 18 and 24 months and then yearly for up to 15 years after therapy for assessment of adverse events/serious adverse events even if the study is terminated or the patient withdraws from the study. Particular attention will be paid to any symptoms (neurological or otherwise) consistent with HSV infection. Patients who are unable to follow-up in person will be contacted by telephone on a yearly basis to monitor for delayed events.

6.7.8 Quality of life **(**optional consent) will be assessed at study enrollment, 1, 3 and 6 month visits by using the PedsQLTM 4.0 Generic Core Scales and the PedsQLTM Brain Tumor Module, WHOQOL Group Quality of Life BREF, and the Brief Symptom Inventory 18.

7.0 Study Test Article:

7.1 Investigational Product Designation

G207 is supplied by Aettis, Inc. in sterile, labeled 1.0 mL cryovials containing 0.12 mL of G207 suspended in the storage buffer, D-PBS/10% glycerin. The vials should remain frozen at -60C or below until use. G207 will be stored in the pediatric hematology-oncology pharmacy at COA prior to being injected. See appendix C for preparation guidelines.

7.2 Dose

A total of 2.4 mL (in 1-4 doses) will be used for treatment. To prepare the dose, the vial should be removed from the -60º C freezer and rubbed gently between gloved hands until all the ice crystals have melted. The vial should then be placed on ice. It should be thawed completely before removing the cap. Care should be taken to ensure that all the liquid is at the bottom of the vial before removing the cap to avoid spillage. If it is suspected that the contents are on the side or top of the vial, the vial can be tapped gently on a flat surface. The appropriate dose level will be prepared according to the dose escalation scheme. The final diluted G207 should be gently withdrawn into the syringe for injection. Complete instructions for dose preparation will be provided prior to initiation of the clinical study.

7.3 Precautions in Handling and Disposal

7.3.1 General Procedures

Sterile technique and Biosafety Level 2 precautions (gown, gloves, mask) will be rigorously followed while preparing the dose. The dose preparations will take place in a biosafety hood. The vial of G207 will be removed from the controlled access freezer and thawed as above in section 8.2. It will be allowed to thaw completely prior to removing cap. Care will be taken to ensure that all of the liquid is at the bottom of the vial prior to removing the cap and that the cap is removed carefully to avoid spilling or contamination.

7.3.2 Disposal Procedures

All materials that have been in contact with the G207 herpes virus are considered to

be infectious biohazards, and must be decontaminated or incinerated prior to disposal. Needles and syringes should be placed into a puncture-resistant, leak-proof container containing disinfectant. All materials that have been in contact with the vector must be incinerated in an institutionally approved biohazard incinerator before disposal

7.3.3 Investigational Drug Accountability

The Investigator must maintain accurate records of dates, quantities, and lots of product(s) received, to whom dispensed (subject-by-subject accounting), and accounts of any product accidentally destroyed. The Investigator must retain all unused, partially used or expired product until the drug manufacturer has confirmed accountability data.

At the conclusion of drug administration, all unused and partially used drug supplies will be returned to Aettis, Inc., An overall summary of all drug supplies received, unused, partially used, wasted, and returned must be prepared at the conclusion of the study.

8.0 Study Conduct:

8.1 Clinical Events: Adverse Medical Experiences and Concurrent Illnesses

8.1.1 Definitions of Untoward Events Occurring During the Study

Throughout the study, all adverse clinical events will be reported. In the event of an adverse event or potential adverse event, the medical monitors will be immediately contacted and appropriate medical intervention will be instituted. The UAB Neuro-Oncology Clinical Trials Monitoring Committee will also be immediately notified. Clinical protocol activities may be suspended or discontinued as necessary for the safety of the patient.

Adverse Experience (AE): A clinical event which is significant, without regard to strength of association with G207 administration. Factors which weigh in the clinical assessment of clinical significance will be recorded in the medical record by the principal investigator. An AE may include changes in signs, symptoms, pre-existing conditions, or laboratory tests. Clinical events which lead to the addition of concomitant medication or an increase in the dose or frequency of concomitant medication are generally considered clinically significant. An abnormal test result is considered an AE if: (1) it is not associated with an already reported AE or diagnosis, or pre-existing condition, and there is an impact on test article administration, or there is a change in concomitant medication, or (2) the abnormal test result indicates a serious or life-threatening condition.

Laboratory Abnormalities: All abnormal laboratory values must be evaluated according to Common Toxicity Criteria. Clinically significant changes from baseline in hematology, serum chemistry, and/or urinalysis values, within or outside of the reference (normal) range, are notable and may constitute an adverse medical experience. All laboratory values found to be significantly above or below the institutional norm must be repeated and evaluated by the Investigator as soon as possible, and documented on the case report form. An abnormal clinical laboratory result corresponding to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 Grade 4 is by definition a life threatening experience.

Serious Adverse Event (SAE): Any clinical event, without regard to strength of association with G207 administration, that is fatal or life-threatening, categorized as Grade 3/4 Toxicity according the CTEP CTCAE v4.0, permanently disabling, or requires

or prolongs hospitalization. Congenital anomalies and new occurrences of cancer also will be considered as serious adverse events.

Unexpected Event: Any clinical event that is not identified in nature, severity or frequency in the risk information supplied by the manufacturer of the investigational agent.

Associated with the Use of the Drug: A reasonable possibility exists that the test article may have directly or indirectly caused the clinical event. The Investigator will be unbiased in assessing the relationship of the event and test article and discuss the possible relatedness to treatment with the Medical Monitor. Factors which weigh in the clinical assessment of causality will be recorded in the medical record by the principal investigator. The following criteria will be used to describe association of AEs and SAEs with G207:

A. Definitely Related: There is a clinically plausible time sequence between the onset of the AE and G207 administration and all other potential causes have been ruled out.

B. Probably Related: There is a clinically plausible time sequence between the onset of the AE and G207 administration; the AE is unlikely to be caused by the concurrent/underlying illness, other drugs, or procedures; and (if applicable) the AE follows a clinically consistent resolution pattern upon withdrawal of G207.

C. Possibly Related: There may or may not be a clinically plausible time sequence between the onset of the AE and G207 administration and investigational drug as a cause cannot be ruled out.

D. Unlikely Related: There is probably NOT a clinically plausible time sequence between the onset of the AE and G207 administration; the AE is likely to be caused by the concurrent/underlying illness, other drugs, or procedures (if applicable) and the AE does not follow a clinically consistent resolution pattern of treatment with G207.

E. Not Related: Another cause of the AE is most plausible; a clinically plausible temporal sequence is inconsistent with the onset of the AE and G207 administration; and/or a causal relationship is considered biologically implausible.

8.2 Reporting of Clinical Events

All clinical events will be recorded on the Case Report Form. In order to avoid vague, ambiguous or colloquial phrases, the event should be recorded using standard medical terminology rather than the subject's own words. A more verbatim account of the event may be recorded in the source document if desired. Concurrent conditions will be recorded on the medical history, physical exam, or laboratory forms. Adverse experiences will be recorded on the AE form. The Investigator will include the date of occurrence, the date of cessation (or the fact that it is still continuing), the intensity, and the relationship to test article administration, any resulting treatment or change in treatment, and the outcome of the event.

For Grade 2-3 unexpected events, the investigator will be unbiased in assessing the relationship of the event and test article and discuss the possible relatedness to treatment with the Medical Monitors. Adverse event and IND safety reports will be filed as soon as possible but in

no case later than 15 calendar days after determination the event qualifies for reporting with institutional authorities (IRB, institutional biosafety committee (IBC)) as well as with the FDA. All serious adverse events (Grade 3 or 4 toxicities) will be reported by fax, e-mail or phone within 24 hours and a written expedited report filed within 7 days.

8.3 Reporting of Serious Life-Threatening Adverse Event

Any serious, life-threatening suspected or unexpected adverse event which occurs during the conduct of this study, regardless of relationship to test article or procedures, will be reported within 24 hours and a written report filed within 7 days after the event to the Medical Monitors, the Investigator's Institutional Review Board (IRB), the UAB CTMC, and the FDA.

8.4 Declaration of Treatment Failure - Patient Taken Off Study

Patients whose tumors show radiographic or clinical evidence of progressive growth will be declared Treatment Failures and may be considered for some other available therapy without restriction, including standard (re-resection, repeat fractionated radiotherapy, and/or chemotherapy) and experimental (biological agents, gene therapy, and novel combinations and administration routes of existing agents) interventions. Patients receiving alternative cancer therapies will continue to be followed in this study. Patients may remove themselves (or may be removed by their guardian) from further participation in this clinical study at personal discretion.

8.5 Insurance and Indemnity

Information on compensation, insurance and indemnity will be supplied to the investigator in the clinical agreement.

8.6 Protocol Deviations

Except in cases that would commonly be considered medical emergencies, the investigator will not deviate from the protocol without prior permission from the oversight committee and the IRB. In the event of a medical emergency, these two regulatory boards will be notified as soon as possible. All other changes in the study procedures will be implemented as amendments and will be approved by the IRB before implementation.

8.7 Data and Safety Monitoring Plan

Because this study is a Phase I trial investigating the safety of a newly developed agent being administered to pediatric patients for the first time and has a relatively small number of patients enrolled at a single institution that will be assuming the full burden of risk, the principal investigator, medical monitors, and UAB Clinical Trials Monitoring committee (CTMC) will be involved in continuous monitoring of the safety data for all patients that receive the investigational drug. The principal investigator will have direct accountability to the CTMC, and will submit monthly, detailed reports to the committee concerning the immediate and long-term follow-up safety of all patients that have received the investigational agent. These reports will be reviewed by the CTMC on a regularly scheduled basis, with additional reviews being conducted both prior to any dose escalation and immediately following any evidence of unacceptable toxicity.

8.8 Retention of Records

To comply with FDA regulations, the Investigator must maintain the records of investigational drug disposition, subject exclusion logs, signed consent forms, electronic Case

Report Forms, all correspondence, and supporting documentation for a minimum of 2 years after the date on which a US marketing application has been approved for the indication investigated in this study or, if no application is to be filed, until 2 years after the investigation is discontinued and the FDA/OBA are notified. The Investigator may withdraw from the responsibility to maintain records and transfer custody of the records to another person who will accept responsibility for them.

9.0 Study Analysis:

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, median, and maximum. Categorical variables will be summarized using frequency counts and percentages. For time-to-event variables, median time-to-event, and other percentiles may be presented. Additional exploratory analyses of the data on some of the secondary endpoints will be conducted as deemed appropriate.

9.1 Analysis of Safety

9.1.1 Safety and Tolerance of G207 Administration (Primary Endpoint #1)

All subjects who receive G207 will be included in the safety analysis. Adverse events will be described and the frequency of events will be both tabulated overall and by dose group. All events with a Grade 3 or above toxicity (where toxicity is defined by the CTCAE v4.0) will be summarized separately and tabulated by event and by relationship to G207, both overall and by dose group. Laboratory analyses (chemistries, hematology, serological/immunological analyses, PCR, cultures, WBC and differential) will consist of measurements of change from baseline over time by patient and overall, with plots of actual values compared to normal values for patients by dose group. Logarithmic transformations may be applied as necessary. Group means and standard errors will be calculated for the various laboratory parameters. Concurrent illnesses will be listed and examined as possible confounders in the treatment response relationship. Concurrent medications will also be listed, as will previous treatments for malignant brain tumors. Effects of concomitant medications and previous treatments for cancer and any potential related side effects will be analyzed and discussed. The MTD or maximal planned dose, if no dose-limiting toxicity is observed, will be established.

9.1.2 Immunologic Response to G207 (Secondary Endpoint #1)

HSV-1 antibody titers will be checked by ELISA on all patients prior to the administration of G207 and at Day 28 and Months 3, 5, 7, 9 and 12. Descriptive statistics of this secondary endpoint will be computed.

9.1.3 Virologic Shedding Following G207 (Secondary Endpoint #2)

Saliva, blood and conjunctival secretions of all patients will be checked by PCR and culture at regular intervals (prior to administration, Day 1, 3 or 4, 7, 14, 28, and Months 3, 5, 7, 9, 12, 18, 24 and yearly thereafter) for evidence of HSV shedding and/or viremia. Descriptive statistics of this secondary endpoint will be computed.

9.2 Clinical Response (Secondary Endpoint #3)

Although not a primary objective of the study, an evaluation of response will be performed. Response rates will be calculated in treated patients that are considered evaluable. All

time-to-event variables (PFS, OS) will be assessed. Descriptive statistics of this secondary endpoint will be computed.

9.2.1 Radiographic Response

Following the treatment, an evaluation of response will be performed. MRI study prior to G207 administration will be considered the baseline. Technical parameters for the baseline scan and evaluation scans will be identical (see appendix D). Two-dimensional measurements obtained at the baseline scan will be compared with the follow-up MRI scans for assessment of response as described in the Response Assessment in Neuro-Oncology (RANO) criteria and summarized below:

Complete Response (CR): Complete disappearance of all abnormal enhancing component of the tumor sustained for at least 4 weeks.

Partial response (PR): ≥50% decrease in the product of the two perpendicular diameters of the target lesion compared to the baseline measurements, taking as reference the initial baseline measurements

Stable Disease (SD): Neither sufficient decrease in the products of the two perpendicular diameters of the target lesion to qualify for PR (taking as reference the initial baseline measurements), nor sufficient increase in the target lesion to qualify for PD, (taking as reference the smallest disease measurement since the treatment started).

Progressive Disease (PD): ≥25% increase in the product of the two perpendicular diameters of the target lesion compared to the baseline measurements, or appearance of new lesions.

The percent of subjects who, at any time point following G207 administration and prior to other cancer therapy, demonstrate PD, SD, PR, CR will be summarized. Patients' radiographic responses will also be reported by evaluation time points and by dosing cohorts. Longitudinal evaluation of region-of-interest based and histogram based analysis of the diffusion parameters (apparent diffusion coefficient and mean diffusivity) and perfusion parameters (K^{trans} and rCBV) will be performed both at the baseline and follow-up MRIs to demonstrate if change of these parameters from baseline is related to the infused dose of virus and if the change from baseline can predict progression-free and overall survival. Changes in clinical disease status and steroid administration will be considered at each time point of imaging evaluation. Measurements will also be analyzed with consideration of any anti-tumor cancer therapies and timeframes administered.

9.2.2 Performance Score

A Modified Lansky or Karnofsky score will be recorded and measured serially with the pre-treatment score. Time to a Karnofsky score of ≤30 (modified Lansky score of ≤30 in children ≤10 years of age) will be explored. Descriptive statistics of this secondary endpoint will be computed.

9.2.3 Progression Free survival

Time after G207 administration to clinical and radiographic disease progression (progression free survival) will be evaluated in all patients receiving G207. Descriptive statistics

of this secondary endpoint will be computed.

9.2.4 Overall Survival

The overall survival for each patient receiving G207 will also be reported. Descriptive statistics of this secondary endpoint will be computed.

9.2.5 Quality of Life (optional consent)

Each patient's self-report (or the caretaker's report, if the patient is <60 months old) of quality of life will be measured with the PedsQOL questionnaire at baseline (before administration of G207) and at specified times thereafter. The primary caregiver will also complete the WHOQOL Group Quality of Life BREF, which measures parent quality of life, and the Brief Symptom Inventory 18, which measures distress. The clinical course of the tumor will be taken into account in the interpretation of the trends that are found. Descriptive statistics of this secondary endpoint will be computed.

References:

1. Markert, JM, Medlock MD, Rabkin SD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther* 7:867-74, 2000
2. Markert, JM, Liechty PG, Wang W, et al. Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. *Mol Ther*, 17:199-207, 2009
3. Markert, JM, Razdan SN, Kuo HC, et al. A Phase 1 Trial of Oncolytic HSV-1, G207, Given in Combination With Radiation for Recurrent GBM Demonstrates Safety and Radiographic Responses. *Mol Ther*. 22:1048-55, 2014
4. Gatta G, Capocaccia R, Coleman MP, Ries LA, Berrino F. Childhood cancer survival in Europe and the United States. *Cancer*. 95:1767-72, 2002
5. Cohen KJ, Pollack IF, Zhou T, et al. Temozolomide in the treatment of high-grade gliomas in children: a report from the Children's Oncology Group. *Neuro Oncol*. 13:317-23, 2011
6. Gajjar A and Pizer B. Role of high-dose chemotherapy for recurrent medulloblastoma and other CNS primitive neuroectodermal tumors. *Pediatr Blood Cancer*. 54:649-51, 2010
7. Diller L, Chow EJ, Gurney JG, et al. Chronic disease in the Childhood Cancer Survivor Study cohort: a review of published findings. *J Clin Oncol*. 27:2339-55, 2009
8. Ribi K, Relly C, Landolt MA, Alber FD, Boltshauser E, Grotzer M.A. Outcome of medulloblastoma in children: long-term complications and quality of life. *Neuropediatrics.* 36:357-65, 2005
9. Kiltie AE, Lashford LS, Gattamaneni HR. Survival and late effects in medulloblastoma patients treated with craniospinal irradiation under three years old. *Med Pediatr Oncol*. 28:348-54, 1997
10. Packer RJ. Sutton LN, Atkins TE, et al. A prospective study of cognitive function in children receiving whole-brain radiotherapy and chemotherapy: 2-year results. *J Neurosurg* 70:707-13,1989
11. Roizmzn B, Sears AE. Herpes Simplex Viruses and their Replication. In Fundamental Virology, 3rd ed, pp 1043-1107. Edited by B.N. Fields, D.M. Knipe & P.M. Howley. Philadelphia: Lippincott-Raven, 1996
12. Chou J, Kern ER, Whitley RJ, Roizman B. Mapping of herpes simplex virus-1 neurovirulence to gamma 134.5, a gene nonessential for growth in culture. *Science* 250;1262-6, 1990
13. Chou J, Roizman, B. Herpes simplex virus 1 gamma(1)34.5 gene function, which blocks the host response to infection, maps in the homologous domain of the genes expressed during growth arrest and DNA damage. *Proc Natl Acad Sci U S A.* 91:5247-51. 1994
14. Martuza RL, Malick A, Markert JM, Ruffner KL, Coen DM. Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* 252:854-6, 1991
15. Markert JM, Coen DM, Malick A, Mineta T, Martuza RL. Expanded spectrum of viral therapy in the treatment of nervous system tumors. *J Neurosurg* 77:590-4, 1992
16. Markert JM, Malick A, Coen DM, Martuza RL. Reduction and elimination of encephalitis in an experimental glioma therapy model with attenuated herpes simplex

mutants that retain susceptibility to acyclovir. *Neurosurgery* 32:597-603, 1993
17. Mineta T, Rabkin SD, Martuza RL. Treatment of malignant gliomas using ganciclovir-hypersensitive, ribonucleotide reductase-deficient herpes simplex viral mutant. *Cancer Res* 54:3963-6, 1994
18. Chambers R. Gillespie GY, Soroceanu L, et al. Comparison of genetically engineered herpes simplex viruses for the treatment of brain tumors in a scid mouse model of human malignant glioma. *Proc Natl Acad Sci U S A*. 92:1411-5, 1995
19. Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nat Med* 1:938-43, 1995
20. Markert JM, Gillespie GY, Weichselbaum RR, Roizman B, Whitley, R. J. Genetically engineered HSV in the treatment of glioma: a review. *Rev Med Virol*. 10:17-30, 2000
21. Whitley RJ, Alford CA, Hirsch MS, et al. Vidarabine versus acyclovir therapy in herpes simplex encephalitis. *N Engl J Med.* 314:144-9, 1986
22. Goldstein DJ, Weller SK. Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: isolation and characterization of an ICP6 lacZ insertion mutant. *J Virol* 62, 196-205 (1988).
23. Gillory LA, Megison ML, Stewart JE, et al. Preclinical evaluation of engineered oncolytic herpes simplex virus for the treatment of neuroblastoma. *PLoS One*. 8:e77753, 2013
24. Megison ML, Gillory LA, Stewart JE, et al. Preclinical evaluation of engineered oncolytic herpes simplex virus for the treatment of pediatric solid tumors. *PLoS One*. 9:e86843, 2014
25. Pressey JG, Haas MC, Pressey CS, Kelly VM, Parker JN, Gillespie GY, Friedman GK. CD133 marks a myogenically primitive subpopulation in rhabdomyosarcoma cell lines that are relatively chemoresistant but sensitive to mutant HSV. *Pediatr Blood Cancer*. 60:45-52, 2013.
26. Friedman GK, Langford C, Coleman J, Cassady K, Parker J, Markert J, Gillespie GY. Engineered herpes simplex virus efficiently infects and kills CD133+ glioma cells that express CD111. *J Neurooncol*. 95:199-209. 2009
27. Friedman GK, Kelly VM, Bray A, Cassady KA, Markert JM, Gillespie GY. Clinically-ready oncolytic engineered herpes simplex viruses kill medulloblastoma neurospheres from patient-derived xenolines. *Neuro Oncol*. 14(Suppl 1):91-92, 2012.
28. Friedman GK, Bharara S, Langford C, Coleman J, Gillespie GY. Engineered herpes simplex virus oncolytic therapy kills both tumor cells and glioma progenitor cells in a pediatric high-grade glioma. Abstracts from the Thirteenth International Symposium on Pediatric Neuro-Oncology. *Neuro Oncol*. 10:508-509, 2008.
30. Friedman GK, Pressey JG, Reddy AT, Markert JM, Gillespie GY. Herpes simplex virus oncolytic therapies for pediatric malignancies. *Mol Ther*. 17:1125-35, 2009
31. Andreansky S, Soroceanu L, Flotte ER. Evaluation of genetically engineered herpes simplex viruses as oncolytic agents for human malignant brain tumors. *Cancer Res* 57:1502-9, 1997
32. Sundaresan P, Hunter WD, Martuza RL, Rabkin SD. Attenuated replication-competent herpes simplex virus 1 mutant G207: safety evaluation in mice. *J Virol* 74:3832-3841, 2000
33. Hunter, W.D. Martuza RL, Feigenbaum F, et al. Attenuated, Replication-Competent Herpes Simplex Virus Type 1 Mutant G207: Safety Evaluation of Intracerebral Injection

in Nonhuman Primates. *J Virol*, 73:6319-6326, 1999

34. Advani S., Sibley G, Hallahan DE, Roizman B, Weichselbaum RR. Enhancement of replication of Genetically Engineered Herpes Simplex Virus by ionizing radiation: a new paradigm for destruction of intractable tumors. *Gene Ther* 5: 160-165, 1998
35. Bradley JD, Kataoka Y, Advani S, et al. Ionizing radiation improves survival in mice bearing intracranial high-grade gliomas injected with genetically modified herpes simplex virus. *Clin Cancer Res* 5: 1517-1522, 1999
36. Stanziale SF, Petrowsky H, Joe JK, Roberts GD, Zager JS, Gusani NJ, Ben-Porat L, Gonen M, Fong Y. Ionizing radiation potentiates the antitumor efficacy of oncolytic herpes simplex virus G207 by upregulating ribonucleotide reductase. *Surgery*. 132:353-9, 2002
37. Xu F, Lee FK, Morrow RA, Sternberg MR, Luther KE, Dubin G et al. Seroprevalence of herpes simplex virus type 1 in children in the United States. *J Pediatr* 151:374–377, 2007
38. Whitley R.J., Kimberlin D.W. and Roizman B. Herpes Simplex Virus. *Clin Infect Dis* 26:541-553, 1998
39. Fontanilla HP, Pinnix CC, Ketonen LM, Woo SY, Vats TS, Rytting ME, Wolff JE, Mahajan A.Palliative reirradiation for progressive diffuse intrinsic pontine glioma. *Am J Clin Oncol* 35:51-57, 2012
40. Nieder C, Astner ST, Mehta MP, Grosu AL, Molls M. Improvement, clinical course, and quality of life after palliative radiotherapy for recurrent glioblastoma. *Am J Clin Oncol* 31:300-305, 2008
41. Bauman GS, Sneed PK, Wara WM, Stalpers LJ, Chang SM, McDermott MW, Gutin PH, Larson DA. Reirradiation of primary CNS tumors. *Int J Radiat Oncol Biol Phys* 36:433-441, 1996.
42. Kimberlin DW, Whitley RJ. Viral infections of the newborn infant. In: Rudolph's Pediatrics, 22nd edition. Rudolph CD, Rudolph AM, Hostetter MK, Lister G, Siegel NJ (eds). McGraw Hill Medical, New York, March, 2011.
43. G, Mescheder A, Jarnagin W, Kemeny N. A herpes oncolytic virus can be delivered via the vasculature to produce biologic changes in human colorectal cancer. *Mol Ther*. 17:389-94, 2009

APPENDICES

Appendix A

Patient Study Schedule

Appendix B

Modified Lansky and Karnofsky Status Determination

Appendix C

Preparation of G207

Appendix D

MRI Sequences

APPENDIX A

Patient Study Schedule

	Pre	Day 0	Day 1	Day 2	Day 3or 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 for adolescent female	X												
CT Scan		X	X#										
Dosing		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.

APPENDIX B

The Modified Lansky Performance Scale (Patients < 10 year)

MODIFIED LANSKY PERFORMANCE SCALE

NORMAL RANGE

100	Fully active.
90	Minor restriction in physically strenuous play.
80	Restricted in strenuous play, tires more easily, otherwise active.

MILD-TO-MODERATE RESTRICTION

70	Both greater restriction of, and less time spent, in active play.
60	Ambulatory up to 50% of the time, limited active play with assistance and supervision.
50	Considerable assistance required for any active play; fully able to engage in quiet play.

MODERATE-TO-SEVERE

40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activity.
20	Limited to very passive activity initiated by other (e.g., television)
10	Completely disabled, not even passive play
0	Unresponsive, comatose

The Karnofsky Performance Status Scale (Patients ≥ 10 year)

100 Normal, no complaints, no evidence of disease
90 Able to carry on normal activity; some minor symptoms
80 Normal activity with some effort; some symptoms
70 Care for self; unable to carry on normal activities
60 Requires occasional assistance; cares for most needs
50 Requires considerable assistance and frequent care
40 Disabled; requires special care and assistance
30 Severely disabled; hospitalized, death not imminent
20 Very sick; active support treatment needed
10 Moribund; fatal processes are rapidly progressing

Appendix C

Preparation of G207 dilutions will take place in the hospital pharmacy.

G207 should be prepared for administration following Biosafety Level 2 Guidelines using a laminar flow biological safety cabinet, hood, gloves, safety glasses and gown.

MATERIALS NEEDED FOR PREPARATION OF G207 DOSE FOR SUBJECT ADMINISTRATION

1. 70% Isopropanol solution
2. MetriSpray, Cavicide or equivalent disinfectant.
3. U-100 Micro-fine IV insulin syringe with fixed 28G 1cm (or 1/2 inch) needle for virus delivery (total of 6 syringes needed)
4. Tuberculin syringe and 18-23 G needle
5. Sterile Vector Diluent (0.9% sodium chloride, USP, preservative-free for injection) cooled to 4°C.
6. Sterile Gloves
7. Alcohol Swabs/Wipes
8. Kim-wipes

EQUIPMENT NEEDED FOR PREPARATION OF G207 DOSE FOR SUBJECT ADMINISTRATION

1. Laminar Flow Biological Safety Cabinet (LFBSC)
2. Rack for vector G207 Stock Vials
3. Refrigerator
4. Wet ice or coolant

METHOD FOR PREPARATION OF G207 DILUTION FOR SUBJECT ADMINISTRATION

1. Remove all items from the LFBSC, wipe down work surfaces with MetriSpray, Cavicide or equivalent disinfectant, followed by 70% isopropanol, allow blower to run for 30 minutes prior to using.
2. Cool virus diluent (sterile preservative-free 0.9% sodium chloride, USP for injection) to approximately 4°C in a refrigerator or on ice.
3. Thaw the appropriate number of vector stock G207 vial(s) based on dosage required by rubbing gently between gloved hands. Continue until the last ice crystals have melted and then place vials on ice. Note the time that the contents have completely thawed. Do not allow the vials to warm.
4. Wipe stock G207 vials dry with Kim-Wipes. Gently mix contents of stock G207

vials.

5. Wipe stock G207 vials with alcohol swab and place in a vial rack in the LFBSC to dry.

6. Dose Preparation Guide

   6.1 Draw up the required amount of sterile preservative-free 0.9% sodium chloride, USP for injection (vector diluent)

   6.2 Remove the required volume of stock G207 from the cryovials. Add stock G207 to the receptacle/vehicle containing the required diluent

   6.3 Gently invert end over end at least 5 times to mix contents

7. Label administration syringe(s) appropriately and load 1 ml diluted G207 into each syringe.

8. Place syringe(s) in sterile syringe holder.

9. Transport syringe container immediately to patient's room for administration. Syringes will be placed in individual syringe pumps. Syringes must be kept at room temperature at all times and infusions completed within 8 hours of thawing the vector. Infusion rates for each individual catheter will be dictated by the number of catheters as follows:

   400 microliters per hour/(number of active catheters).

10. Start and stop time of G207 injection will be documented.

11. All reactions or complications encountered during the administration of G207 should be documented.

12. All remaining biological material must be chemically disinfected or autoclaved. All instruments coming in contact with G207 must be autoclaved. All other items coming in contact with G207 must be incinerated or autoclaved.

13. Waste Disposal responsibility must be managed according to the institutional SOP on disposal of hazardous waste.

Appendix D

All MRI will be performed on a 3T MRI scanner. The MRI schedule will be: baseline (prior to virus injection), Day 3, Day 28, month 3, 5, 7, 9 and 12. Unscheduled MRI scans may be done if needed to evaluate post-administration neurological decline. Sequences needed for the biopsy and resection will be determined by the operating neurosurgeon.

SAG T1
FOV
FH (mm) = 230;
AP (mm) = 230;
RL (mm) = 154;
Echoes = 1;
partial echo = "no";
TE ="user defined";
(ms) =10;
Flip angle (deg) = 70;
TR = "range";
minimum (ms) = 600;
maximum (ms) = 700;
Total scan duration = "04:09.6";
SAR / head = "< 99 %";
Whole body / level = "< 0.2 W/kg / normal";

Ax T1
FOV
AP (mm) = 230;
RL (mm) = 183.28125;
FH (mm) =141.5;
Slice thickness (mm) = 5;
TE ="user defined";
(ms) = 10;
Flip angle (deg) = 70;
TR = "range";
minimum (ms) = 600;
maximum (ms) = 700;
Total scan duration = "03:19.2";
SAR / head = "< 62 %";
Whole body / level = "< 0.1 W/kg / normal";

Ax T2
FOV
AP (mm) = 230;
RL (mm) = 182.954544;
FH (mm) = 141.5;

Slice thickness (mm) = 5;
Reconstruction matrix = 512;
TE = "user defined"
(ms) =125;
angle (deg) = 120;
echo enhancement = "no";
bright fat reduction = "no";
TR = "user defined";
(ms) = 11000;
Fast Imaging mode = "TSE";
shot mode = "multishot";
TSE factor = 31;
SAR / head = "< 33 %";
Total scan duration = "04:36.0";
Whole body / level = "< 0.1 W/kg / normal";

AX FLAIR
FOV
AP (mm) = 230;
RL (mm) = 182.954544;
FH (mm) = 141.5;
slices = 22;
slice gap = "user defined";
gap (mm) = 1.5;
Fast Imaging mode = "TSE";
shot mode = "multishot";
TSE factor = 31;
TE = "user defined";
(ms) = 125;
Refocusing control = "yes";
angle (deg) = 120;
echo enhancement = "no";
bright fat reduction = "no";
TR = "user defined";
(ms) =11000;
Total scan duration = "03:51.0";
SAR / head = "< 33 %";
Whole body / level = "< 0.1 W/kg / normal";

Ax DWI
FOV
RL (mm) = 230;
AP (mm) = 230;
FH (mm) = 149;
Slice thickness (mm) = 4;

Fast Imaging mode = "EPI";
shot mode = "single-shot"
Echoes = 1;
TE = "shortest";
Flip angle (deg) = 90;
TR = "shortest";
Diffusion mode = "DWI";
nr of b-factors = 2;
b-factor order = "ascending";
max b-factor = 1000;
average high b = "yes";
Total scan duration = "00:37.9";
SAR / head = "< 35 %";
Whole body / level = "< 0.1 W/kg / normal";

VENBOLD
FOV
AP (mm) = 200;
RL (mm) = 200;
FH (mm) = 125;
Reconstruction matrix = 512;
Scan mode = "3D";
TE = "shortest";
Flip angle (deg) = 10;
TR = "shortest";
Total scan duration = "05:46.8";
SAR / head = "< 4 %";
Whole body / level = "0.0 W/kg / normal";

T1 mapping (2, 10, 15, 20, 30degrees)
FOV
AP (mm) = 256;
RL (mm) = 168;
FH (mm) = 80;
TE = "user defined"
(ms) = 2.48000002;
Flip angle (deg) = 2, 10, 15, 20, 30;
TR = "user defined";
(ms) = 4.88000011;
Total scan duration = "00:11.5"; "00:05.7"; "00:11.5"; "00:11.5"; "00:11.5";
SAR / head = "< 1 %";
Whole body / level = "0.0 W/kg / normal";

DCE perfusion MRI
FOV
AP (mm) = 256;

RL (mm) = 168;
FH (mm) = 80;
Echoes = 1;
partial echo = "no";
shifted echo = "no";
TE = "user defined";
(ms) = 2.48000002;
Flip angle (deg) = 20;
TR = "user defined";
(ms) = 4.88000011;
Dynamic study = "individual";
dyn scans = 60;
dyn scan times = "shortest";
FOV time mode = "default";
Total scan duration = "05:43.2";
SAR / head = "< 60 %";
Whole body / level = "< 0.1 W/kg / normal";

SAG T1 THRIVE
Coil selection = "SENSE-Head-8";
FOV
FH (mm) = 250;
AP (mm) = 250;
RL (mm) = 166.200012;
Echoes = 1;
partial echo = "no";
shifted echo = "no";
TE = "shortest";
Flip angle (deg) = 10;
TR = "shortest";
Total scan duration = "04:58.6";
SAR / head = "< 21%";
Whole body / level ="0.0 W/kg / normal";

DSC PERFUSION
FOV
RL (mm) = 224;
AP (mm) = 224;
FH (mm) = 140;
Voxel size RL (mm) = 2.32999992;
AP (mm) = 2.32999992;
Slice thickness (mm) = 4;
Recon voxel size (mm) = 1.75;
Fold-over suppression = "no";

Reconstruction matrix = 128;

slice gap = "user defined";
gap (mm) = 0;
slice orientation = "transverse";
fold-over direction = "AP";
fat shift direction = "P";
TE = "user defined";
(ms) = 20;
Flip angle (deg) = 60;
TR = "shortest";
Dynamic study = "individual";
dyn scans = 60;
dyn scan times = "shortest";
Act. TR/TE (ms) = "2202 / 40";
Dyn. scan time = "00:02.2";
SAR / head = "< 27 %";
Whole body / level = "< 0.1 W/kg / normal";

Ax T1+C
FOV
RL (mm) = 224;
AP (mm) = 224;
FH (mm) = 140;
Echoes = 1;
partial echo ="no";
TE = "user defined";
(ms) = 12;
Flip angle (deg) = 70;
TR = "range";
minimum (ms) = 400;
maximum (ms) = 750;
Total scan duration = "04:08.3";
SAR / head = "< 100 %";
Whole body / level = "< 0.2 W/kg / normal";

Phase 1 Clinical Trial of HSV G207 Alone or with a Single Radiation Dose in Children with Recurrent Supratentorial Brain Tumors

IND No.: IND 16294, approved January 14, 2015

Site: **University of Alabama at Birmingham**
Principal Investigator: Gregory K. Friedman, M.D., Dept. of Pediatrics, Hematology/Oncology

Co-Investigators: James M. Johnston Jr, M.D., Dept. of Surgery, Div. of Neurosurgery
James M. Markert, M.D., Dept. of Neurosurgery
John Fiveash, M.D., Dept. of Radiation Oncology
Asim K. Bag, M.D., Dept. of Radiology, St. Jude Research Hospital
Elizabeth Alva, M.D., Dept. of Pediatrics, Hematology/Oncology
Avi Madan-Swain, Ph.D., Dept. of Pediatrics, Hem./Oncology

Medical Monitors: Clinical Trials Review Committee
Tony McGrath, M.D., Dept. of Pediatrics
Shannon Ross, M.D., Dept. of Pediatrics

Table of Contents

Appendices

Study Synopsis

Title of Study:	Phase 1 Clinical Trial of HSV G207 Alone or With a Single Radiation Dose in Children with Recurrent Supratentorial Brain Tumors
Principal Investigator:	Gregory K. Friedman, M.D., Dept. of Pediatrics, Hematology/Oncology
Co-Investigators:	James M. Johnston Jr, M.D., Dept. Neurosurgery James M. Markert, M.D., Dept. of Neurosurgery John Fiveash, M.D., Dept. of Radiation Oncology Asim K. Bag, M.D., Dept. of Radiology, St. Jude Research Hospital Elizabeth Alva, M.D., Dept. of Pediatrics, Hematology/Oncology Avi Madan-Swain, Ph.D., Dept. of Pediatrics, Hem./Oncology
Medical Monitors:	Clinical Trials Review Committee Tony McGrath, M.D., Dept. of Pediatrics Shannon Ross, M.D., Dept. of Pediatrics
Study Center:	University of Alabama at Birmingham **Children's of Alabama**
Clinical Phase:	Phase 1 (open-label)
Study Rationale:	Outcomes for children with recurrent supratentorial brain tumors are extremely poor
Study Objectives:	Primary: To assess the safety and tolerability of G207 administered intratumorally via controlled rate alone or followed by a single dose of radiation within 24 hours of G207 administration in children with recurrent or progressive malignant supratentorial brain tumors. To establish a maximum tolerated dose (MTD) or maximal planned dose if no dose-limiting toxicity is observed. Secondary: 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 by assessing radiographic response, performance scale, progression-free and overall survival, immune response, and presence of G207 in blood, saliva, and conjunctiva.
Investigational Drug:	G207 is a genetically altered herpes simplex virus that has been demonstrated to be aneurovirulent secondary to deletions of both copies of the $\gamma_1$34.5 gene. After stereotactic biopsy to confirm tumor recurrence, up to 4 silastic catheters will be passed to predefined

coordinates of enhancing tumor, if present. Catheters may be placed in non-enhancing regions if residual tumor is confirmed in those locations. Subsequently, patients will be inoculated with 2.4 ml of G207 over 6 hours alone or followed by a single 5 Gy fraction of radiation within 24 hours of virus inoculation.

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

Treatment Indication: Progressive or recurrent growth of malignant supratentorial brain tumor after initial surgery, radiotherapy, and/or chemotherapy. Patients with brainstem, cerebellar or intraventricular tumors will not be eligible.

Study Design: This study will be a Phase 1, open-label study of HSV G207 to assess its safety and tolerability. A traditional 3 + 3 design will be used. The following dose escalation scheme will be used:

Dose Level	Patients	Dose (pfu)	Volume	# Loci
-1	3	$1x10^6$	2.4 ml	1-4
1	3 (+3)	$1x10^7$	2.4 ml	1-4
2	3 (+3)	$1x10^8$	2.4 ml	1-4
3	3 (+3)	$1x10^7$ + 5 Gy radiation	2.4 ml	1-4
4	3 (+3)	$1x10^8$ + 5 Gy radiation	2.4 ml	1-4

The first three patients enrolled will be entered at the starting dose (dose level 1). If no significant toxicities are encountered, subsequent doses will be escalated as outlined above. If significant toxicity is encountered in one patient at the starting dose, 3 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 1×10^6 plaque-forming units (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 maximally tolerated dose (MTD). There will be a minimum 28 day observation period between the first and second patient in each cohort and a 28 day observation period from one cohort to the next to allow for evaluation of potential toxicity. It will take an estimated 24-30 months to complete patient accrual.

Safety Analysis: Patient monitoring will be appropriately tapered from the initial intensive monitoring in the Pediatric Intensive Care Unit immediately after biopsy and catheter placement, to an inpatient room, and then to regular clinic follow-up visits. In addition to regularly scheduled screening tests looking specifically for G207 shedding and viremia, other routine evaluations will include vital

sign measurements, neurological exams, neurological performance scores, magnetic resonance imaging studies (MRIs), chemistry and hematology laboratories, urinalysis, and quality of life questionnaires (quality of life measures are optional consent). Two medical safety monitors, who have expertise in herpes encephalitis, will be integrally involved in the assessment of toxicity throughout the study. Safety/tolerability will be assessed by adverse events and laboratory tests. Descriptive statistics will be provided for selected demographic, safety data overall and by dose group. Additional exploratory analyses of the data will be conducted as deemed appropriate.

Study Duration: Patients will have close scheduled follow up for 24 months following treatment with G207. Annual follow up appointments will be made for patients who survive longer than 24 months. 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.

1.0 Introduction:

Primary CNS tumors are the most common solid neoplasm of childhood and the leading cause of cancer related death in pediatric patients. Approximately 25% of children with brain tumors will not be long-term survivors of their disease despite aggressive treatment with combinations of surgery, radiation, and chemotherapy. 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 lacking genes essential for replication in normal brain cells. G207 has been shown to be efficacious in the treatment of both human and rodent tumors in murine models. Safety has been established following pre-clinical investigation involving intracranial inoculation in the highly HSV-sensitive owl monkey (*Aotus nancymai*). A Phase I study in adults with recurrent glioblastoma multiforme (GBM) demonstrated safety of intratumoral injections of G207 (1). Patients in this study were treated with doses escalating from 1×10^6 pfu to 3×10^9 without serious adverse events or toxicity. In a Phase Ib study in adults with recurrent GBM, patients were safely injected with two doses of the G207 (totaling 1.15×10^9 pfu) pre- and post-tumor resection (2). A follow-up phase I trial of G207 given in combination with a single 5 Gy dose of radiation for recurrent GBM demonstrated safety and radiographic responses (3). A maximum tolerated dose was not reached in all 3 trials.

2.0 Background and Rationale:

2.1 Disease Processes Involved in 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 a leading cause of cancer related morbidity and mortality (4). Although survival rates for low-grade, localized tumors have improved, there remains a significant subset of patients with high-grade, unresectable and/or disseminated tumors that have very poor outcomes despite conventional treatment approaches including surgery, chemotherapy and radiation. For patients with high-grade recurrent tumors, traditional treatment usually only provides a brief interval of disease control (5,6). Furthermore, patients who survive their disease after traditional therapy often have long-term sequelae such as hormone dysfunction, neurosensory impairment, and neurocognitive changes that are attributed to the treatment (7-10).

2.2 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 post-mitotic, non-dividing cell population of the brain. Many vectors have been considered for neoplastic therapy, including naked DNA, liposomes, and viruses. Attenuated HSV has a number of potential advantages as a neoplastic therapy for pediatric brain tumors. While traditional chemotherapy requires cancer cells to be dividing, HSV can enter a non-dividing cancer cell, replicate, and lyse the cell.

HSV-1 is an enveloped, double-stranded DNA virus (11). It has a genome of approximately 150 kb encoding for at least 80 genes. The genome is divided into long and short unique regions, each flanked by characteristic repeated sequences. Genetically altered, conditionally replicating HSV has been generated containing a variety of gene deletions and their effectiveness as therapeutic agents has been examined *in vitro* and *in vivo* in numerous models of metastatic and primary tumors. These experiments provide valuable information on the efficacy and safety in the

animal models. HSV-1 vectors have the advantage of being neurotropic. Genes essential for HSV-1 replication in the brain have been identified (12,13). Studies for brain tumor therapy have included alterations in one or more of the viral genes thymidine kinase, DNA polymerase, uracil DNA glycosylase, ribonucleotide reductase and $\gamma_1$34.5 (14-20). Thymidine kinase, DNA polymerase, uracil DNA glycosylase and ribonucleotide reductase are all viral enzymes necessary for nucleotide synthesis and replication. It has been clearly demonstrated experimentally that by altering these genes, either by deletion, insertion, or point mutation in the coding region, viruses will not be able to replicate in post-mitotic cells such as neurons. The viruses, however, are able to utilize cellular enzymes and factors present in dividing tumor cells to infect and kill them.

There are two copies of $\gamma_1$34.5 gene; one on each side of the two inverted repeat regions of the HSV genome. This gene encodes a critical bifunctional protein that enables the virus to replicate in neurons and also blocks a normal host response to infection. This gene is felt to be responsible for the neurovirulence of HSV. In the absence of this protein, the virus fails to induce HSV encephalitis even when injected in high amounts into the CNS of susceptible animal models. Additionally, a portion of the latency activated transcripts is encoded on the DNA strand opposite the $\gamma_1$34.5 genes. Thus the capacity to express genes necessary for the virus to establish latency is lost when the $\gamma_1$34.5 gene is altered. The deletion of the neurovirulence gene produced a greater survival of nude mice with human gliomas and retained the tk gene, making it susceptible to acyclovir which is considered standard of care treatment for HSV infections (21). Thus, in the unlikely event that a mutant virus produces toxicity in normal brain tissue, effective antiviral agents are readily available.

2.3 G207 Development and Preclinical Studies

G207 is a modified HSV that contains 1) deletions of both copies of $\gamma_1$34.5 and 2) a viral ribonucleotide reductase disabled secondary to disruption of the U_L39 gene by insertion of the *E. coli LacZ* coding region (16). Both mutations had previously been independently investigated in animal models. The second modification was based on the observation that a virus deficient in ribonucleotide reductase (22), a non-essential enzyme for HSV replication in dividing cells, showed severe attenuation in non-dividing cells while remaining proficient in killing proliferating Vero cells and human tumor cells in culture (21). These features were incorporated into G207 in order to maximally decrease the neurovirulence and yet maintain the ability of the virus to replicate in tumor cells. Other favorable properties of G207 are its easy detection due to the *lacZ* reporter gene, temperature sensitivity, and hypersensitivity to the antiviral drug acyclovir due to the retained tk gene, and interruption of the UL39 gene (19). Genetically altered viruses such as G207 with multiple mutations offer the safest approach for any proposed clinical trial.

A variety of pediatric tumors, including neural and glial tumors, express the primary HSV entry receptor CD111 and are sensitive to engineered HSV both *in vitro* and *in vivo* (23-27). When comparing the sensitivities of adult and pediatric glioma xenograft lines (xenolines) to G207, Friedman et al. demonstrated that the pediatric xenoline was more sensitive than 6 adult xenolines tested (28). Both pediatric glioma cells and primitive neuroectodermal tumor (PNET)/medulloblastoma cells were sensitive to G207 with a low dose of virus required to kill 50% of cells *in vitro*. Furthermore, brain tumor stem cells (also known as brain tumor-initiating cells) from pediatric brain tumors were sensitive to $\gamma_1$34.5-deleted HSV suggesting that engineered HSV may be able to target this chemotherapy and radiotherapy resistant population (26,27) In athymic nude mice bearing intracranial tumors, oHSV prolonged survival significantly with

several long-term survivors, including malignant pediatric brain tumors (26,30-31). G207 significantly prolonged survival of nude mice bearing human tumors either subcutaneously or intracranially when injected at titers of 10^6-10^7 plaque-forming units (pfu).

G207 has also been inoculated in young BALB/c mice via four different routes: intracerebrally, intracerebroventricularly, intravenously, and intrahepatically with no adverse effects or symptoms of disease (32). In addition, 10^9 pfu of G207 injected intracerebrally into the HSV sensitive owl monkey, *Aotus nancymai*, did not produce any deleterious side effects, whereas 10^3 pfu of HSV-1(F) virus was lethal (33). MRI analysis of G207 treated monkeys showed no CNS abnormalities. Neuropathologic evaluation showed local inflammation at the site of injection, but no other abnormalities. HSV DNA was detected throughout the brain by PCR analysis, but the unremarkable clinical course of the monkeys has not provided any evidence supporting the clinical significance of this finding. Histopathological evaluation of multiple organs revealed no evidence of altered cellular architecture or HSV immunoreactive cells.

The fundamental difference between G207 and other vector systems is that G207 is a conditionally replicating vector capable of replication in tumor cells, but not in the surrounding brain tissue. Conditionally replicating HSV is desirable when considering that, following resection, more than 10^9 tumor cells remain *in situ*. Other methods of treatment, e.g., a retroviral vector with the HSV tk, will not likely be able to target this many cells, especially considering the ongoing cellular proliferation of the remaining glioma cells. The ability of G207 to selectively replicate may allow for the delivery of sufficient vector into the tumor bed to potentially destroy most of the cells that remain after conventional therapy.

As G207 represents an excellent vector for evaluation in clinical studies for the treatment of malignant brain tumors, a G207 Master Viral Bank and clinical lot of virus have been produced by MediGene, Inc. (Now owned by Treovir, Inc.) in accordance with Good Manufacturing Practice (GMP) regulations and tested according to the Points to Consider for the Testing of Cell Lines Used in the Manufacture of Biological Products.

2.4 Genetically-engineered HSV-1 and Radiation

A number of pre- clinical studies have combined the selective oncolytic activity of genetically modified viruses with radiotherapy. Advani et al. first reported the interaction between the modified HSV-1 R3616 (the parent molecule of G207) and radiation (34). In nude mice with subcutaneous U87 malignant glioma xenografts implanted in the flank, R3616 injection followed by fractionated radiotherapy not only in improved viral replication and distribution, but significantly increased tumor regression (observed in 22 of 33 mice receiving combined therapy versus 4 of 33 mice receiving R3616 alone). Similarly, a later study used immunohistochemistry staining of intracranial glioma xenografts in nude mice to demonstrate a 2- to 5-fold enhancement of viral replication when inoculation of R3616 was followed with fractionated radiotherapy (35). Longer survival times were observed for the mice in this study receiving the combined therapy, and the interaction of radiation and R3616 was significantly shown to be synergistic by statistical analysis based on a proportional hazards model.

Markert et al. observed that although there was not a statistically significant difference in the median survival of nude mice with implanted human malignant gliomas that received only G207 versus G207 combined with radiation ($p=0.09$), the latter group was the only group with a majority of long- term survivors (70% in the group receiving combination therapy versus 20-30% in the groups receiving either G207 or radiation alone) (20). Of note, this study used two 5 Gy

fractionated doses of radiotherapy given at 4 and then 24 hours, doses lower than the prior studies and more feasible for delivery in a clinical setting. Further preliminary work has compared the survival in mice receiving G207 followed by a single 5 Gy dose of irradiation given at varying time intervals (4 hours, 24 hours, and 120 hours). The group receiving radiation at 24 hours has demonstrated markedly improved survival over the groups receiving radiation at 4 or 120 hours (60% versus 30-40% at 91 days) (G.Y. Gillespie, personal communication).
Importantly, none of the pre-clinical *in vivo* studies observed any mortality that could be attributed to a potential adverse synergism of these two modalities.

Several mechanisms have been proposed to explain the observed *in vivo* synergism of G207 and radiation. Because viruses with varying mutations have shown enhancement in response to radiation (Advani et al. observed a similar response in a second HSV-1 mutant), it appears unlikely that the irradiated cells are simply increasing production of a single protein which substitutes for a missing viral gene function, although Stanziale, et al, showed that radiation potentiated the antitumor efficacy of G207 by upregulating ribonucleotide reductase (34, 36). Since the HSV mutants replicate more efficiently in dividing tumor cells, it has been hypothesized that the enhanced viral replication may be accountable to a higher proportion of tumor cells in S phase after radiation. Other theories suggest that tumor cells may modify cytokine expression in response to radiation, or that the local inflammatory response to radiation may somehow facilitate increased viral replication within the tumor cells (20). Although the mechanisms underlying this synergy have not been fully elucidated, the results of the pre-clinical trials nonetheless provide strong rationale to begin investigating the safety of these combined modalities in humans.

2.5 Previous Clinical Experience

A Phase I clinical trial in adults was completed in March 2000 under BB-IND-7393 with HSV G207 (1). The genetically altered virus was supplied as clinical grade virus by the study sponsor, MediGene, Inc. Twenty-one adult patients with recurrent malignant glioma (GBM or anaplastic astrocytoma) were treated in seven cohorts of three each by stereotactic intratumoral injection of HSV G207 in a standard dose escalation fashion. Doses ranged from 1 $x10^6$ pfu as a single injection in 100 to 300 μl up to 3 x 10^9 pfu given at 200 μl each in five sites. While some patients developed complications frequently seen in patients with high-grade gliomas, no toxicity or serious adverse events could unequivocally be ascribed to G207. In fact, a toxic dose level was not reached during this trial because available viral production techniques limited the total dose that could be administered. There was also radiographic and neuropathologic evidence suggestive of anti-tumor activity and long-term presence of viral DNA in some cases. In a phase Ib study, 6 patients with recurrent GBM received two doses (one administered pre-tumor resection and one post resection in the resection cavity) of G207 totaling 1.15 x 10^9 pfu (2). Viral replication was demonstrated and radiographic and neuropathologic evidence suggestive of antitumor response was reported. No patient developed HSV encephalitis or required treatment with acyclovir.

A third Phase I study examined the safety of stereotactic intratumoral administration of G207 followed within 24 hours with a single 5 Gy radiation dose in patients with recurrent malignant gliomas. Treatment was well tolerated and no dose limiting toxicities were experienced. Importantly, 6 of 9 patients had a stable or partial response for at least one time point. The safety established in these three studies forms the basis to extend HSV G207 to the pediatric population. Pediatric patients may 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, especially encephalitis.

2.6 Safety Information

The safety of G207 has been addressed both by the deliberate molecular engineering of the virus (as discussed in Section 2.3), as well as by extensive *in vitro* and *in vivo* testing of precursors to the clinical material, as well as on the clinical material. *In vitro* testing has included the following:

SUMMARY OF G207 TESTING

G207 Master Virus Bank (MVB)	
Test	*Result*
Bulk Cell Harvest	
Sterility	Pass
Mycoplasma	Negative
Final Filled Vials	
Sterility (21 CFR 610.12)	Pass

Lot Release (7/3/97)	
Test	*Result*
Bulk Cell Harvest	
Sterility	Pass
Mycoplasma	Negative
Final Filled Vials: Sublot A	
Sterility (21 CFR 610.12)	Pass
General Safety (21 CFR 610.11)	Pass
LAL	9.8 EU/mL
Final Filled Vials: Sublot B	
Sterility (21 CFR 610.12)	Pass
General Safety (21 CFR 610.11)	Pass
LAL	< 1.0 EU/mL
Bacteria/Fungistasis USP XXII	Pass

HSV infection is widespread among healthy adults and children alike. Influenced by socioeconomic and geographic factors, HSV seropositivity in children five years of age is 20-30%; this increases to 40-80% by the second decade of life (37). In the Phase I study with G207 in adults, only 1 of 5 seronegative patients demonstrated seroconversion after G207 administration. Importantly, no specific clinical decline seemed to be attributable to this seroconversion, therefore suggesting that children may not be at an increased risk secondary to their lower incidence of seropositivity. Although neonates are more susceptible to the development of HSV encephalitis (HSE) than adults (the sensitivity of a human neonate is generally equivalent to the sensitivity of *Aotus nancymai*), other pediatric patients beyond the neonatal period develop HSE at an incidence similar to that in adults (one in 200,000 individuals).

Although HSE most often presents in neonates with generalized symptoms, HSE in adults and older children typically produces specific disturbances of the normal functions of the involved brain. It is unknown, however, if pediatrics patients will be more susceptible than adults to developing HSE or other herpes related infection after treatment with G207. Patients will be

closely monitored during the clinical studies for indications of HSE (see Section 7.4) as well as other possible side effects with the administration of G207. In the event of the development of HSE, the tk gene, which confers sensitivity to gancyclovir and acyclovir, remains intact, allowing for medical management with high-dose acyclovir according to established methods (14). In general, children tend to have more complete recovery from HSE than adults who are >30 years of age (38).

Unlike whole brain radiotherapy, inoculation of G207 is a focal intervention, and as such is not expected to produce any neurodevelopmental insult in the pediatric patient greater than that which would be expected for other standard focal interventions in patients with this disease (surgery or focal radiation). The single 5 Gy dose of radiation proposed is to enhance virus replication and cancer cell killing (see Section 2.3.2) and was well tolerated in the adult Phase I study when combined with G207 (3). Palliative fractionated reirradiation at total doses up to 18 to 35 Gy has been safely used in children and adults with a variety of recurrent brain tumors (39-41). The patient population to be studied will have been heavily pretreated most likely with radiation and chemotherapy. They are therefore likely to have neurocognitive deficits upon entry into this study.

3.0 Study Objectives:

3.1 Primary Objective:

To assess the safety and tolerability of G207 administered intratumorally via controlled rate infusion alone or followed by a single dose of radiation within 24 hours of G207 administration in children with recurrent or progressive malignant supratentorial brain tumors. Safety/tolerability will be assessed by adverse events and laboratory tests. The dose escalation scheme for this study was based on the adult phase I study in which the maximum planned dose of 3 $x10^9$ was administered without significant adverse effects. The MTD or maximal planned dose, if no dose-limiting toxicity is observed, will be determined.

3.2 Secondary objective:

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 measuring radiographic response, determining patient performance scale, progression-free and overall survival, measuring antiviral immune response and presence of G207 in blood, saliva, and conjunctiva. The impact of this new therapy on patients' quality of life will also be assessed (optional consent).

4.0 Study Design:

4.1 Overview

Pediatric patients with recurrent or progressive supratentorial malignant brain tumors who have failed surgery, radiotherapy and who may or may not have had chemotherapy or other form of treatment will be eligible for the study. An MRI must demonstrate progression of residual tumor at least eight weeks after radiation treatment. Last chemotherapy must be 3 or more weeks from entry and patients must have normal hematologic function. Patients must be on a stable dose of steroids for at least one week prior to injection. HSV immune status will be determined both pre- and post-treatment.

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 (biological agents, gene therapy, and novel combinations and administration routes of existing agents) interventions. The patient's caretaker(s) will be provided with a summary of this information as contained in the informed consent form, and will be given as much time as needed to make a deliberate decision concerning the involvement of their child in this trial. While those who do not decide to enroll in this trial will be provided with the best standard of care available, those consenting to enrollment will undergo the following procedures.

Under general anesthesia, patients will undergo stereotactic image-guided craniotomy, stereotactic biopsy and intraoperative histopathological analysis to document tumor cells, followed by placement of 1-4 catheters for G207 inoculation. Treatment with G207 will only proceed if viable, recurrent tumor is present on the frozen section. Three patients will be entered at each dose. The initial dose will be 1×10^7 pfu of G207 in a volume of 2.4 ml for the first three patients. If no significant (Grade 3 or 4) toxicities are encountered within 28 days of completing this treatment cohort, a subsequent dose level of 1×10^8 will be used. If no dose limiting toxicities are seen at either dose level, the initial dose level (1×10^7) will be repeated followed by a single 5 Gy fraction of radiation directed to the tumor within 24 hours of G207 inoculation. If deemed safe, a final dose level of 1×10^8 combined with a single dose of 5 Gy of radiation will be used. If two or more patients have toxicity at the starting dose, a cohort will be added at 1×10^6. If two or more patients have toxicity at this lower dose, the study will be terminated. The maximally tolerated dose (MTD) will be defined as the dose immediately below any dose that causes Grade 3/4 toxicities in 2 patients. There will be a minimum 28 day observation period between the first and second patient of each cohort, a seven-day observation period between patient two and three enrolled in a cohort, and a 28 day observation period from one cohort to the next to allow for evaluation of potential toxicity. Patients will be followed closely with potential effects of HSV being monitored closely. 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-operatively, and at 1, 3, 5, 7, 9 and 12 months. PCR and cultures will also be performed at the same time points to check for HSV in saliva and conjunctival secretions and PCR for HSV in blood. MRI will be done on day 3 or 4 after treatment and at months 1, 3, 5, 7, 9 and 12. 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 (as described above). Nonetheless, patients who have received G207 will continue long-term follow-up even after receiving other cancer therapies.

4.2 Sample Size Considerations

This Phase I study is conducted using a traditional (3+3) dose escalation design with four dosing cohorts. Thus the sample size for this study is up to 24 patients. In each cohort, 3 patients will be initially enrolled. Once the cohort of three patients has completed the toxicity and safety evaluation, additional patients will be enrolled into the next cohort or current cohort expanded following the dose escalation rules. Sample size has been determined based on practical consideration of the traditional 3+3 Dose Escalation design. Although accrual is expected to take 24-30 months for 12 patients, the actual time necessary for accrual is difficult to estimate due to an inability to predict the degree of toxicity that will be observed.

4.3 Statistical Considerations

This study is a Phase I clinical trial, standard dose-escalation study of safety and toxicity. A standard 3 + 3 design will be used. 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, 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, median, 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.

4.4 Treatment Summary

Dosing will start at an initial dose level of 1×10^7 pfu of G207, and will then escalate by one log increment to a dose of 1×10^8 pfu. If there are no unacceptable dose-limiting toxicities, the dose levels will be repeated in combination with a single 5 Gy dose of radiation. Subjects will be enrolled sequentially. Treatment will be escalated as follows:

Dose Level	Patients	Dose (pfu)	Volume	# Loci
-1	3	$1x10^6$	2.4 ml	1-4
1	3 (+3)	$1x10^7$	2.4 ml	1-4
2	3 (+3)	$1x10^8$	2.4 ml	1-4
3	3 (+3)	$1x10^7$ + 5 Gy radiation	2.4 ml	1-4
4	3 (+3)	$1x10^8$ + 5 Gy radiation	2.4 ml	1-4

There will be a minimum 28 day observation period between the first and second patient of each cohort, a seven-day observation period between patient two and three enrolled in a cohort, and a 28 day observation period from one cohort to the next to allow for evaluation of potential toxicity. A dose limiting toxicity (DLT) is defined as any Grade 3 or 4 toxicity (NCI CTCAE v.5.0) that is considered as being possibly, probably or likely related to G207 within 30 days of G207 inoculation with the following exceptions:

1) Neurologic deterioration will not be considered dose-limiting if it resolves back to the patient's baseline within two 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.
3) Any Grade 4 seizure that improves within 48 hours of initiation of supportive care measures.

Due to the possibility of pseudoprogression, a well-known entity seen in oncolytic viral therapy, as outlined in section 6.4.7., neurological changes may be treated with supportive care agents, bevacizumab and/or steroids. Improvement of symptoms with supportive care agents will not be considered a dose limiting toxicity (DLT). 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 if symptoms improve to baseline within seven days of completing treatment. If one patient has a DLT at the starting dose, up to 3 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 x 10^6. If two or more patients have toxicity at this lower dose, the study will be terminated. If significant toxicity is encountered in one patient at subsequent dose levels, patient numbers will be increased to six for that dose level. If no further toxicity is observed, the dose escalation will continue. If 2 patients in a cohort have any Grade 3 or 4 non-hematologic toxicity, as defined by NCI CTCAE v.5, that is considered as being possibly, probably or likely related to G207, the dose level immediately below will be defined as the MTD. If only 3 patients had previously received the newly determined MTD (because no Grade 3/4 toxicity was observed), a second cohort of 3 patients will receive that dose to ensure that it is indeed safe. If there are no DLTs in the highest cohort, 3 additional patients will be added to ensure safety at that dose.

4.5 Stopping Rules

The study will be stopped if the MTD is established or if the lowest proposed dose causes unacceptable toxicity. The medical monitors and/or principal investigator will apply the stopping rules. 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 hours, HSV encephalitis, or mental status changes requiring intubation. The pause will be in effect to allow assessment by the medical monitors, principal investigator, UAB Comprehensive Cancer Center Clinical Trials Monitoring Committee (CTMC), and the FDA to review the events to decide if the study should be terminated or can be resumed.

4.6 Data and Safety Monitoring

All patient data will be maintained on UAB computers with appropriate password protection. A nurse clinician with expertise in clinical trials and the principal investigator will be responsible for all data collection and maintenance. This project will be conducted under the oversight of the UAB Comprehensive Cancer Center for which a Data and Safety Monitoring Plan has been established. This study will be under the supervision of the UAB CTMC which acts as a Data Safety and Monitoring Committee for patients with brain tumors who are enrolled in clinical trials at UAB. There is no Data and Safety Monitoring Board for this Phase I study.

5.0 Study Population:

5.1 Inclusion Criteria

Patients meeting the following inclusion criteria will be eligible for the study:

5.1.1 Age ≥ 36 months and < 19 years

5.1.2 Pathologically proven malignant supratentorial brain tumor (including glioblastoma multiforme, giant cell glioblastoma, anaplastic astrocytoma, primitive neuroectodermal tumor, ependymoma, atypical teratoid/rhabdoid tumor, germ cell tumor, or other high-grade malignant tumor) which is progressive or recurrent despite standard care including surgery, radiotherapy, and/or chemotherapy. A pathologically proven secondary malignant tumor without curative treatment options is eligible.

5.1.3 Lesion must be ≥ 1.0 cm in diameter and surgically accessible as determined by

MRI

5.1.4 Patients must have fully recovered from acute treatment related toxicities of all prior chemotherapy, immunotherapy or radiotherapy prior to entering this study.

5.1.5 Myelosuppressive chemotherapy: patients must have received their last dose at least 3 weeks prior (or at least 6 weeks if nitrosurea)

5.1.6 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). For viral therapy, patients must have received viral therapy ≥ 3 months prior to study entry and have recovered from all acute toxicities potentially related to the agent

5.1.7 Monoclonal antibodies: patient must have received last dose ≥ 21 days prior.

5.1.8 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 ≥ 28 days prior to study entry.

5.1.9 Autologous bone marrow transplant: Patients must be ≥ 3 months since transplant prior to study entry.

5.1.10 Normal hematological, renal and liver function (Absolute neutrophil count > 1000/mm3, Platelets > 100,000/mm3, PT or PTT < 1.3 x control, Creatinine within normal institutional limits OR creatinine clearance >60 mL/min/1.73 m2 for patients with creatinine levels above institutional normal, Total Bilirubin < 1.5 mg/dl, Transaminases < 3 times above the upper limits of the institutional norm)

5.1.11 Patients < 10 years, Modified Lansky score ≥ 60; patients ≥ 10 years, Karnofsky score ≥ 60

5.1.12 Patient life expectancy must be at least 8 weeks

5.1.13 Written informed consent in accordance with institutional and FDA guidelines must be obtained from patient or legal guardian

5.2 Exclusion Criteria

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

5.2.1 Any treatment outside the allowable guidelines outlined in section 5.1.

5.2.2 Acute infection, granulocytopenia or medical condition precluding surgery

5.2.3 Pregnant or lactating females

5.2.4 Prior history of encephalitis, multiple sclerosis, or other CNS infection

5.2.5 Tumor involvement which would require ventricular, cerebellar or brainstem inoculation or would require access through a ventricle in order to deliver treatment

5.2.6 Required steroid increase within 1 week prior to injection

5.2.7 Known HIV seropositivity

5.2.8 Concurrent therapy with any drug active against HSV (acyclovir, valacyclovir, penciclovir, famciclovir, gancyclovir, foscarnet, cidofovir) or any immunosuppressive drug therapy (except dexamethasone or prednisone).

6.0 Study Methods and Procedures:

6.1 Designated Laboratories/Central MRI Review

6.1.1 Routine chemistry and hematology will be performed by a CLIA certified laboratory.

6.1.2 All viral culture and viral serology will be performed by a Diagnostic Virology laboratory.

6.1.3 MRI/CT will be reviewed by a designated pediatric neuroradiologist.

6.1.4 All pathology will be reviewed by a pediatric neuropathologist.

6.2 Screening Evaluations

Prior to treatment, patients will undergo physical examination, neurologic evaluation and routine preoperative clinical laboratory testing as well as collection of saliva and conjunctival secretions for HSV culture and PCR and blood for HSV PCR. After having undergone a preoperative evaluation consistent with inclusion in the study, the study participant will undergo a contrast-enhanced MRI scan. Sedation will be used during the MRIs as necessary for the patient's comfort. Review of these MRI scans will be necessary to determine if the tumor location and size is such that the patient may be included in the study. Tumor size will be determined using the maximal 2-dimensional cross-sectional tumor measurements, transverse x width, using either T1 or T2 weighted images. This imaging study will be obtained a maximum of 14 days prior to the patient's stereotactic inoculation. Also obtained within this timeframe will be assessment of quality of life by a neuropsychologist.

6.3 Intratumoral Inoculation with G207

After informed consent has been obtained and all screening procedures completed according to the protocol, craniotomy and biopsy may commence. The patient will undergo a contrast enhanced MRI study using a frameless stereotactic system protocol. Utilizing the frameless stereotactic system, children under general anesthesia will undergo a stereotactic guided craniotomy. 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 4 silastic catheters (PIC-030 or equivalent: (30cm x 2.0mm outer diameter, 1.00mm inner diameter) Neuro-Infusion Catheter, equipped with a stainless steel stylet and a 6 French compression hub) will be passed to stereotactically predefined coordinates of enhancing tumor, if present. Catheters may be placed in non-enhancing regions if residual tumor is confirmed in those locations. 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 (PICU). While in the PICU, vital signs (temperature, blood pressure, pulse, respiratory rate) and neurologic function (Glasgow Coma Scale and limited neurological exam) will be monitored every hour overnight according to the PICU standard for intensive monitoring. The following day, a

postoperative 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, mal-placed 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 400 microliters (0.4 ml) per hour for 6 hours. However, the infusion will actually last 6 hours and 36 minutes to allow flushing of the saline solution from the catheter(s). The infusion rate for the flush will be 400 microliters (0.4 ml) per hour for 36 minutes per catheter. After the 36 minute flush, the infusion rate will be changed to the appropriate rate for the G207 infusion. Following the flush, the rate of administration through each individual catheter shall be determined by the equation:

400 microliters (0.4 ml) per hour/number of active catheters.

The total volume administered from each syringe will be determined by the number of catheters actively infusing, using a volume of 400 microliters (0.4 ml) per hour as a guide. Refer to table below.

Table : Plan for Loading rate and Infusion Rate for G207 HSV

Number of Catheters	Initial Loading Rate for First 36 mins per catheter	Final Infusion Rate per catheter
1	0.4 cc/hour	0.4cc/hour
2	0.4 cc/hour	0.2cc/hour
3	0.4 cc/hour	0.13cc/hour (for 2 catheters); 0.14cc/hr (for 1 catheter)
4	0.4 cc/hour	0.1cc/hour

For the study drug infusion, the study drug syringe will be connected to the infusion tubing, that has been flushed with sterile PBS + 10% glycerol (Alanza). The infusion tubing (PIT-400, Sophysa) will then be primed with 4 ml of the diluted G207 virus preparation in the syringe and connected to the catheter. The infusion period will begin with the 36 minute flush of the saline solution from the catheter. After completion of the flush the pump will be reprogramed to the appropriate rate for the study drug infusion and the infusion initiated for 6 hours. 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 400 microliters (0.4 ml) per hour. Infusion may extend up to 8 hours, if needed due to catheter malfunction.

6.4 Patient Management Following Virus Infusion

At the start of the virus infusion, vital signs and neurologic function will be monitored every hour x 6, then every two hours overnight for the first 24 hours. At least one hour after

completion of the virus infusion, the catheter(s) may be removed at the bedside by the neurosurgeon team. Patients that appear stable after removal of the catheter(s) may be moved to a general inpatient room, and the frequency of monitoring will be determined by the attending physician(s) based on the medical condition of the patient.

6.4.1 FOCAL NEUROLOGICAL DEFICITS: General neurologic worsening (decreased state of consciousness) including focal neurological deficits or specific neurological worsening (paresis, dysphasia, etc., depending upon location of tumor), could be due to edema, hematoma, hydrocephalus, or encephalitis. An MRI or CT will be done to help determine the cause.

6.4.2 CEREBRAL EDEMA: This is common in tumor patients and occurs post-operatively and usually responds to standard measures for the treatment of increased intracranial pressure. Although medical management with systemic steroids is often very effective, patients occasionally require a ventriculostomy or surgical shunting procedure.

6.4.3 HEMATOMA: PT, PTT, and platelet count will be obtained. A small hematoma noted on a scheduled MRI may simply be watched and the patient treated as above for edema and then rescanned to exclude an enlarging lesion. A large hematoma or one associated with progressive neurologic deterioration may require operative evacuation.

6.4.4 ENCEPHALITIS: Patients who develop fever >101.5F and/or alterations in mental status/waning Glasgow Coma Scale, or seizures will immediately undergo general physical and neurologic examination. If the patient has fever without any neurologic signs/symptoms, source for systemic infection will be investigated. If no source for fever can be found and fever persists for greater than 12 hours, evaluation for encephalitis will take place. Patients who have neurologic changes as above will also undergo immediate evaluation for encephalitis. CT or MRI will be performed. If this study demonstrates an increase in the area of hemorrhagic necrosis extending beyond the borders of the tumor Post-operative bed, a stereotactic biopsy will be taken to assess for presence of G207 or HSV-1(F) virus. This will consist of a minimum of 2-3 needle core biopsies which will undergo standard hematoxylin and eosin (H&E) staining, as well as immunostaining for HSV-1, PCR and culture, CD68, leukocyte common antigen, glial fibrillary acidic protein (GFAP), and X-gal staining for the beta-gal product. This will be reviewed by a Pediatric Neuropathologist. While waiting for biopsy results, the patient will be empirically started on intravenous acyclovir at a dose of 30 mg/kg divided every 8 hours. At the time of biopsy, a sample of CSF will be obtained if safe and checked for HSV by PCR to rule out HSV meningitis. Patients whose clinical course is suggestive of HSV encephalitis but imaging studies do not show a change from baseline, will undergo lumbar puncture for CSF examination as well. Those patients will be treated with empiric acyclovir as well. Patients who have tissue, CSF, or strong clinical evidence for HSV encephalitis will be treated for a 14-day course of acyclovir therapy. The medical monitor will be consulted immediately for any suspected cases of encephalitis and be integrally involved in evaluation and treatment decisions.

6.4.5 DISSEMINATED HSV: Disseminated multi-organ disease attributed to HSV is exceedingly uncommon, even in the immunocompromised host. Such disease has been encountered in newborns with disease identified as 'disseminated multi-organ involvement' and occurs in about 25% of newborns with this disease (incidence 1:10,000 deliveries) (42). In all circumstances, disseminated disease is associated with a systemic host response of fever, and findings compatible with sepsis, including hepatic dysfunction. To monitor for these rare events, most importantly, serial physical examinations will be performed to assess for fever and symptoms of sepsis or hepatic dysfunction. In addition, serial blood specimens will be obtained and cultured for infectious HSV at days 1, 3 or 4, 7, 14, 28; months 3, 5, 7, 9, 12, 18, 24; and yearly thereafter. Any positive cultures will be analyzed by PCR to discriminate between wild type HSV-1 and mutant G207 HSV. Should a patient develop fever, rash, or unexplained elevations in liver function tests in conjunction with a concomitant increase of infectious HSV by plaque assay in blood specimens (exceeding prior values by 2 logs), patients will undergo a 21 day course of treatment with acyclovir at a dose of 30mg/kg divided every 8 hours for presumed disseminated HSV infection. The medical monitor will be consulted immediately for any suspected cases of disseminated HSV and be integrally involved in evaluation and treatment decisions. Parenthetically, it should be noted that oncolytic HSV therapy for metastatic colon carcinoma involving the liver led to detectable HSV DNA in the blood but in the ABSENCE of clinical symptomatology (43); thus, increasing levels as well as fever will both be required to therapeutically intervene. Should two Grade 3 or 4 toxicities attributable to disseminated HSV infection occur in the same dose level, the prior dose level shall be declared the MTD and enrollment will be halted.

6.4.6 VIRAL SHEDDING DUE TO CSF LEAK OR BODY FLUID VIRAL CONTAMINATION: Patients with CSF leak will receive the standard of care by neurosurgery to close the leak. HSV PCR of the CSF will be performed prior to closure. Any abnormal fluid collection at the surgical site will be assessed by neurosurgery and HSV culture and PCR of the fluid will be performed. Any signs of encephalitis or HSV dissemination will be managed as outlined above. Patients will be assessed for HSV shedding from body fluids (saliva, conjunctival secretions, and blood) routinely throughout the study (see Appendix A). Any patients with body fluid viral contamination will be placed on contact precautions until there are two negative follow-up samples.

6.4.7 PSEUDOPROGRESSION: Pseudoprogression is a well-known entity in oncolytic viral therapy, immunotherapies, and the treatment of malignant brain tumors (44, 45). Patients with possible pseudoprogression may be watched or treated with bevacizumab, which has been shown to ameliorate symptoms of pseudoprogression or radiation necrosis, and/or dexamethasone at the discretion of the investigators (46-49). If the patient improves with bevacizumab and/or additional steroid administration (to be used if bevacizumab is contraindicated or insufficient) and the MRI changes in lesion size/and or enhancement also improves, the patient will be determined to have not progressed but to have had pseudoprogression, and will continue follow-up within the trial under the previously defined schedule. Should neurologic symptoms and/or imaging changes progress despite steroid

administration on follow-up imaging, the patient will be determined to have progressed and the date of progression shall be assigned to the date of the initial scan that demonstrated findings consistent with progressive disease as defined in section 9.2.1. For patients that are symptomatic despite bevacizumab and/or steroids, surgical resection of the lesion may be recommended to remove necrotic tissue and any residual tumor.

6.5 Radiation Planning and Treatment

For patients receiving radiation, a 100 SAD linear accelerator with at least 6 MV beam energy will be utilized for radiation treatment.

6.5.1 Simulation and Immobilization

All patients will undergo simulation and radiation treatment. Simulation may occur at the screening visit or on postoperative day 1. The patient will generally be placed in the supine position, but prone positioning is allowed. A custom aquaplast immobilization device will be fabricated and axial CT images will be collected at a slice thickness less than 3 mm.

6.5.2 Target Volumes and Treatment Planning

The postoperative 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 RT planning are shown in the table below and follow ICRU-62 conventions. The goal of treatment planning is coverage of the PTV1 and 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 chiasms) should be limited to 3 Gy or less unless coverage of the GTV would be compromised. The 2 mm margin for setup and registration error may be dosimetrically compromised in cases which gross tumor is very close to critical structures such as the brainstem or optic apparatus. In no case should gross disease receive less than the prescription dose. For tumors that do not have T1 abnormality, high risk portions of the T2 or FLAIR abnormality should serve as the GTV.

Volume	**Abbreviation**	**Definition**	**Dose**
Gross Tumor Volume	GTV	T1 gross residual disease	-
Clinical Target Volume	CTV	T2 or FLAIR abnormality	-
Planning Target Volume 1	PTV1	GTV plus 2 mm	5 Gy
Planning Target Volume 2	PTV2	CTV plus 2 mm	3 Gy

Generally, it is expected that multiple axial or non-axial beams (5-9) will be required to generate an acceptable plan. Field segmentation will often allow differential dosing to the PTV1 and PTV2 without computer optimization. Computer optimization and intensity modulated radiation therapy are allowed but not required. Plans utilizing computer optimization will need ion chamber measurement before delivery.

The treating radiation oncologist must review all treatment and setup/localization films before treatment.

6.6 Supportive Care Guidelines

Appropriate supportive care during the duration of the study includes the following:

6.6.1 Bevacizumab or steroid administration for neurologic symptoms arising from increased edema or intracranial pressure if deemed appropriate and safe in the determination of the investigator. Bevacizumab may be given at least 14 days after initial surgery for biopsy and catheter placement to allow adequate time healing of the surgical incision. Bevacizumab will be given every two weeks at a dose of 5-10 mg/kg IV. Dosage adjustments or cessation of drug may be made should adverse events related to bevacizumab occur (e.g. uncontrolled hypertension, proteinuria, bleeding, thrombosis, etc.) and are made on a case-by-case basis 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.

6.6.2 Proton pump inhibitors or H2 antagonists for control of steroid-induced gastric irritation

6.6.3 Anti-epileptic medicines for control of partial or generalized seizures

6.6.4 Post-operative neurological intensive care that is routine for the neurosurgical interventions involved in the administration of G207

6.6.5 Other than a restriction on medications with anti-HSV activity (to be given only for the management of an adverse event), there are not any limitations on concomitant medications that patients may receive for other co-morbidities.

6.7 Study Evaluations by Time Point (Appendix A)

6.7.1 Prescreening/Screening
Vital signs (temperature, blood pressure, pulse, respiratory rate)
History and Physical including complete neurological status on exam
Modified Lansky or Karnofsky status determination (Appendix B)
Complete metabolic panel (CMP), magnesium, phosphorous, Complete blood count with differential and platelets (CBC) including lymphocyte markers
Cystatin C (if creatinine is elevated from institutional normal value)
HIV serology
HSV antibody titer
HSV detection (HSV culture saliva, conjunctival secretions; HSV PCR saliva, conjunctival secretions, blood) to rule out viral shedding and/or viremia
Serum pregnancy (adolescent females) and urinalysis
PT/PTT
MRI with and without contrast of brain to evaluate tumor and assess for placement of catheter(s)

6.7.2 Day -1 evaluation
Biopsy and placement of catheter(s)
Vital signs and neurologic function per PICU standard

6.7.3 Day 0 Evaluations

CT scan to confirm placement of the catheters
G207 infusion
Vital signs and neurologic function (Glasgow Coma Scale and neurological exam) will be monitored every hour x 6, then every 2 hours overnight for the first 24 hours.

6.7.4 Day 1 - 4 Evaluations
Vital signs and neurologic function each day
Modified Lansky or Karnofsky status determination
HSV detection Day 1, and Day 3 or 4 (HSV culture saliva, conjunctival secretions; HSV PCR saliva, conjunctival secretions, blood)
CMP, magnesium, phosphorous on Day 2
CBC with lymphocyte markers on Day 2
MRI with and without contrast of the brain on Day 3 or 4. This MRI will be the first in a series of MRIs, and will be used a baseline for evaluation of potential clinical response. Should there be medical indication prior to 72 hours, MRI will be performed as deemed necessary by the Principal Investigator or medical monitors.
For patients receiving radiation therapy on Day 1, they will have a CT scan for radiation simulation

6.7.5 Day 7 Evaluations
Vital signs
History and Physical including complete neurological status on exam
Modified Lansky or Karnofsky status determination
CMP, magnesium, phosphorous
CBC with lymphocyte markers
HSV detection (HSV culture saliva, conjunctival secretions; HSV PCR saliva, conjunctival secretions, blood)

6.7.6 Day 14 Evaluations
Vital signs
History and Physical including complete neurological status on exam
Modified Lansky or Karnofsky status determination
CBC with lymphocyte markers
HSV detection (HSV culture saliva, conjunctival secretions; HSV PCR saliva, conjunctival secretions, blood)

6.7.7 Day 28 (+/- 7 days) Evaluation
Vital Signs
History and Physical including complete neurological status on exam
CMP, magnesium, phosphorous, CBC with lymphocyte markers
Modified Lansky or Karnofsky score
HSV antibody titer
HSV detection (HSV culture saliva, conjunctival secretions; HSV PCR saliva, conjunctival secretions, blood)
MRI with/without contrast

6.7.8 Month 3, 5, 7, 9 and 12 (+/- 14 days) Evaluations
Vital Signs

History and Physical including complete neurological status on exam
CBC with lymphocyte markers
Modified Lansky or Karnofsky score
HSV antibody titer by ELISA
HSV detection (HSV culture saliva, conjunctival secretions; HSV PCR saliva, conjunctival secretions, blood)
MRI with/without contrast of brain

6.7.9 Months 18, 24, and yearly Follow-up

All surviving study patients will receive follow-up clinic visits at 18 and 24 months to include the same evaluations in 6.7.8 and then yearly for up to 15 years after therapy for assessment of adverse events/serious adverse events possibly related to G207 even if the study is terminated or the patient withdraws from the study. Particular attention will be paid to any symptoms (neurological or otherwise) consistent with HSV infection. Patients who are unable to follow-up in person will be contacted by telephone on a yearly basis to monitor for delayed events.

6.7.10 Quality of life **(**optional consent) will be assessed at study enrollment, 1, 3, 5, and 12 month visits by using the PedsQLTM Brain Tumor Module, WHOQOL Group Quality of Life BREF, and the Brief Symptom Inventory 18.

6.7.11 Blood sample (optional consent). An extra teaspoon of blood will be drawn in a lavender top tube during a routine blood draw at screening, day 2, 7, 14, 28 and month 3, 5, 7, 9, 12, 18, 24, and then yearly thereafter.

7.0 Study Test Article:

7.1 Investigational Product Designation

G207 is supplied by Treovir, Inc. in sterile, labeled 1.0 mL cryovials containing 0.12 mL of G207 suspended in the storage buffer, D-PBS/10% glycerin. The vials should remain frozen at -60C or below until use. G207 will be stored in the pediatric hematology-oncology pharmacy prior to being injected. See appendix C for preparation guidelines.

7.2 Dose

A total of 2.4 mL (in 1-4 doses) will be used for treatment. To prepare the dose, the vial should be removed from the -60º C freezer and rubbed gently between gloved hands until all the ice crystals have melted. The vial should then be placed on ice. It should be thawed completely before removing the cap. Care should be taken to ensure that all the liquid is at the bottom of the vial before removing the cap to avoid spillage. If it is suspected that the contents are on the side or top of the vial, the vial can be tapped gently on a flat surface. The appropriate dose level will be prepared according to the dose escalation scheme. The final diluted G207 should be gently withdrawn into the syringe for injection. Complete instructions for dose preparation will be provided prior to initiation of the clinical study.

7.3 Precautions in Handling and Disposal

7.3.1 General Procedures

Sterile technique and Biosafety Level 2 precautions (gown, gloves, mask) will be

rigorously followed while preparing the dose. The dose preparations will take place in a biosafety hood. The vial of G207 will be removed from the controlled access freezer and thawed as above in section 7.2. It will be allowed to thaw completely prior to removing cap. Care will be taken to ensure that all of the liquid is at the bottom of the vial prior to removing the cap and that the cap is removed carefully to avoid spilling or contamination.

7.3.2 Disposal Procedures

All materials that have been in contact with the G207 herpes virus are considered to be infectious biohazards, and must be decontaminated or incinerated prior to disposal. Needles and syringes should be placed into a puncture-resistant, leak-proof container containing disinfectant. All materials that have been in contact with the vector must be incinerated in an institutionally approved biohazard incinerator before disposal

7.3.3 Investigational Drug Accountability

The Investigator must maintain accurate records of dates, quantities, and lots of product(s) received, to whom dispensed (subject-by-subject accounting), and accounts of any product accidentally destroyed. The Investigator must retain all unused, partially used or expired product until the drug manufacturer has confirmed accountability data.

At the conclusion of drug administration, all unused and partially used drug supplies will be returned to Treovir, Inc., An overall summary of all drug supplies received, unused, partially used, wasted, and returned must be prepared at the conclusion of the study.

8.0 Study Conduct:

8.1 Clinical Events: Adverse Medical Experiences and Concurrent Illnesses

8.1.1 Definitions of Untoward Events Occurring During the Study

Adverse Experience (AE): An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (e.g., abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug and from any route of administration, formulation, or dose, including an overdose. An illness or condition present at study entry is considered a pre-existing condition and will not be considered an AE unless the pre-existing condition worsens during the course of the study. This change may be considered an AE. An AE that occurs after signing informed consent but prior to the administration of study drug will be considered not related to study treatment. AE's that have a grade increase to 4 or 5 will be considered an SAE and reported as required. All AE's, irrespective of relationship to study drug, which occur or worsen after signing of consent form until 30 days after administration of study drug, must be reported and documented on the AE Case Report Form (CRF). All medications given for an AE will be reported on the Concomitant Medication CRF. Hospitalizations that are expected due to the disease and those that are unrelated to the study drug or disease will be reported as AE's unless it meets the requirements for a serious adverse event (SAE). Hospitalizations due to merely diagnostic reasons, examinations as a matter of routine or planned surgery and due to social indication do not represent a hospitalization in the sense of the term "serious".

Laboratory Abnormalities: All abnormal laboratory values must be evaluated according to

Common Toxicity Criteria. Clinically significant changes from baseline in hematology, serum chemistry, and/or urinalysis values, within or outside of the reference (normal) range, are notable and may constitute an adverse event. All laboratory values found to be significantly above or below the institutional norm must be repeated and evaluated by the Investigator as soon as possible, and documented on the case report form. An abnormal clinical laboratory result corresponding to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 Grade 4 is by definition a life threatening experience.

Serious Adverse Event (SAE): Any clinical event that is fatal or life-threatening, permanently disabling, or requires or prolongs hospitalization. Congenital anomalies and new occurrences of cancer also will be considered as serious adverse events.

Expected Adverse Events Associated with G207: G207 caused no dose limiting toxicities in adult studies and the adverse events observed following treatment were not thought to be attributable to the study drug. Adverse events occurring after the administration of G207 included the following, which are therefore expected to possibly occur after G207:

- Headache
- Nausea
- Vomiting
- Diarrhea
- Anorexia
- Weakness
- Fatigue
- Lethargy
- Seizure
- Abnormal mentation
- Anemia
- Thrombocytopenia
- Leukopenia/neutropenia
- Fever
- Transaminitis
- Skin infection
- Varicella zoster infection
- Pseudoprogression (transient enlargement of tumor on MRI)
- Hematoma

Unexpected Event: Any clinical event that is not identified in nature, severity or frequency in the risk information included above under expected adverse events.

Associated with the Use of the Drug: A reasonable possibility exists that the test article

may have directly or indirectly caused the clinical event. The Investigator will be unbiased in assessing the relationship of the event and test article and discuss the possible relatedness to treatment with the Medical Monitor. Factors which weigh in the clinical assessment of causality will be recorded in the medical record by the principal investigator. The following criteria will be used to describe association of AEs and SAEs with G207:

A. Definitely Related: There is a clinically plausible time sequence between the onset of the AE and G207 administration and all other potential causes have been ruled out.

B. Probably Related: There is a clinically plausible time sequence between the onset of the AE and G207 administration; the AE is unlikely to be caused by the concurrent/underlying illness, other drugs, or procedures; and (if applicable) the AE follows a clinically consistent resolution pattern upon withdrawal of G207.

C. Possibly Related: There may or may not be a clinically plausible time sequence between the onset of the AE and G207 administration and investigational drug as a cause cannot be ruled out.

D. Unlikely Related: There is probably NOT a clinically plausible time sequence between the onset of the AE and G207 administration; the AE is likely to be caused by the concurrent/underlying illness, other drugs, or procedures (if applicable) and the AE does not follow a clinically consistent resolution pattern of treatment with G207.

E. Not Related: Another cause of the AE is most plausible; a clinically plausible temporal sequence is inconsistent with the onset of the AE and G207 administration; and/or a causal relationship is considered biologically implausible.

8.2 Reporting of Clinical Events

Adverse experiences will be recorded on the AE form. The Investigator will include the date of occurrence, the date of cessation (or the fact that it is still continuing), the intensity, and the relationship to test article administration, any resulting treatment or change in treatment, and the outcome of the event.

8.3 Reporting of Serious Life-Threatening Adverse Event

Any serious and unexpected suspected adverse reaction which occurs during the conduct of this study, regardless of relationship to test article or procedures, will be reported within 24 hours to the Medical Monitors and UAB CTMC, and a written report filed within 7 days after the event to the Medical Monitors, the Investigator's Institutional Review Board (IRB), the UAB CTMC, and the FDA.

8.4 Declaration of Treatment Failure - Patient Taken Off Treatment or Off Study

Patients whose tumors show radiographic or clinical evidence of progressive growth will be declared Treatment Failures and may be considered for some other available therapy without restriction, including standard (re-resection, repeat fractionated radiotherapy, and/or chemotherapy) and experimental (biological agents, gene therapy, and novel combinations and administration routes of existing agents) interventions. Patients receiving alternative cancer therapies will continue to be followed in this study; details of subsequent therapy, progression, and survival will continue to be collected. Patients (or guardians of patients) who withdraw consent

from further participation in this clinical study will be considered off study.

Off study criteria also includes:

- Patient determined to be ineligible
- Patient no longer meets eligibility criteria after enrollment but before therapy
- Subject death

8.5 Insurance and Indemnity

Information on compensation, insurance and indemnity will be supplied to the investigator in the clinical agreement.

8.6 Protocol Deviations

Except in cases that would commonly be considered medical emergencies, the investigator will not deviate from the protocol without prior permission from the oversight committee and the IRB. In the event of a medical emergency, these two regulatory boards will be notified as soon as possible. All other changes in the study procedures will be implemented as amendments and will be approved by the IRB before implementation.

8.7 Data and Safety Monitoring Plan

Because this study is a Phase I trial investigating the safety of a newly developed agent being administered to pediatric patients for the first time and has a relatively small number of patients enrolled at a single institution that will be assuming the full burden of risk, the principal investigator, medical monitors, and UAB Clinical Trials Monitoring committee (CTMC) will be involved in continuous monitoring of the safety data for all patients that receive the investigational drug. The principal investigator will have direct accountability to the CTMC, and will submit monthly, detailed reports to the committee concerning the immediate and long-term follow-up safety of all patients that have received the investigational agent. These reports will be reviewed by the CTMC on a regularly scheduled basis, with additional reviews being conducted both prior to any dose escalation and immediately following any evidence of unacceptable toxicity.

8.8 Retention of Records

To comply with FDA regulations, the Investigator must maintain the records of investigational drug disposition, subject exclusion logs, signed consent forms, electronic Case Report Forms, all correspondence, and supporting documentation for a minimum of 2 years after the date on which a US marketing application has been approved for the indication investigated in this study or, if no application is to be filed, until 2 years after the investigation is discontinued and the FDA/OBA are notified. The Investigator may withdraw from the responsibility to maintain records and transfer custody of the records to another person who will accept responsibility for them.

9.0 Study Analysis:

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, median, and maximum. Categorical variables will be summarized using frequency counts and percentages. For time-to-event variables, median time-

to-event, and other percentiles may be presented. Additional exploratory analyses of the data on some of the secondary endpoints will be conducted as deemed appropriate.

9.1 Analysis of Safety

9.1.1 Safety and Tolerance of G207 Administration (Primary Endpoint #1)

All subjects who receive G207 will be included in the safety analysis. Adverse events will be described and the frequency of events will be both tabulated overall and by dose group. All events with a Grade 3 or above toxicity (where toxicity is defined by the CTCAE v5.0) will be summarized separately and tabulated by event and by relationship to G207, both overall and by dose group. Laboratory analyses (chemistries, hematology, serological/immunological analyses, PCR, cultures, WBC and differential) will consist of measurements of change from baseline over time by patient and overall, with plots of actual values compared to normal values for patients by dose group. Logarithmic transformations may be applied as necessary. Group means and standard errors will be calculated for the various laboratory parameters. Concurrent illnesses will be listed and examined as possible confounders in the treatment response relationship. Concurrent medications will also be listed, as will previous treatments for malignant brain tumors. Effects of concomitant medications and previous treatments for cancer and any potential related side effects will be analyzed and discussed. The MTD or maximal planned dose, if no dose-limiting toxicity is observed, will be established.

9.1.2 Immunologic Response to G207 (Secondary Endpoint #1)

HSV-1 antibody titers will be checked by ELISA on all patients prior to the administration of G207 and at Day 28 and Months 3, 5, 7, 9 and 12. Descriptive statistics of this secondary endpoint will be computed.

9.1.3 Virologic Shedding Following G207 (Secondary Endpoint #2)

Saliva, blood and conjunctival secretions of all patients will be checked by PCR and culture at regular intervals (prior to administration, Day 1, 3 or 4, 7, 14, 28, and Months 3, 5, 7, 9, 12, 18, 24 and yearly thereafter) for evidence of HSV shedding and/or viremia. Descriptive statistics of this secondary endpoint will be computed.

9.2 Clinical Response (Secondary Endpoint #3)

Although not a primary objective of the study, an evaluation of response will be performed. Response rates will be calculated in treated patients that are considered evaluable. All time-to-event variables (PFS, OS) will be assessed. Descriptive statistics of this secondary endpoint will be computed.

9.2.1 Radiographic Response

Following the treatment, an evaluation of response will be performed. MRI study prior to G207 administration will be considered the baseline. Technical parameters for the baseline scan and evaluation scans will be identical (see appendix D). Two-dimensional measurements obtained at the baseline scan will be compared with the follow-up MRI scans for assessment of response primarily as described in the immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria and summarized below:

Complete Response (CR): Disappearance of all enhancing disease for $\geq$ 4 weeks;

no new lesions; stable or improved T2/FLAIR; no more than physiological steroids; clinically stable or improved.

Partial response (PR): ≥ 50% decrease in the sum of biperpendicular diameters of enhancing disease for ≥4 weeks; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved.

Stable Disease (SD): Does not qualify for complete response, partial response, or progressive disease; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved.

Progressive Disease (PD): ≥ 25% increase in the sum of biperpendicular diameters of enhancing disease; or new lesions; or substantial worsened T2/FLAIR; or substantial clinical decline. Due to the possibility of pseudoprogression, a well-known entity seen in oncolytic viral therapy and immunotherapy, 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. During the interval, the patient may be watched or treated with bevacizumab and/or dexamethasone at the discretion of the investigators. If follow-up imaging confirms disease progression, the date of actual progression will be back-dated to the date of initial radiographic progression. The appearance of new lesions 6 months or less from the initiation of immunotherapy alone does not define progressive disease. When a tumor cannot be classified strictly by iRANO criteria, T2/FLAIR abnormality may be used for two-dimensional measurements and response may be determined by consensus among the treating physicians.

The percent of subjects who, at any time point following G207 administration and prior to other cancer therapy, demonstrate PD, SD, PR, CR will be summarized. Patients' radiographic responses will also be reported by evaluation time points and by dosing cohorts. Longitudinal evaluation of region-of-interest based and histogram based analysis of the diffusion parameters (apparent diffusion coefficient and mean diffusivity) and perfusion parameters (K^{trans} and rCBV) will be performed both at the baseline and follow-up MRIs to demonstrate if change of these parameters from baseline is related to the infused dose of virus and if the change from baseline can predict progression-free and overall survival. Changes in clinical disease status and steroid administration will be considered at each time point of imaging evaluation. Measurements will also be analyzed with consideration of any anti-tumor cancer therapies and timeframes administered.

9.2.2 Performance Score

A Modified Lansky or Karnofsky score will be recorded and measured serially with the pre-treatment score. Time to a Karnofsky score of ≤30 (modified Lansky score of ≤30 in children ≤10 years of age) will be explored. Descriptive statistics of this secondary endpoint will be computed.

9.2.3 Progression Free survival

Time after G207 administration to clinical and radiographic disease progression (progression free survival) will be evaluated in all patients receiving G207. Descriptive statistics

of this secondary endpoint will be computed.

9.2.4 Overall Survival

The overall survival for each patient receiving G207 will also be reported. Descriptive statistics of this secondary endpoint will be computed.

9.2.5 Quality of Life (optional consent)

Each patient's self-report (or the caretaker's report, if the patient is <60 months old) of quality of life will be measured with the PedsQOL questionnaire at baseline (before administration of G207) and at specified times thereafter. The primary caregiver will also complete the WHOQOL Group Quality of Life BREF, which measures parent quality of life, and the Brief Symptom Inventory 18, which measures distress. The clinical course of the tumor will be taken into account in the interpretation of the trends that are found. Descriptive statistics of this secondary endpoint will be computed.

References:

1. Markert, JM, Medlock MD, Rabkin SD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther* 7:867-74, 2000
2. Markert, JM, Liechty PG, Wang W, et al. Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. *Mol Ther*, 17:199-207, 2009
3. Markert, JM, Razdan SN, Kuo HC, et al. A Phase 1 Trial of Oncolytic HSV-1, G207, Given in Combination With Radiation for Recurrent GBM Demonstrates Safety and Radiographic Responses. *Mol Ther*. 22:1048-55, 2014
4. Gatta G, Capocaccia R, Coleman MP, Ries LA, Berrino F. Childhood cancer survival in Europe and the United States. *Cancer*. 95:1767-72, 2002
5. Cohen KJ, Pollack IF, Zhou T, et al. Temozolomide in the treatment of high-grade gliomas in children: a report from the Children's Oncology Group. *Neuro Oncol*. 13:317-23, 2011
6. Gajjar A and Pizer B. Role of high-dose chemotherapy for recurrent medulloblastoma and other CNS primitive neuroectodermal tumors. *Pediatr Blood Cancer*. 54:649-51, 2010
7. Diller L, Chow EJ, Gurney JG, et al. Chronic disease in the Childhood Cancer Survivor Study cohort: a review of published findings. *J Clin Oncol*. 27:2339-55, 2009
8. Ribi K, Relly C, Landolt MA, Alber FD, Boltshauser E, Grotzer M.A. Outcome of medulloblastoma in children: long-term complications and quality of life. *Neuropediatrics.* 36:357-65, 2005
9. Kiltie AE, Lashford LS, Gattamaneni HR. Survival and late effects in medulloblastoma patients treated with craniospinal irradiation under three years old. *Med Pediatr Oncol*. 28:348-54, 1997
10. Packer RJ. Sutton LN, Atkins TE, et al. A prospective study of cognitive function in children receiving whole-brain radiotherapy and chemotherapy: 2-year results. *J Neurosurg* 70:707-13,1989
11. Roizmzn B, Sears AE. Herpes Simplex Viruses and their Replication. In Fundamental Virology, 3rd ed, pp 1043-1107. Edited by B.N. Fields, D.M. Knipe & P.M. Howley. Philadelphia: Lippincott-Raven, 1996
12. Chou J, Kern ER, Whitley RJ, Roizman B. Mapping of herpes simplex virus-1 neurovirulence to gamma 134.5, a gene nonessential for growth in culture. *Science* 250;1262-6, 1990
13. Chou J, Roizman, B. Herpes simplex virus 1 gamma(1)34.5 gene function, which blocks the host response to infection, maps in the homologous domain of the genes expressed during growth arrest and DNA damage. *Proc Natl Acad Sci U S A.* 91:5247-51. 1994
14. Martuza RL, Malick A, Markert JM, Ruffner KL, Coen DM. Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* 252:854-6, 1991
15. Markert JM, Coen DM, Malick A, Mineta T, Martuza RL. Expanded spectrum of viral therapy in the treatment of nervous system tumors. *J Neurosurg* 77:590-4, 1992
16. Markert JM, Malick A, Coen DM, Martuza RL. Reduction and elimination of encephalitis in an experimental glioma therapy model with attenuated herpes simplex mutants that retain susceptibility to acyclovir. *Neurosurgery* 32:597-603, 1993

17. Mineta T, Rabkin SD, Martuza RL. Treatment of malignant gliomas using ganciclovir-hypersensitive, ribonucleotide reductase-deficient herpes simplex viral mutant. *Cancer Res* 54:3963-6, 1994
18. Chambers R. Gillespie GY, Soroceanu L, et al. Comparison of genetically engineered herpes simplex viruses for the treatment of brain tumors in a scid mouse model of human malignant glioma. *Proc Natl Acad Sci U S A*. 92:1411-5, 1995
19. Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nat Med* 1:938-43, 1995
20. Markert JM, Gillespie GY, Weichselbaum RR, Roizman B, Whitley, R. J. Genetically engineered HSV in the treatment of glioma: a review. *Rev Med Virol*. 10:17-30, 2000
21. Whitley RJ, Alford CA, Hirsch MS, et al. Vidarabine versus acyclovir therapy in herpes simplex encephalitis. *N Engl J Med.* 314:144-9, 1986
22. Goldstein DJ, Weller SK. Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: isolation and characterization of an ICP6 lacZ insertion mutant. *J Virol* 62, 196-205 (1988).
23. Gillory LA, Megison ML, Stewart JE, et al. Preclinical evaluation of engineered oncolytic herpes simplex virus for the treatment of neuroblastoma. *PLoS One*. 8:e77753, 2013
24. Megison ML, Gillory LA, Stewart JE, et al. Preclinical evaluation of engineered oncolytic herpes simplex virus for the treatment of pediatric solid tumors. *PLoS One*. 9:e86843, 2014
25. Pressey JG, Haas MC, Pressey CS, Kelly VM, Parker JN, Gillespie GY, Friedman GK. CD133 marks a myogenically primitive subpopulation in rhabdomyosarcoma cell lines that are relatively chemoresistant but sensitive to mutant HSV. *Pediatr Blood Cancer*. 60:45-52, 2013.
26. Friedman GK, Langford C, Coleman J, Cassady K, Parker J, Markert J, Gillespie GY. Engineered herpes simplex virus efficiently infects and kills CD133+ glioma cells that express CD111. *J Neurooncol*. 95:199-209. 2009
27. Friedman GK, Kelly VM, Bray A, Cassady KA, Markert JM, Gillespie GY. Clinically-ready oncolytic engineered herpes simplex viruses kill medulloblastoma neurospheres from patient-derived xenolines. *Neuro Oncol*. 14(Suppl 1):91-92, 2012.
28. Friedman GK, Bharara S, Langford C, Coleman J, Gillespie GY. Engineered herpes simplex virus oncolytic therapy kills both tumor cells and glioma progenitor cells in a pediatric high-grade glioma. Abstracts from the Thirteenth International Symposium on Pediatric Neuro-Oncology. *Neuro Oncol*. 10:508-509, 2008.
30. Friedman GK, Pressey JG, Reddy AT, Markert JM, Gillespie GY. Herpes simplex virus oncolytic therapies for pediatric malignancies. *Mol Ther*. 17:1125-35, 2009
31. Andreansky S, Soroceanu L, Flotte ER. Evaluation of genetically engineered herpes simplex viruses as oncolytic agents for human malignant brain tumors. *Cancer Res* 57:1502-9, 1997
32. Sundaresan P, Hunter WD, Martuza RL, Rabkin SD. Attenuated replication-competent herpes simplex virus 1 mutant G207: safety evaluation in mice. *J Virol* 74:3832-3841, 2000
33. Hunter, W.D. Martuza RL, Feigenbaum F, et al. Attenuated, Replication-Competent Herpes Simplex Virus Type 1 Mutant G207: Safety Evaluation of Intracerebral Injection in Nonhuman Primates. *J Virol*, 73:6319-6326, 1999

34. Advani S., Sibley G, Hallahan DE, Roizman B, Weichselbaum RR. Enhancement of replication of Genetically Engineered Herpes Simplex Virus by ionizing radiation: a new paradigm for destruction of intractable tumors. *Gene Ther* 5: 160-165, 1998
35. Bradley JD, Kataoka Y, Advani S, et al. Ionizing radiation improves survival in mice bearing intracranial high-grade gliomas injected with genetically modified herpes simplex virus. *Clin Cancer Res* 5: 1517-1522, 1999
36. Stanziale SF, Petrowsky H, Joe JK, Roberts GD, Zager JS, Gusani NJ, Ben-Porat L, Gonen M, Fong Y. Ionizing radiation potentiates the antitumor efficacy of oncolytic herpes simplex virus G207 by upregulating ribonucleotide reductase. *Surgery*. 132:353-9, 2002
37. Xu F, Lee FK, Morrow RA, Sternberg MR, Luther KE, Dubin G et al. Seroprevalence of herpes simplex virus type 1 in children in the United States. *J Pediatr* 151:374–377, 2007
38. Whitley R.J., Kimberlin D.W. and Roizman B. Herpes Simplex Virus. *Clin Infect Dis* 26:541-553, 1998
39. Fontanilla HP, Pinnix CC, Ketonen LM, Woo SY, Vats TS, Rytting ME, Wolff JE, Mahajan A.Palliative reirradiation for progressive diffuse intrinsic pontine glioma. *Am J Clin Oncol* 35:51-57, 2012
40. Nieder C, Astner ST, Mehta MP, Grosu AL, Molls M. Improvement, clinical course, and quality of life after palliative radiotherapy for recurrent glioblastoma. *Am J Clin Oncol* 31:300-305, 2008
41. Bauman GS, Sneed PK, Wara WM, Stalpers LJ, Chang SM, McDermott MW, Gutin PH, Larson DA. Reirradiation of primary CNS tumors. *Int J Radiat Oncol Biol Phys* 36:433-441, 1996.
42. Kimberlin DW, Whitley RJ. Viral infections of the newborn infant. In: Rudolph's Pediatrics, 22nd edition. Rudolph CD, Rudolph AM, Hostetter MK, Lister G, Siegel NJ (eds). McGraw Hill Medical, New York, March, 2011.
43. G, Mescheder A, Jarnagin W, Kemeny N. A herpes oncolytic virus can be delivered via the vasculature to produce biologic changes in human colorectal cancer. *Mol Ther*. 17:389-94, 2009
44. Carceller F, Fowkes LA, Khabra K, Moreno L, Saran F, Burford A, Mackay A, Jones DT, Hovestadt V, Marshall LV, Vaidya S, Mandeville H, Jerome N, Bridges LR, Laxton R, Al-Sarraj S, Pfister SM, Leach MO, Pearson AD, Jones C, Koh DM, Zacharoulis S. Pseudoprogression in children, adolescents and young adults with non-brainstem high grade glioma and diffuse intrinsic pontine glioma. *J Neurooncol* 129:109-21, 2016.
45. Zamora C, Lopez M, Cunningham F, Collichio F, Castillo M. Imaging Manifestations of Pseudoprogression in Metastatic Melanoma Nodes Injected with Talimogene Laherparepvec: Initial Experience. *AJNR Am J Neuroradiol*. 2017 Apr 20.
46. Miyatake S, Furuse M, Kawabata S, Maruyama T, Kumabe T, Kuroiwa T, Ono K. Bevacizumab treatment of symptomatic pseudoprogression after boron neutron capture therapy for recurrent malignant gliomas. Report of 2 cases. Neuro Oncol 15:650-655, 2013.
47. Levin VA, Bidaut L, Hou P, Kumar AJ, Wefel JS, Bekele BN, Grewal J, Prabhu S, Loghin M, Gilbert MR, Jackson EF. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. *Int J Radiat Oncol Biol Phys* 79:1487-95, 2011.
48. Liu AK, Macy ME, Foreman NK. Bevacizumab as therapy for radiation necrosis in four

children with pontine gliomas. *Int J Radiat Oncol Biol Phys* 75:1148-54, 2009.48.
49. Foster KA, Ares WJ, Pollack IF, Jakacki RI. Bevacizumab for symptomatic radiation-induced tumor enlargement in pediatric low grade gliomas. *Pediatr Blood Cancer* 62:240-245, 2015.

APPENDICES

Appendix A

Patient Study Schedule

Appendix B

Modified Lansky and Karnofsky Status Determination

Appendix C

Preparation of G207

Appendix D

MRI Sequences

APPENDIX A

Patient Study Schedule

	Pre	Day -1	Day 0	Day 1	Day 2	Day 3or 4	Day 7	Day 14	Day 28	Month 3	Month 5	Month 7	Month 9	Month 12, 18, 24 †‡
History and Physical	X						X	X	X	X	X	X	X	X
Complete metabolic panel, magnesium and phosphorous	X				X		X		X					
Complete blood count with differential and platelets including lymphocyte markers	X				X		X	X	X	X	X	X	X	X
Cystatin C	X%													
HIV Serology	X													
HSV Antibody Titer	X								X	X	X	X	X	X
HSV Detection (culture and PCR for Saliva and conjunctival secretions, and PCR for Blood)**	X			X		X	X	X	X	X	X	X	X	X
Serum Pregnancy for adolescent female	X													
CT Scan			X	X#										
Biopsy and placement of catheter(s)		X												
Dosing			X											
Radiation				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
Blood sample***	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. Quality of life evaluation will be performed at screening, 1, 3, 5, and 12 months.

% Only for patients with elevated creatinine level on complete metabolic panel

Only for patients who receive radiation therapy. Patients may get simulation CT scan at screening or Day 1.

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

† After the 24 month visit, studies will be performed 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 that might be related to G207 for up to 15 years after treatment with G207. Particular attention will be paid to any symptoms (neurological or otherwise) consistent with HSV infection.

& Only for patients in dose level 3 and 4

APPENDIX B

The Modified Lansky Performance Scale (Patients < 10 year)

MODIFIED LANSKY PERFORMANCE SCALE

NORMAL RANGE

100	Fully active.
90	Minor restriction in physically strenuous play.
80	Restricted in strenuous play, tires more easily, otherwise active.

MILD-TO-MODERATE RESTRICTION

70	Both greater restriction of, and less time spent, in active play.
60	Ambulatory up to 50% of the time, limited active play with assistance and supervision.
50	Considerable assistance required for any active play; fully able to engage in quiet play.

MODERATE-TO-SEVERE

40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activity.
20	Limited to very passive activity initiated by other (e.g., television)
10	Completely disabled, not even passive play
0	Unresponsive, comatose

The Karnofsky Performance Status Scale (Patients ≥ 10 year)

100 Normal, no complaints, no evidence of disease
90 Able to carry on normal activity; some minor symptoms
80 Normal activity with some effort; some symptoms
70 Care for self; unable to carry on normal activities
60 Requires occasional assistance; cares for most needs
50 Requires considerable assistance and frequent care
40 Disabled; requires special care and assistance
30 Severely disabled; hospitalized, death not imminent
20 Very sick; active support treatment needed
10 Moribund; fatal processes are rapidly progressing

Appendix C

Preparation of G207 dilutions will take place in the hospital pharmacy.

G207 should be prepared for administration following Biosafety Level 2 Guidelines using a laminar flow biological safety cabinet, hood, gloves, safety glasses and gown.

MATERIALS NEEDED FOR PREPARATION OF G207 DOSE FOR SUBJECT ADMINISTRATION

1. 70% Isopropanol solution
2. MetriSpray, Cavicide or equivalent disinfectant.
3. U-100 Micro-fine IV insulin syringe with fixed 28G 1cm (or 1/2 inch) needle for virus delivery (total of 6 syringes needed)
4. Tuberculin syringe and 18-23 G needle
5. Sterile Vector Diluent (0.9% sodium chloride, USP, preservative-free for injection) cooled to 4°C.
6. Sterile Gloves
7. Alcohol Swabs/Wipes
8. Kim-wipes

EQUIPMENT NEEDED FOR PREPARATION OF G207 DOSE FOR SUBJECT ADMINISTRATION

1. Laminar Flow Biological Safety Cabinet (LFBSC)
2. Rack for vector G207 Stock Vials
3. Refrigerator
4. Wet ice or coolant

METHOD FOR PREPARATION OF G207 DILUTION FOR SUBJECT ADMINISTRATION

1. Remove all items from the LFBSC, wipe down work surfaces with MetriSpray, Cavicide or equivalent disinfectant, followed by 70% isopropanol, allow blower to run for 30 minutes prior to using.
2. Cool virus diluent (sterile preservative-free 10% Glycerol in Phosphate Buffered Saline, pH 7.0-7.4, USP (Alanza)) to approximately 4°C in a refrigerator or on ice.
3. Thaw the appropriate number of vector stock G207 vial(s) based on dosage required by rubbing gently between gloved hands. Continue until the last ice crystals have melted and then place vials on ice. Note the time that the contents have completely thawed. Do not allow the vials to warm.

4. Wipe stock G207 vials dry with Kim-Wipes. Gently mix contents of stock G207 vials.

5. Wipe stock G207 vials with alcohol swab and place in a vial rack in the LFBSC to dry.

6. Dose Preparation Guide

   6.1 Draw up the required amount of sterile preservative-free 10% Glycerol in Phosphate Buffered Saline, pH 7.0-7.4, USP for injection (vector diluent)

   6.2 Remove the required volume of stock G207 from the cryovials. Add stock G207 to the receptacle/vehicle containing the required diluent

   6.3 Gently invert end over end at least 5 times to mix contents

7. Label administration syringe(s) appropriately and load 1 ml diluted G207 into each syringe.

8. Place syringe(s) in sterile syringe holder.

9. Transport syringe container immediately to patient's room for administration. Syringes will be placed in individual syringe pumps. Syringes must be kept at room temperature at all times and infusions completed within 8 hours of thawing the vector. Infusion rates for each individual catheter will be dictated by the number of catheters as follows:

400 microliters per hour/(number of active catheters).

10. Start and stop time of G207 injection will be documented.

11. All reactions or complications encountered during the administration of G207 should be documented.

12. All remaining biological material must be chemically disinfected or autoclaved. All instruments coming in contact with G207 must be autoclaved. All other items coming in contact with G207 must be incinerated or autoclaved.

13. Waste Disposal responsibility must be managed according to the institutional SOP on disposal of hazardous waste.

Appendix D

All MRI will be performed on a 3T MRI scanner. The MRI schedule will be: baseline (prior to virus injection), Day 3 or 4, Day 28, month 3, 5, 7, 9, 12, 18, 24, and then yearly thereafter. Unscheduled MRI scans may be done if needed to evaluate post-administration neurological decline. Sequences needed for the biopsy and resection will be determined by the operating neurosurgeon.

SAG T1
FOV
FH (mm) = 230;
AP (mm) = 230;
RL (mm) = 154;
Echoes = 1;
partial echo = "no";
TE ="user defined";
(ms) =10;
Flip angle (deg) = 70;
TR = "range";
minimum (ms) = 600;
maximum (ms) = 700;
Total scan duration = "04:09.6";
SAR / head = "< 99 %";
Whole body / level = "< 0.2 W/kg / normal";

Ax T1
FOV
AP (mm) = 230;
RL (mm) = 183.28125;
FH (mm) =141.5;
Slice thickness (mm) = 5;
TE ="user defined";
(ms) = 10;
Flip angle (deg) = 70;
TR = "range";
minimum (ms) = 600;
maximum (ms) = 700;
Total scan duration = "03:19.2";
SAR / head = "< 62 %";
Whole body / level = "< 0.1 W/kg / normal";

Ax T2
FOV
AP (mm) = 230;
RL (mm) = 182.954544;
FH (mm) = 141.5;

Slice thickness (mm) = 5;
Reconstruction matrix = 512;
TE = "user defined"
(ms) =125;
angle (deg) = 120;
echo enhancement = "no";
bright fat reduction = "no";
TR = "user defined";
(ms) = 11000;
Fast Imaging mode = "TSE";
shot mode = "multishot";
TSE factor = 31;
SAR / head = "< 33 %";
Total scan duration = "04:36.0";
Whole body / level = "< 0.1 W/kg / normal";

AX FLAIR
FOV
AP (mm) = 230;
RL (mm) = 182.954544;
FH (mm) = 141.5;
slices = 22;
slice gap = "user defined";
gap (mm) = 1.5;
Fast Imaging mode = "TSE";
shot mode = "multishot";
TSE factor = 31;
TE = "user defined";
(ms) = 125;
Refocusing control = "yes";
angle (deg) = 120;
echo enhancement = "no";
bright fat reduction = "no";
TR = "user defined";
(ms) =11000;
Total scan duration = "03:51.0";
SAR / head = "< 33 %";
Whole body / level = "< 0.1 W/kg / normal";

Ax DWI
FOV
RL (mm) = 230;
AP (mm) = 230;
FH (mm) = 149;
Slice thickness (mm) = 4;

Fast Imaging mode = "EPI";
shot mode = "single-shot"
Echoes = 1;
TE = "shortest";
Flip angle (deg) = 90;
TR = "shortest";
Diffusion mode = "DWI";
nr of b-factors = 2;
b-factor order = "ascending";
max b-factor = 1000;
average high b = "yes";
Total scan duration = "00:37.9";
SAR / head = "< 35 %";
Whole body / level = "< 0.1 W/kg / normal";

VENBOLD
FOV
AP (mm) = 200;
RL (mm) = 200;
FH (mm) = 125;
Reconstruction matrix = 512;
Scan mode = "3D";
TE = "shortest";
Flip angle (deg) = 10;
TR = "shortest";
Total scan duration = "05:46.8";
SAR / head = "< 4 %";
Whole body / level = "0.0 W/kg / normal";

T1 mapping (2, 10, 15, 20, 30degrees)
FOV
AP (mm) = 256;
RL (mm) = 168;
FH (mm) = 80;
TE = "user defined"
(ms) = 2.48000002;
Flip angle (deg) = 2, 10, 15, 20, 30;
TR = "user defined";
(ms) = 4.88000011;
Total scan duration = "00:11.5"; "00:05.7"; "00:11.5"; "00:11.5"; "00:11.5";
SAR / head = "< 1 %";
Whole body / level = "0.0 W/kg / normal";

DCE perfusion MRI
FOV
AP (mm) = 256;

RL (mm) = 168;
FH (mm) = 80;
Echoes = 1;
partial echo = "no";
shifted echo = "no";
TE = "user defined";
(ms) = 2.48000002;
Flip angle (deg) = 20;
TR = "user defined";
(ms) = 4.88000011;
Dynamic study = "individual";
dyn scans = 60;
dyn scan times = "shortest";
FOV time mode = "default";
Total scan duration = "05:43.2";
SAR / head = "< 60 %";
Whole body / level = "< 0.1 W/kg / normal";

SAG T1 THRIVE
Coil selection = "SENSE-Head-8";
FOV
FH (mm) = 250;
AP (mm) = 250;
RL (mm) = 166.200012;
Echoes = 1;
partial echo = "no";
shifted echo = "no";
TE = "shortest";
Flip angle (deg) = 10;
TR = "shortest";
Total scan duration = "04:58.6";
SAR / head = "< 21%";
Whole body / level ="0.0 W/kg / normal";

DSC PERFUSION
FOV
RL (mm) = 224;
AP (mm) = 224;
FH (mm) = 140;
Voxel size RL (mm) = 2.32999992;
AP (mm) = 2.32999992;
Slice thickness (mm) = 4;
Recon voxel size (mm) = 1.75;
Fold-over suppression = "no";

Reconstruction matrix = 128;

slice gap = "user defined";
gap (mm) = 0;
slice orientation = "transverse";
fold-over direction = "AP";
fat shift direction = "P";
TE = "user defined";
(ms) = 20;
Flip angle (deg) = 60;
TR = "shortest";
Dynamic study = "individual";
dyn scans = 60;
dyn scan times = "shortest";
Act. TR/TE (ms) = "2202 / 40";
Dyn. scan time = "00:02.2";
SAR / head = "< 27 %";
Whole body / level = "< 0.1 W/kg / normal";

Ax T1+C
FOV
RL (mm) = 224;
AP (mm) = 224;
FH (mm) = 140;
Echoes = 1;
partial echo ="no";
TE = "user defined";
(ms) = 12;
Flip angle (deg) = 70;
TR = "range";
minimum (ms) = 400;
maximum (ms) = 750;
Total scan duration = "04:08.3";
SAR / head = "< 100 %";
Whole body / level = "< 0.2 W/kg / normal";

Summary of Changes to the Protocol

Protocol Version	Summary of Changes
v07.30.15 to v02.02.16	Corrected clerical errors. Infusion time changed from two consecutive 3-hour infusion to one 6-hour infusion. Response criteria changed from Response Assessment in Neuro-Oncology (RANO) criteria to the immunotherapy RANO criteria (iRANO).
v02.02.16 to v06.05.16	Corrected clerical errors. The term injection was changed to infusion. Clarified that only HSV PCR (and not cultures) would be performed on blood. Adjusted the expected accrual time. Clarified that cystatin C only used if serum creatinine abnormal. Clarified that tumors requiring access through a ventricle to deliver treatment are ineligible similar to ventricular inoculation. Clarified the definition of adverse event, serious adverse event (SAE), serious unexpected suspected adverse reaction, and the timing for reporting these. Clarified timing of study procedures after informed consent is signed. Updated timing of inpatient vital signs and neurologic function. Clarified that catheters can be removed ≥ 1 hour post-G207 and that then stable patients can be transferred from the PICU. Added that bevaziumab and/or dexamethasone can be used for pseuodprogression or neurologic symptoms. Clarified when radiation simulation may occur. Added that study blood samples (optional consent) are drawn with standard blood draws. Removed specific references to hospital affiliations.
v06.05.17 to v11.03.17	Clarified catheters can be passed to predefined coordinates of enhancing and non-enhancing tumor if biopsy confirms presence of tumor cells.
v11.03.17 to v06.06.18	Corrected clerical errors. Changed a co-investigator. Added prior participation in viral therapy to the eligibility criteria. Bevacizumab dose adjusted to 5-10 mg/kg. Clarified study labs required for HSV detection, time points for quality of life measures, and follow-up visits at 18, 24 months and yearly thereafter.
v06.06.18 to v05.13.19	Corrected clerical errors. Added a non-UAB affiliation (St. Jude Children's Research Hospital) for co-investigator. Changed Clinical Trials Review Committee (CTRC) chair's name. Clarified pseudoprogression management. Clarified the eligibility of pathologically proven secondary malignant tumor. Clarified period for last dose of bevacizumab before eligibility. Changed the infusion flush time from 35 to 36 minutes to make infusion amount an integer and not a fraction. Clarified infusion rate for 3 catheters in total volume of 2.4 mL. Revised Patient Study Schedule to include simulation for radiation.
v05.13.19 to v04.20.2020	Corrected clerical errors. Removed Clinical Trials Review Committee (CTRC) chair's name. Changed G207 company name from Aettis to Treovir, Inc. Clarified the definition of dose-limiting toxicity for consistency with recently opened G207 cerebellar study. Clarified period for last dose of monoclonal antibody before eligibility. Clarified volume for priming each infusion tubing. Added additional language regarding radiation treatment for non-enhancing tumors. Clarified patients taken off treatment and off-study. Clarified that T2/FLAIR abnormality may be used for two-dimensional measurements and response may be determined by consensus among the treating physicians when a tumor cannot be classified strictly by iRANO criteria

Summary of Changes to the Statistical Analysis Plan

Protocol Version	Summary of Changes
v07.30.15 to v02.02.16	Response criteria was changed from Response Assessment in Neuro-Oncology (RANO) criteria to the immunotherapy RANO criteria (iRANO). This change was made because the immunotherapy criteria were released and were more appropriate criteria for G207, an immunovirotherapy. The change was made prior to any patient being treated on the study.
v02.02.16 to v04.20.2020	Clarified that T2/FLAIR abnormality may be used for two-dimensional measurements and response may be determined by consensus among the treating physicians when a tumor cannot be classified strictly by iRANO criteria. This clarification was made because some pediatric malignant brain tumors do not enhance, patients with biopsy-confirmed non-enhancing recurrent tumors were eligible for treatment, and non-enhancing tumors cannot be strictly classified by iRANO.