Phase 1 Study of M032 (NSC 733972), a Genetically Engineered HSV-1 Expressing IL-12, in Patients with Recurrent/Progressive *Glioblastoma Multiforme*, Anaplastic Astrocytoma, or Gliosarcoma

Study Protocol & Statistical Analysis Plan

NCT02062827

Date of Last Protocol Approval
10 August 2023

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TITLE: A Phase 1 Study of M032 (NSC 733972), a Genetically Engineered HSV-1 Expressing IL-12, in Patients with Recurrent/Progressive *Glioblastoma Multiforme*, Anaplastic Astrocytoma, or Gliosarcoma

Version: Amendment 12 Version 2.9 Date: Sep 20, 2021
Version: Amendment 11 Version 2.8 Date: Jan 20, 2021
Version: Amendment 10 Version 2.7 Date: Aug 25, 2020
Version: Amendment 9 Version 2.6 Date: Apr 6, 2020
Version: Amendment 8 Version 2.5 Date: Dec 10, 2019
Version: Amendment 7 Version 2.4 Date: Apr 5, 2019
Version: Amendment 6 Version 2.3 Date: Feb 22, 2019
Version: Amendment 5 Version 2.2 Date: Jan 15, 2019
Version: Amendment 4 Version 2.1 Date: May 30, 2018
Version: Amendment 3 Version 2 Date: April 28, 2017
Version: Amendment 2 version 1.8 Date: August 26, 2015
Version: Amendment 2 version 1.7 Date: April 20, 2015
Version: Amendment 1 version 1.6 Date: July 22, 2014
Version: Revision 1.5 Date: August 20, 2013
Version: 1.1 Date: August 20, 2013
Version: 1.0 Date: December 15, 2011

SPONSOR: **NIH/NCI**

IND#: 14,946

NIH Grant: **1R01CA217179**

NCI Supplied Agent: M032 (NSC 733972, supplied by NeXT program

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Changes between Amendment 11 version 2.8 dated 20 Jan, 2021 and Amendment 12 version 2.9 dated 20 Sep 2021

Section/location	Change	Rationale
8	• **Adding an unscheduled visit**	**Administrative change**
Study synopsis	• **In a previous amendment it was stated that 4-20 participants are to be enrolled and treated in addition to the first 4 in the seed model (Section 4.1.2). The enrollment number is being increased to 24 to clarify that the previously stated 4-20 participants are in addition to the first 4.**	**Administrative change**

STUDY SYNOPSIS

Objective:	To obtain safety information in small cohorts of individuals (three patients per group), with cohorts to receive escalating doses of M032. Safety will be assessed at each dose level before proceeding to the next dose. Biologic secondary objectives include characterization of the *in situ* activity of M032 after intratumoral inoculation and of the local and systemic immune responses to M032. As a clinical secondary objective, patients will be followed serially by MRI for potential clinical response to M032. The clinical strategy takes advantage of the virus' ability to infect and lyse tumor cells and the potential for enhancement of this effect by the induction of an anti-tumor immune response by IL-12 as well as an anti-angiogenic response by this cytokine.
Treatment Indication:	Progressive growth of *glioblastoma multiforme*, anaplastic astrocytoma or gliosarcoma after radiation therapy.
Clinical Phase:	Phase 1 (open-label)
Design:	Single dose of M032 infused through catheters into region(s) of tumor defined by MRI; Up to 3 patients/dosage level; dosage escalation proceeds only after a minimum of 24 days of observation, if incidence of Grade III/IV toxicities is acceptable. Dose increases or reductions will be determined using a modified Continual Reassessment Method (CRM); extent of dose changes for subsequent subjects will be increased up to, but not exceeding the next higher dose level (1 log). Dose modifications will utilize a modified CRM for each successive subject until an MTD or the maximal planned dose is reached.
Study Duration Per Patient:	12 months
Subject Population:	8 to 24 patients with recurrent/progressive *glioblastoma multiforme*, anaplastic astrocytoma or gliosarcoma depending on toxicities. (Numbers may vary slightly pending acceptance of previously treated patients to update the CRM)
Study Medication and Dosage:	Dose escalations of up to, but not exceeding the next highest dose level (1 log). Dose escalations from 1 x 10^5 to 1 x 10^9 plaque-forming units of M032, a genetically engineered herpes simplex virus type 1 expressing human IL-12 (NSC 733972).
Safety Evaluations:	Follow-up evaluations using routine laboratory analyses and clinical measurements of neurological function and evidence of M032-related toxicity. Studies to evaluate the possibility of M032 shedding will also be conducted. Patients will be observed closely during the planned post-treatment hospitalization period, followed by outpatient evaluations done at 10 days, then months 1, 2, 3, 4, 5, 6, 9 and 12, subject to disease progression.
Study Endpoints	Primary: CRM-estimated highest safe dose or maximal planned dose if no dose-limiting toxicity observed. Secondary: Time to progression, survival, biologic assessments.

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1. OBJECTIVES

1.1 Lay Abstract

M032 is a second-generation oncolytic herpes simplex virus (oHSV) that is conditionally replication competent; that is, similar to G207, a first generation oHSV, it can replicate in tumor cells, but not in normal cells, thus killing the tumor cells directly through this process. Replication of M032 in the tumor itself not only kills the infected tumor cells, but causes the tumor cell to act as a factory to produce new virus. These virus particles are released as the tumor cell dies, and can then proceed to infect other tumor cells in the vicinity, and continue the process of tumor kill. In addition to this direct oncolytic activity, the virus carries a therapeutic payload--acting as a gene therapy vector, too--and causes the tumor cell to synthesize and secrete an immunity-stimulating protein called Interleukin-12 (IL-12) before it is killed. This IL-12 is released and promotes an immune response against surviving tumor cells, which increases the antitumor effect of the therapy. The IL-12 that is expressed can also produce an anti-angiogenic effect, by interfering with the production of new tumor blood vessels necessary to allow tumor growth. Anti-angiogenic therapies potentially starve the tumor of necessary oxygen and nutrients. Thus, the M032 oHSV produces three different potential mechanisms for antitumor effects. The virus has also been genetically engineered to minimize the production of any toxic effects for the patient receiving the therapy.

1.2 Primary Objective

To determine, in patients who would not be eligible for resection of recurrent glioma, the safety and tolerability of stereotactic intracerebral injections of escalating doses of M032 virus (NSC 733972), and to determine the maximally tolerated dose (MTD) of M032.

1.3 Secondary Objectives

To obtain preliminary information about the potential benefit of M032 in the treatment of patients with recurrent malignant gliomas (progression-free survival, overall survival).

1.4 Tertiary Objectives

1.4.1 To delineate the local and systemic immune response to M032 administration.

1.4.2 To characterize the *in situ* biologic activity of M032 after intratumoral inoculation, where possible.

2. BACKGROUND

2.1 Safety and efficacy of non-virulent, oncolytic Δγ$_1$34.5 HSV

The efficacy of herpes simplex virus (HSV) as a treatment for brain tumors has been demonstrated experimentally. The earliest studies used a thymidine kinase (*tk*) deficient HSV vector following the demonstration that tk deficient viruses grow relatively slowly in a variety of cell lines, and exhibit reduced pathogenicity *in situ*. Martuza and colleagues demonstrated that subcutaneous and intracerebral tumors regressed following treatment with varying titers of a *tk*$^-$ HSV [1-2]. This HSV vector, which consisted of a 360 base pair (bp) deletion within the tk gene (resulting in inactivation of the gene), was able to reduce the growth rate of a variety of human tumors including gliomas in murine models.

Additional modified viruses based on the HSV backbone have been developed and tested with encouraging results [3-6]. Viruses containing deletions in viral DNA polymerase, the neurovirulence gene ($\gamma_1$34.5), or HSV-1/HSV-2 intertypic recombinants also retained the capability of killing cultured human tumors [7]. Specifically, 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 the antiviral drug acyclovir which is routinely used to treat HSV infections [8].

A series of studies was carried out to investigate variations in the mutation of the neurovirulence gene $\gamma_1$34.5. Two viruses derived from HSV strain F have been tested exhaustively [5, 9-10]. The first, R3616, has a 1 kb deletion in each copy of the $\gamma_1$34.5 gene, whereas R4009 has a stop codon inserted in the coding region. Using a severe combined immunodeficiency (SCID) mouse model, Whitley and colleagues demonstrated that both viruses, designated Δγ$_1$34.5 HSV, were effective agents for the treatment of human gliomas introduced intracerebrally into mice. Moreover, injection of up to 10^7 plaque-forming units (pfu) of R3616 virus

intracerebrally into immunocompetent mice resulted in no deleterious effects, whereas 100 pfu of the parental HSV-1(F) virus was lethal. Additionally, a mutation in a more neurovirulent herpes HSV strain (strain 17) has been evaluated extensively [11-12]. Consisting of a 760-bp deletion in both copies of the $\gamma_1$34.5 gene, the mutant HSV 1716 has also been shown to be aneurovirulent in immunocompetent mice at 10^6 pfu and to cause regression of human brain tumors in mice with corresponding increased survival.

Martuza and colleagues generated G207, 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 [6, 13]. G207 significantly prolonged survival of nude mice bearing human tumors either subcutaneously or intracranially when injected at titers of 10^6-10^7 pfu. In addition, virus injected intracerebrally into HSV sensitive *Aotus* simian primates did not produce any deleterious side effects, whereas $<10^3$ pfu of HSV-1(F) virus was lethal.

Clinical Trials Utilizing Oncolytic Δγ$_1$34.5 HSV in Human Malignant Glioma

G207 and 1716 have both been used in human trials. A dose-escalating phase 1 study of G207 was completed in patients with recurrent, progressive malignant glioma [14]. The trial was conducted at University of Alabama at Birmingham and Georgetown University Medical Center. Twenty-one patients were enrolled in a total of seven dose-escalating cohorts, with three patients per cohort. Patients were stereotactically inoculated with G207 in the enhancing portions of their tumors. Five separate loci were inoculated in the final cohort; all previous cohorts were inoculated in a single locus within the enhancing tissue only. No toxicity definitively related to G207 was observed at doses up to 3×10^9 plaque forming units (pfu). In fact, a toxic dose level was not attained during this trial. This was due to viral processing techniques available at that time, limiting the total dose that could be administered.

In a Phase IB study, six patients with recurrent, resectable malignant glioma were enrolled at the University of Alabama at Birmingham, in a trial examining a split dose administration strategy of G207 [15]. Patients underwent stereotactic inoculation of G207 into their tumors, followed two to five days later by resection of the tumor and reinoculation of G207 into the tumor cavity. 1×10^8 pfu was inoculated initially, followed by a second dose of 1×10^9 pfu. No dose limiting toxicities were seen in the trial, although one patient suffered a twelve hour period of mental status changes, contralateral paresis, and an elevated temperature when a protocol deviation resulted in an inadvertent partial dose of virus being administered intraventricularly. The patient fully recovered quickly. One of the six patients went nearly two years before remote recurrence of her *glioblastoma multiforme* resulted in her death.

A third study in 9 patients with recurrent malignant glioma was recently completed in which G207 was being administered followed by a single dose of 5Gy of radiation. Nine patients were followed without any dose limiting toxicity. Results of this trial are pending completion of a manuscript for submission for publication.

In a phase 1 study utilizing a similar oncolytic HSV vector, nine patients with relapsed malignant glioma received intratumoral inoculation of 1716. Although 1716 carries only a deletion of the $\gamma_1$34.5 gene (it does not carry the additional mutation of the U_L39 gene found in G207), it also caused no dose-limiting toxicity at all doses tested (maximum dose,10^5 pfu) [16]. Both the G207 and 1716 studies used modified HSV strains that retained the tk gene, thus allowing potential response to standard anti-viral therapy if encephalitis occurred. Two additional studies of 1716 have been published, neither of which demonstrated any dose limiting toxicities [17-18].

M002 – a murine interleukin-12 expressing Δγ$_1$34.5 HSV

Parker and colleagues have generated M002 from the parent virus R3659, which is similar to both G207 and 1716 [19]; **Figure 1**. R3659 resembles G207 and 1716 except that it carries a 501-bp deletion within the wild-type tk gene and has, therefore, been constructed with a copy of the HSV-1 thymidine kinase gene at the locus of each deleted $\gamma_1$34.5 gene. Additionally, although R3659 is derived from the same wild type parent strain as G207, R3659 more closely resembles 1716 in that it does not carry the additional mutation of G207 in the U_L39 gene.

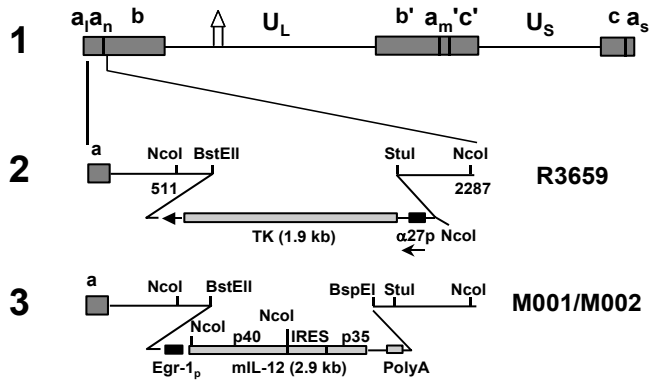
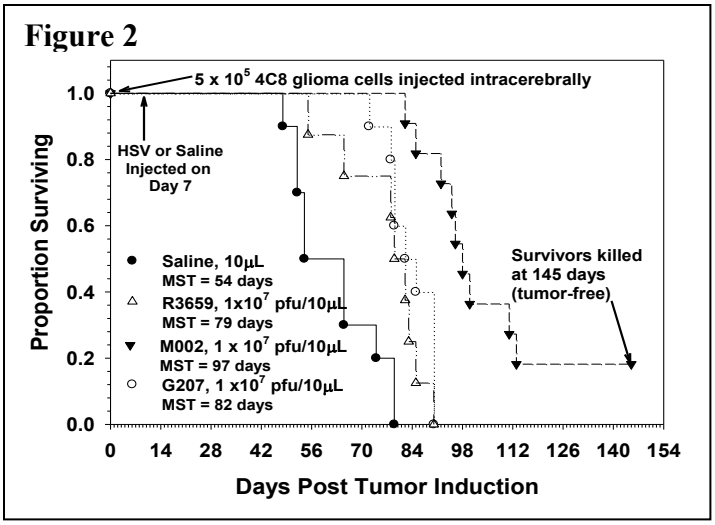


Figure 1: Schema #1 is a linear representation of the genomic structure of wild-type Strain F HSV-1 from which the other viruses were constructed. Schema #2 represents an intermediate construct in which the wild-type thymidine kinase gene is deleted from its normal position (U_L23 – up arrow in #1) and used to replace the diploid gene, $\gamma_1 34.5$, in the inverted repeat segments. Schema #3 depicts the murine IL12 gene construct used to replace the heterotopically tk gene. The IL12 construct is described in the adjacent text. M001 represents the isolate prior to replacing the tk gene in its orthotopic location in the $U_L$segment (M002).

M002 contains deletions of both copies of $\gamma_1 34.5$ and an insertion in the $\gamma_1 34.5$ locus of the gene encoding murine IL-12 (mIL-12) [20]. The two cDNA sequences encoding the p35 and p40 chains of mIL-12 are co-expressed by the Egr-1 promoter and linked by an IRES sequence. Since $\gamma_1 34.5$ is a diploid gene, two copies of the mIL-12 cassette are present in the virus. Because the insertion of the mIL-12 gene left the modified R3659 virus tk-, the deletion in the native *tk* gene locus was repaired. For this reason M002 remains sensitive to acyclovir.

M002 has been extensively evaluated *in vivo* with animal models [20-22]. mIL-12 has been shown to be expressed in physiologically relevant concentrations in various tumors of neuroectodermal origin (from 800-3200 picograms per ml per 24 hours per 5 x 10^5 cells). When compared to either the parental virus R3659 which expresses no foreign genes or the clinically evaluated HSV G207, M002 produces increased survival in murine intracranial tumor models (**Figure 2**) and increased tumor growth inhibition in subcutaneous models (**Figure 3**).

Figure 2: Immunocompetent C57BL/6xDBA/2 F1 hybrid mice were injected intracranially with 4C8 mouse glioma cells, followed in 7 days by saline or the viruses shown. Median survivals were determined by Kaplan-Meier plots and are shown on the Figure. Log rank analyses of these survival values confirmed the significantly prolonged survival afforded mice treated with M002, compared with R3659 ($p = 0.00007$) or G207 ($p = 0.0003$). In addition, about 20% of the M002-treated mice were apparently "cured" with no evidence of remaining 4C8 glioma cells histologically when the experiment was terminated.



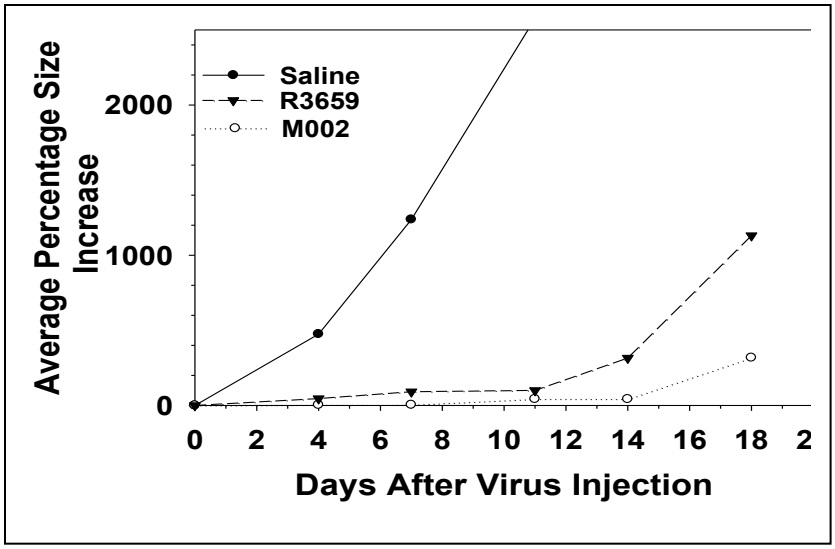


Figure 3: Immunocompetent BALB/c mice were injected subcutaneously with 1 x 10^5 Neuro-2A cells. When tumors attained 200mm^3 in volume, they were injected with 50 µl of saline or 5 x 10^7 pfu of HSV R3659 or M002. Tumor volumes were measured every 3 days and the average percentage increase in volume was calculated relative to the treatment date.

Preclinical studies have documented the increase in efficacy of IL-12 expressing oncolytic HSV in a syngeneic intracranial model of neuroblastoma and a second model of syngeneic intracranial murine malignant glioma when compared to a parent $\gamma_1$34.5 deleted virus not expressing IL-12 [21]; further studies in both immunocompetent mice bearing syngeneic neuroblastomas and nude mice bearing human U87 malignant glioma xenografts have demonstrated the superiority of IL-12 expressing oncolytic HSV specifically compared to G207, the virus previously used in clinical trials. Immunohistochemistry shows increased inflammatory response, particularly CD4+ and CD8+ cells and macrophages, suggesting that an anti-tumor immune response may be partially responsible for the increase [21].

Although M002 has not been used in human clinical studies, the safety of M002 has been addressed both by the deliberate molecular engineering of the virus as described above as well as *in vivo* testing in HSV-sensitive owl monkey, *Aotus nancymai.* Murine IL-12 has been demonstrated to be active on Aotus lymphocytes, validating this as an appropriate model for preclinical safety testing (JM Markert, unpublished data). A purified extract (made according to GMP specifications) of M002 has been injected intracerebrally into three *Aotus nancymai* at titers up to 4.8 x 10^8 pfu with no adverse effects. One of these *Aotus* is alive and well more than 5.5 years after inoculation. A second animal was alive and well more than 5 years after inoculation when a positive routine TB skin test resulted in the intentional sacrifice of this and another untreated animal in the same colony; necropsy later revealed no evidence of tuberculosis infection or viral toxicity. In contrast, 10^3 pfu of HSV-1(F) virus is lethal. MRI analysis of M002 treated monkeys showed no CNS abnormalities. Neuropathologic evaluation is pending and will be performed at one year post-inoculation. Two additional *Aotus* have also been injected with lower doses of M002 (non-purified standard laboratory purifications of 1 x 10^7 and 1 x 10^8 pfu, respectively). One of these animals died from iatrogenic antibiotic-induced renal failure and the second from anesthetic complications; brains from both animals were fixed and serially sectioned, then stained. No evidence of encephalitis or HSV-related toxicity was found.

M032 – the human Interleukin-12 expressing Δ$\gamma_1$34.5 HSV

Typically, murine proteins have not been used in human gene therapy but mIL-12 retains bioactivity in human cells. Unfortunately, human IL-12 has no activity in murine systems, which prevented the use of this protein in preclinical (murine) brain tumor models. Reconstruction of the vector to express hIL-12 instead of murine IL-12 was recommended by the reviewers of our RAID application. This new vector was constructed (**Figure 4**) and extensively characterized, and shown to be similar to M002 in terms of IL-12 production, replication, and neurotoxicity in murine models (JM Markert, GY Gillespie, unpublished data) [20, 22]. Moreover we have determined that the recombinant human IL-12 produced by M032 HSV-infected cells is biologically active and able to stimulate the proliferation of primed Aotus peripheral blood lymphocytes as evidenced by tritated thymidine uptake [41].

FIGURE 4

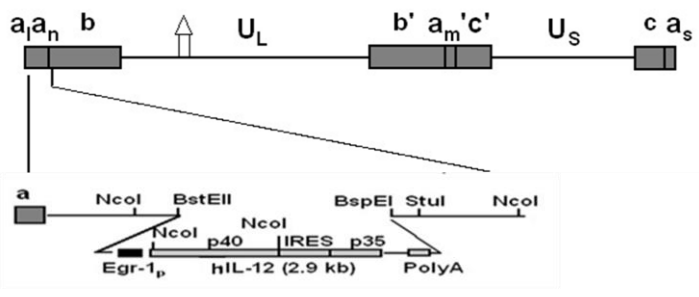


Figure 4: The $\gamma_1$34.5 gene is diploid, one copy in each of two inverted repeat segments. Construction of M032 was achieved by replacing both copies of the $\gamma_1$34.5 gene by homologous recombination using flanking sequences cloned into the 5' and 3' ends of human IL-12 construct described above. Terminal repeat insertion only shown for simplicity. The M032 HSV construct was repaired to express native HSV thymidine kinase at the U_L24 position (Up arrow); the virus is sensitive to anti-viral drugs such as acyclovir or valgancyclovir.

As M032 represents an excellent vector for evaluation in clinical studies for the treatment of *glioblastoma multiforme*, the NIH Rapid Access to Investigational Drug (RAID) Program agreed to produce a M032 Master Viral Bank, manufacture a toxicology lot of the virus, conduct biodistribution and toxicology studies in the HSV-hypersensitive non-human primate, *Aotus nancymae*, and to produce and qualify a clinical lot of virus 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 [23]. These and other documents, including this protocol, were used to obtain an IND from the FDA, #14946.

The Biodistribution and Toxicology studies were conducted by intracerebral injection of 100 microL of M032 HSV into 30 of 36 Aotus nancymae monkeys, distributed evenly by gender. The remaining 6 monkeys were injected with saline as a control. Two doses of HSV were tested – a low dose of 1 x 10^6 plaque-forming units (PFU) and a high dose of 1 x 10^8 PFU. On a dose/gram of brain basis, these doses would correspond to human equivalent doses of 5 x 10^7 PFU and 5 x 10^9 PFU. Two male and two female monkeys in each of the low dose and high dose groups were killed at 3, 31 and 91 days and a complete necropsy was performed, including PCR analysis for presence and copy number of HSV in various organs and body sites. There were no serious adverse events or adverse events that could be attributed directly to the test article, M032.

However, one animal was killed at 16 days due to a progressively moribund condition. This animal was losing weight, not eating and progressively lethargic, without clear clinical evidence of viral encephalitis (no fever). A complete necropsy did not reveal any histopathological changes indicative of HSV encephalitis and there were no substantially elevated levels of HSV as determined by quantitative PCR in the brain or in other organs, compared to the levels detected in all other monkeys. While there was nothing to support a diagnosis of HSV encephalitis or HSV infection in other organs of the body, nevertheless, neither could it be excluded that the test article was not a contributing cause of this monkey's deteriorating condition 16 days after the injection. In summary, the HSV M032 that will be used in this clinical trial has been qualified for safety and stability and has been determined to be safe for intracerebral administration in HSV-hypersensitive non-human primates at a dose that far exceeds that we intend to employ here.

2.2 Malignant Glioma

Malignant gliomas are the most common primary brain tumors of humans, accounting for 30% of all primary central nervous system (CNS) tumors in adults. There are three types of malignant gliomas: (i) anaplastic astrocytoma, (ii) *glioblastoma multiforme* and (iii) gliosarcoma. Primary malignant brain tumors in the United States are estimated to occur at an incidence of 14.7 per 100,000 people, and 10,000—15,000 new cases are diagnosed annually [24]. *Glioblastoma multiforme* (GBM), the most malignant type of brain tumor, has been refractory to improvements in treatment; the outcome of conventional treatments is poor, with a median survival of one year.

Gliomas develop from the unsuppressed growth of neural support cells called astrocytes, usually resulting in the first clinical symptoms such as memory loss, visual impairment, or seizures. Presently, the most common modalities for the treatment of brain tumors are surgical resection, chemotherapy and/or radiation therapy, depending on the severity of the tumor and type of malignancy. The major problem associated with conventional treatment stems from tumor recurrence.

2.3 Rationale

Many vectors have been considered for neoplastic therapy, including naked DNA, liposomes, and viruses. Viral vectors include those derived from retrovirus, adenovirus (AdV), adeno-associated virus (AAV) and HSV type 1. Each of the vectors has advantages and disadvantages for treating glioma *in situ*.

Recombinant HSV have been generated containing a variety of gene deletions. Their effectiveness as gene therapeutic agents have been examined *in vitro* and *in vivo* in numerous models of metastatic and primary tumors. These experiments have provided valuable safety information in various animal models. HSV vectors are neurotropic, for they have evolved to coexist within neuronal tissue (latency). The identification of genes essential for replication in the brain [8, 25] has opened new therapeutic avenues for currently incurable brain tumors. It has been clearly demonstrated experimentally that by altering these genes, either by deletion, insertion, or point mutation in the coding region, the virus will kill dividing tumor cells but is otherwise avirulent following delivery into post-mitotic brain tissue. Thus, it is possible to develop a genetically stable engineered virus with the ability to selectively replicate in glioma cells. This property is desirable when considering that, following resection, more than 10^9 tumor cells may remain *in situ*. Other methods of treatment, e.g., a retroviral vector with the HSV *tk* gene, have been unable to target this many cells, especially in light of the ongoing cellular proliferation of the remaining glioma cells. However, the ability of the modified HSV to selectively replicate within the tumor may allow for the delivery of sufficient virus into the tumor bed to potentially destroy tumor cells remaining after conventional therapy.

As described in **Section 2.1**, one conditionally replicating HSV, G207, has been tested by intracerebral administration in humans at doses up to 3×10^9 pfu without evidence of toxicity that could be attributed to the vector. However, because the selectively replicating recombinant HSV can still carry other recombinant genes, the opportunity exists to combine its direct oncolytic effects with additional gene therapy effects. The approach utilized with this study agent, M032, depends upon the modified virus' direct oncolytic activity in combination with the recruitment of a targeted inflammatory response to kill the malignant tumor cells.

This "double-barrel" effect is accomplished by the expression of IL-12 in physiologically relevant concentrations from cells infected with the recombinant HSV [20-22]. Murine IL-12 is known to cross-react with the human IL-12 receptor and stimulate an immune response, but human IL-12 does not cross react with the murine receptor; thus all *in vivo* preclinical efficacy studies have necessarily been performed in murine models using M002, a variant of the virus that expresses murine IL-12 [20-22]. IL-12 is a cytokine with potent antitumor qualities [26-28]. By induction of interferon-γ production [29], IL-12 acts to enhance the cytolytic activity of natural killer cells and cytotoxic T lymphocytes [30]. IL-12 also induces a T_H-1 type immune response [31], which may provide a more durable antitumor effect than other cytokine anti-neoplastic approaches. Furthermore, IL-12 has also been demonstrated to have *in vivo* anti-angiogenic activity which may contribute to its antitumor effects [32-33]. It has been shown to produce important antiglioma activity in different rodent models [34-35]. In preclinical models, M002 has been shown to be more effective than G207 or similar viruses as an inhibitor of tumor growth. Immunohistochemistry studies suggest that this increased efficacy may be due to an anti-tumor immune response induced by the secretion of IL-12 by the infected tumor cells [36]. A potential advantage of IL-12 is its ability to stimulate an antitumor response in the absence of IL-2. This is important in patients with malignant glioma, who suffer from a decreased ability to respond to IL-2. Nonetheless, the local immunomodulatory effects of IL-12 in the immunosuppressive milieu of human malignant gliomas is currently unknown, and will only be understood through a phase 1 study designed to assess biologic activity.

While early clinical studies of systemic IL-12 have produced evidence of toxicity [35], its effects when administered locally are not well known. As discussed in **Section 2.1**, preclinical models support the safety of intracranial inoculation with M032 in two relevant species (mouse, non-human primate).

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed *glioblastoma multiforme*, anaplastic astrocytoma, or gliosarcoma.

3.1.2 Prior therapy: Patients must have failed external beam radiotherapy to the brain, and if eligible and tolerated, undergone appropriate treatment with temozolomide chemotherapy. All radiation and additional chemotherapies must have been completed at least 4 weeks prior to enrollment. Prior therapy with nitrosoureas must have been completed at least 6 weeks prior to enrollment.

3.1.3 Age ≥18 years (age of majority for clinical trials in Alabama). Because no dosing or adverse event data are currently available on the use of M032 in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric phase 1 single-agent trials.

3.1.4 Karnofsky Performance Status (KPS) ≥70% (see Appendix B).

3.1.5 Life expectancy of greater than 4 weeks.

3.1.6 Patients must have normal organ and marrow function as defined below:

- leukocytes…….....≥3,000/μl
- absolute neutrophil count .....≥1,500/μl
- platelets .....≥100,000/μl
- total bilirubin.....within normal institutional limits
- AST(SGOT)/ALT(SGPT)…..…..≤2.5 X institutional upper limit of normal
- creatinine within normal institutional limits

OR

creatinine clearance……..≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.1.7 Residual lesion must be ≥1.0 cm and ≤ 5.5 cm in diameter without bilateral extension through the corpus callosum as determined by MRI as this is a locally delivered treatment. These parameters will be re-evaluated on imaging done on the day of catheter implantation and if the lesion no longer meets the criteria, the patient will not undergo catheter implantation or treatment with M032.

3.1.8 The effects of M032 on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception prior to study entry and for the first six months after receiving M032. Because it is currently unknown if M032 can be transmitted by sexual contact, a barrier method of birth control must be employed and for six (6) months following the administration of the study drug. Should a woman become pregnant while participating in this study, she should inform her treating physician immediately. For two weeks after receiving M032, subjects should avoid intimate contact with pregnant women, infants and young children and individuals with decreased immunity (ability to fight infection). Subjects should also refrain from donating blood during the trial.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.1.10 Females of childbearing potential must not be pregnant; this will be confirmed by a negative serum pregnancy test within 14 days prior to starting study treatment.

3.1.11 Steroid use is allowed as long as dose has not increased within 2 weeks of scheduled M032 administration. Whenever possible, the patient should be on a steroid dose that is equivalent to a dexamethasone dose of ≤ 2mg daily at the time of treatment.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy, cytotoxic therapy, immunotherapy within 4 weeks prior to entering the study (6 weeks for nitrosoureas), surgical resection within 4 weeks prior to entering the study, or have received experimental viral therapy or gene therapy at any time (e.g., adenovirus, retrovirus or herpes virus protocol). However, this does not preclude re-treatment with M032 at a later date.

3.2.2 Patients who have not recovered from adverse events due to therapeutic interventions administered more than 4 weeks earlier.

3.2.3 Patients may not be receiving any other investigational agents.

3.2.4 History of allergic reactions attributed to compounds of similar biologic composition to M032 or to IL-12.

3.2.5 Tumor involvement which would require ventricular, brainstem, basal ganglia, or posterior fossa inoculation or would require access through a ventricle in order to deliver treatment. Also, since M032 is a local treatment, patients whose tumors have bilateral extension through the corpus callosum, those with actively growing multifocal disease by MRI, and/or CSF dissemination/ leptomeningeal disease, are ineligible, .

3.2.6 Prior history of encephalitis, multiple sclerosis, or other CNS infection.

3.2.7 Required steroid increase within 2 weeks of scheduled M032 administration. When possible, the patient should be on a dexamethasone equivalent dose of ≤ 2mg daily at the time of treatment.

3.2.8 Active herpes lesion.

3.2.9 Concurrent therapy with any drug active against HSV (acyclovir, valacyclovir, penciclovir, famcyclovir, gancyclovir, foscarnet, cidofovir).

3.2.10 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or any other medical condition that precludes surgery. Also, psychiatric illness/social situations that would limit compliance with study requirements.

3.2.11 Excluded patient groups:

Pregnant women are excluded from this study because M032 is a viral oncolytic therapy with unknown potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with M032, breastfeeding women will not be included in the study.

Because patients with immune deficiency will be unable to mount the anticipated immune response underlying this therapeutic rationale, HIV-seropositive patients are excluded from this study. Other treatment studies for this disease that are less dependent on the patients' immune response are more appropriate for HIV-seropositive patients.

3.2.12 Patients with known history of allergic reaction to IV contrast material that is not amenable to pre-treatment by UAB protocol.

3.2.13 Patients with pacemakers, ferro-magnetic aneurysm clips, metal infusion pumps, metal or shrapnel fragments or certain types of stents.

3.2.14 Receipt of Gliadel Therapy.

3.2.15 Receipt of Bevacizumab (Avastin) therapy within 4 weeks of scheduled M032 administration. (Receipt of Bevacizumab (Avastin) greater than 4 weeks of scheduled M032 administration does not exclude patient.

3.2.16 Any other reason the investigator deems subject is unfit for participation in the study

3.3 Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. The proposed study population, assuming the maximum number of patient enrollments occurs, is illustrated in the table below.

Gender	Asian or Pacific Islander American Indian or Alaskan Native	*Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown (Non-white)	*Total
Female	--	1	1	8	--	10
Male	--	1	0	9	--	10
TOTAL	--	2	1	17	--	20

*Based on percentages of patients with recurrent malignant gliomas treated at UAB 2000-2015

4. TREATMENT PLAN

4.1 Study Summary

This will be an open-label, CRM-directed, dose-escalating Phase I safety trial conducted at a single site (UAB) in patients with a confirmed diagnosis of progressing MG after TMZ chemoradiation therapy. Treatment will be administered on an inpatient basis. Expected adverse events caused by M032 are described in **Section 5**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

These subjects will include eligible patients for whom surgical resection of their recurrent glioma is considered to be infeasible or would not likely afford greater benefit than the potential risks associated with surgical resection. All patients will be required to be placed on an anticonvulsant at the time of screening; those patients already on an anti-convulsant (anti-epileptic drug/AED) will have a second anti-convulsant added per the discretion of the investigator. This anti-convulsant will be continued for a minimum of 28 days unless the patient develops a reaction to the medication. If this develops, all attempts will be made to implement an alternative agent. On Day 0, these subjects will be treated under monitored local anesthesia, or at the surgeon's discretion, under general anesthesia. The patients will undergo a contrasted MRI scan to determine the site(s) for stereotactic biopsy and inoculation of the test agent. Patients will then undergo stereotactic biopsy of their tumor. While evidence of radiation damage or necrosis may be present on the frozen section, inoculation with M032 will only proceed if viable, recurrent glioma is also present on the frozen section.

These patients will undergo placement of one to four stereotactically placed catheters, which will be primed in the OR with 0.4 ml preservative-free normal sterile saline solution per catheter. The wound will be closed, the catheters aseptically exteriorized and the subject allowed to recover overnight from the procedure in the Neurosurgery ICU. If the patient's condition is stable they may be transferred the next morning, Day 1, to a Clinical Research Unit bed for M032 administration and monitoring until discharge. After pathology results confirm recurrent tumor, and a CT confirms appropriateness of catheter placement, infusion of M032 may begin. If a catheter is determined, by the investigator, to be out-of-position and cannot be properly positioned by simple partial withdrawal at the bedside (as determined by the surgeon investigator), this/these catheter(s) will not be used for infusion. The catheters will be removed 6-18 hours after the completion of the infusion.

Patients will then receive the entire M032 dose by intratumoral infusion over a 6 hour period (infusion may extend up to 12 hours, if needed due to catheter malfunction). The total amount of M032, 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 an approved syringe 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 M032 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.

Each catheter will be placed in a different enhancing area of the tumor and labelled. If, in the surgeon's judgment, the tumor location or other properties are not suitable for all four catheters to be placed, fewer catheters will be placed, ranging from number one to four.

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 M032 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 will then be primed with 4.6 ml of the diluted M032 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.

After the initiation of the infusion, the subjects' vital signs (temperature, blood pressure, pulse, respiratory rate) and neurologic function and limited neurological exam will be monitored every hour for the first twelve hours, then every two hours for the next 12 hours, then every 4 hours for the next 24 hours. Thereafter, the frequency of monitoring will be determined by the attending physician(s) based on the medical condition of the patient.

After discharge the subject will continue to be closely followed for evidence of adverse events, with outpatient follow-up evaluations scheduled at Day 10 and months 1, 2, 3, 4, 5, 6, 9 and 12, or more often if medically indicated. HSV detection will be performed at additional time points. The visits at months 2, 4 and 5 will consist of sample collections and a study coordinator visit only. Due to the complicated procedure for obtaining samples remotely at visits 2, 4 and 5, if a patient is unable to travel then, and if determined feasible by the PI, an attempt can be made to have the specimens collected by the patients' referring physician and sent to UAB for analysis.

4.1.1 Continual Reassessment Method

Dr. Gary R. Cutter and Dr. Inmaculada B. Aban, Professors in the Department of Biostatistics, UAB School of Public Health, with expertise in CRM calculations will apply the software developed by Dr. Steven Piantadosi, (Cedars-Sinai) who will serve as a clinical trial statistical consultant to advise and assist in the implementation of the Continual Reassessment Method in determining dose alterations. In addition, Drs. Aban and Cutter, will provide support in clinical trial and data management, and data analysis.

Rationale for the Use of a Modified Continual Reassessment Method: The description of the determination of MTD from the CRM that we are employing is adapted from that used previously in a Phase I/II study of the poly (ADP-ribose) polymerase-1 (PARP-1) inhibitor BSI-201 in patients with newly diagnosed malignant glioma conducted under the New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium. To estimate the MTDs in terms of clinical toxicities, a modified continual reassessment method (CRM), based on that described by Piantadosi et al [45], will be employed. The CRM has been shown to be less biased and more efficient for estimating the MTD than traditional dose-finding models. The efficiency of the CRM stems from its explicit use of biological knowledge in the form of a parametric dose toxicity model. In the CRM, only a starting dose is specified and the dose is escalated or deescalated based on the toxicities of the previous cohort.

This is advantageous because the investigators can choose the next dose level based on all available clinical and statistical information rather than on relatively arbitrary predetermined doses. One disadvantage of the CRM is uncertainty of the dose levels that will be used in the trials, because the specific dose levels are not defined at the start of the trial.

Details of CRM Design: The primary statistical outcome for this study is the occurrence of serious clinical toxicity. Investigators would like to employ a dose of drug that yields approximately a 1/3 chance of serious toxicity. In our opinion, this represents the best chance at a beneficial therapeutic ratio for this unusually difficult disease to treat. This target probability of toxicity is chosen based on knowledge of previous trials of oHSV in

glioma, but is fundamentally subjective. The relationship between dose of drug and probability of toxicity is assumed to follow a two-parameter logistic model, both before and during the dose finding given by:

$$P(toxicity|dose) = \frac{1}{1 + e^{-\beta(dose - d_{50})}}$$

where β is the "slope" of the curve and d_{50} the midpoint (or dose that yields a 50% response). The parameters β and *d50* govern the dose finding process, and are estimated, denoted by $\hat{\beta}$ and $\hat{d}_{50}$, initially by clinical judgment and afterwards by the observed data. This represents a second subjective component of dose finding designs.

Classical dose-ranging designs incorporate this component of subjectivity by setting out, in advance of the experiment, a set of doses to employ. A strictly Bayesian approach to the CRM requires a joint prior probability distribution for the parameters β and d_{50}. However, we have found it easier for clinicians to render their clinical judgment about β and d_{50} in the form of pseudo-data. Specifically, we ask for two points on the dose toxicity curve: the dose thought to yield a 10% probability of toxicity (d_{10}) and the dose thought to yield a 90% probability of toxicity (d_{90}). Two points, d_{10}, d_{90}, are required to initiate the fit for a two-parameter model. The exact points chosen are arbitrary, but we have found these generally work well or are easily adapted. The d_{10} point can often be discarded after some data have been obtained. The d_{90} point is necessary to obtain a fit of the model, but is usually relatively unimportant because it characterizes a region of the model that does not heavily influence prediction of the next dose. In this sense, d_{90} is a nuisance parameter – necessary for model fitting, but relatively uninfluential on the dose escalation.

Table 1: Initializing Data used to start the CRM

Log Dose	N	r	Weight
4	1	0.05	0.5
5	1	0.10	0.5
6	1	0.2	0.5
7.5	1	0.5	0.5
9	1	0.8	0.5
10	1	0.9	0.5
12	1	1	0.5
15	1	1	0.5

(Initial recommended dose based on these data=6.7842)

At both d_{10} and d_{90}, the investigator specifies a numerator (number of responses) and denominator (number of subjects treated), such that numerator/denominator = 0.1 or 0.9 as the case may be. The denominators are always taken to be small numbers, e.g. 1, to represent weak evidence and reduce the influence of these points compared to real subject data. Three values for d_{90} will be chosen in this protocol with weights of one tenth that of real subject data. This allows flexibility in the upper end of the dose toxicity curve. This adaptation to the CRM came about after subject data demonstrated that d_{90} was incorrect in previous studies, because doses approaching d_{90} were tried with no toxicities observed. This situation required that d_{90} be moved to a higher dose or the fitted dose toxicity curve became too steep and no dose escalations resulted.

We assume the dose for d_{10} = 1 x 10^4 pfu for M032 (**Table 1**). A set of three values will be chosen for d_{90} =1 x 10^{15} pfu. These values for d_{90} spread probability mass over a wider range and may eliminate the need to increase d_{90} if the data are inconsistent with a single value. Based on these starting points, our desire is to employ a dose of virus to yield no more than 33% chance of clinical toxicity. The initial starting dose will be assumed to be 1 x 10^5 PFU and the maximum dose to be tested would be 1 x 10^9 PFU. Further assumptions would be that the maximum number of subjects would be 204 and that the dose cohort size would be 1. Further, the next CRM recommended log dose would be rounded to the nearest 0.5 or full dose. Moreover, the number of consecutive subjects at the maximum tolerated dose would be 10 or a number until 20 subjects are treated, whichever comes first. This will allow us to collect more information of the safety of the MTD. If a second toxicity is observed for patients on the highest dose, then the next lower dose is used and declared MTD if 10 consecutive subjects on the same dose are toxicity free or if n=20 is reached. If more toxicities are found, CRM will be used in the de-escalation of the dose.

The outcome space for the first CRM iterations would look like the values shown as six sample scenarios in **Table 2** using a starting dose **1 x 10^5 pfu**. We will not allow dose escalations to exceed 10 times the maximum dose already administered. In this way, we prevent a too rapid dose escalation or testing high doses without a reasonable degree of certainty concerning their safety. Although the values in **Table 2** appear reasonable to us currently, we emphasize that they should not be taken literally. Indeed, if we receive approvals (DSMB, IRB, FDA), we will update the CRM model using the four patients previously enrolled and treated. Some of the data

$$L(\beta, d_{50}) = \sum_{i=1}^{k} \left[\log \binom{n_i}{r_i} - n_i \log\left(1 + e^{-\beta(d_i - d_{50})}\right) - (n_i - r_i) \log\left(1 + e^{-\beta(d_i - d_{50})}\right) \right]$$

could suggest that the current value for d_{10} and/or d_{90} is inappropriate. We would not proceed blindly with a CRM (or any other) dose escalation in such a circumstance. All testing doses will be the estimated dose round-down to the nearest 10th.

For each subsequent level, the investigators will evaluate the recommended dose-level. It is possible that the model may recommend large interval increases in dose level with which the investigators do not feel comfortable. In these cases, the investigators will use this information and their best clinical judgment to assign a next dose level. We emphasize that the dose levels recommended by the CRM should not be taken literally.

Model based dose escalation methods, especially the CRM, are able to account for ordinal or quantitative toxicity assessments, and use this information to guide subsequent dose changes. In particular, the binomial likelihood is

where *i* indexes the dose level, *p* is the probability of toxicity (equation 1 above), *d* is dose, *n* the number of subjects treated at each dose, and *r* the number of toxicities. While *n* is constrained to be an integer, *r* does not have to be integral. In particular, *r* can be taken to be the sum of ordinal or quantitative toxicity measures provided that $r < n$. The maximization of equation 2 with respect to the parameters (model fitting) can then proceed in the usual fashion. Furthermore, the ordinal scores need not be the same for all types of toxicity. For example, we might not want alopecia to have the same effect on dose reduction as neurologic toxicities.

Table 2: Six Different CRM-defined Dose Escalation Scenarios

Subject #	S1	S2	S3	S4	S5	S6
1	5	5	5	5	5	5
2	6	6	6	6	6	6
3	7	7	7	7	7	7
4	7.5	7.5	7.5	7.5	7.5	7.5
5	8	8	8	8	8	8
6	8	8	8	8	8	**8**
7	8.5	8.5	8.5	8.5	**8.5**	7.5
8	9	**9**	9	9	8	8
9	9	8.5	**9**	**9**	8.5	8
10	9	8.5	9	**9**	8.5	8
11	9	8.5	9	8	8.5	8.5
12	9	8.5	9	8.5	9	8.5
14	9	8.5	9	8.5	9	9
15	9	8.5	9	8.5	9	9
16	9	8.5	**9**	8.5	9	9
17	9	8.5	8.5	8.5	9	9
18	9	8.5	8.5	8.5	9	9
19		8.5	8.5	8.5	9	9
20			8.5	8.5	9	9
MTD	**9**	**8.5**	**8.5**	**8.5**	**9**	**9**

(yellow cells= maximum tolerated dose (MTD); **magenta cells =toxicity**; white cells=no toxicity)

As subjects are treated at doses estimating the true MTD, the recommended subsequent dose levels will begin to converge. The criteria to declare the MTD will be when 2 recommended doses are within 10% of one another. It is possible that the dose escalation could continue indefinitely and that the MTD is not reached. If 10 subjects are treated in the full dose range in this dose-finding study and the MTD has not been reached, we will take pause, evaluate the data in consultation with the DSMB, and determine whether to continue the dose-escalation or terminate the Phase I portion of the study. If a MTD is not being reached based on clinical toxicities (too many or too few), the biological indicator (increased PFS or OS) may factor in the choice of the M032 dose for the ultimate planned phase II trial.

A maximum of 10 subjects will be treated at the putative MTD to have better estimation of ≤ 33% DLT rate.

No enrollment of special or vulnerable populations is anticipated.

4.1.2 Estimated Number of Patients

Moving forward, we anticipate as few as four and as many as 20 additional patients could be enrolled and treated in this trial. We expect approval of the use of the four already enrolled and treated patients to seed the model, which would bring the overall total range from 8- 24 patients. If the highest planned dose is successfully administered to subjects and assuming one subject per CRM-calculated dose level (10), and an additional 10 subjects will be entered at the CRM-estimation of the safe target dose (10+10 = 20). If all subjects develop DLTs, the CRM model would de-escalate both through the initial and the two lower dose levels involving no more than 4 subjects and the trial would be halted (Section **4.5**) with a minimal accrual of 4 subjects. Assuming that no DLTs will be encountered, we expect that under optimal conditions only 20 subjects (10+10) will be enrolled and treated in this study. These numbers will be modified if the new patient data for seeding the model is approved and could even decrease.

Thus, the maximum number of subjects that we expect to enroll and treat in this trial would be 20, assuming that the CRM will adjust each dose after the first 2 or 3 subjects and as more subjects are enrolled to encompass the initial test range. On the other hand, the minimum number of subjects who could be enrolled in this trial would be 4 assuming 2 DLTs at Dose Level 1, and the CRM recommending one or two lower doses.

4.2 Definition of Dose-Limiting Toxicity

The Cancer Therapy Evaluation Program (CTEP) has published Common Terminology Criteria for Adverse Events, version 4.0 for the grading of adverse events experienced in clinical trials for anti-neoplastic agents. Any grade 3 or greater non hematologic toxicity as defined by NCI CTCAE v 4, and considered as being possibly, probably or likely related to M032 will be considered a Dose Limiting Toxicity (DLT). However, specific symptoms including seizures, neurologic deficits such as weakness, dysphasia or sensory loss are prominent in this patient population due to the fact that these tumors frequently disrupt the function of normal areas of eloquent brain. Neurologic symptoms from involvement of eloquent brain are commonplace; corticosteroid, radiation, and chemotherapies also produce pre-existing symptoms in almost all cases. The pre-existing conditions listed in the **Table** on the following page are acceptable for inclusion in the clinical trial, provided the subjects meet the other inclusion and exclusion criteria.

For all of these pre-existing conditions, DLTs will be defined as progressive involvement of neurological dysfunction not attributable to tumor progression and/or the development of life threatening symptomatology associated with the original baseline status.

Neurologic deterioration will not be considered dose-limiting if it resolves back to the patient's baseline within two weeks 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 fourteen days of completing treatment. Because of the site of M032 inoculation, important additional events that will be considered dose-limiting toxicities if they are likely related to M032 include death, stroke, hematoma requiring surgery, untreatable neurologic deterioration, unresponsive systemic infection, and disseminated HSV infection.

Management of the above adverse events is outlined in **Section 5**.

The maximally tolerated dose (MTD) is the highest dose level below the maximally administered dose when dose escalation decisions are made according to the guidelines above. Enrollment will be stopped when the MTD is determined or the maximally planned dose is acceptably tolerated by six patients.

There will be a minimum twenty-four (24) day observation period between the first patient at each dose level and subsequent patients enrolled to allow for evaluation of potential toxicity. If no dose-limiting toxicity is seen with the first patient, the twenty-four day waiting period will be waived for the second and third patients at that dose level. If the incidence of DLT at any given dose level meets the criteria for dose escalation, there will be a waiting period of a minimum of 24 days before dose escalation occurs. The Medical Monitor, in conjunction with a duly chartered external Data and Safety Monitoring Board comprised of an Infectious Disease expert, a neurosurgeon familiar with oncolytic virus therapy, a neuro-oncologist familiar with oncolytic virus therapy and an expert in the use of molecular biology methods to detect and quantify Herpes Simplex Viruses in clinical

specimens, will evaluate the safety of each dose tested to determine whether the protocol may proceed to the next dose level.

CTCAE#	Body System	Event name	Definition of Grade 3 Toxicity	Rationale for inclusion
222	General disorders and administration site conditions	Gait disturbance	Disabling; limiting self care ADL	Many subjects with malignant glioma have gait disturbance as a result of chronic corticosteroid administration
494	Musculoskeletal and connective tissue disorders	Muscle weakness left-sided	Limiting self care ADL; disabling	Malignant glioma can produce muscle weakness due to interruption of motor function
495	Musculoskeletal and connective tissue disorders	Muscle weakness lower limb	Limiting self care ADL; disabling	Malignant glioma can produce muscle weakness due to interruption of motor function
496	Musculoskeletal and connective tissue disorders	Muscle weakness right-sided	Limiting self care ADL; disabling	Malignant glioma can produce muscle weakness due to interruption of motor function
498	Musculoskeletal and connective tissue disorders	Muscle weakness upper limb	Limiting self care ADL; disabling	Malignant glioma can produce muscle weakness due to interruption of motor function
527	Nervous system disorders	Ataxia	Severe symptoms; limiting self care ADL; mechanical assistance indicated	Malignant glioma itself and many of the anti-epileptics drugs used to treat seizures associate with this disease can produce ataxia that requires mechanical assistance
529	Nervous system disorders	Central nervous system necrosis	Severe symptoms; medical intervention indicated	Accepted treatments for malignant glioma (for example radiation therapy) can produce CNS necrosis that requires medical intervention such as corticosteroid administration
531	Nervous system disorders	Cognitive disturbance	Severe cognitive disability; significant impairment of work/school/life performance	Both malignant glioma and its accepted therapies (for example radiation therapy) can produce impairment of work/school/life performance
532	Nervous system disorders	Concentration impairment	Severe impairment in attention or decreased level of concentration; limiting self care ADL	Both malignant glioma and its accepted therapies (for example radiation therapy) can produce impairment in attention or decreased level of concentration that affects self care ADL
536	Nervous system disorders	Dysesthesia	Severe sensory alteration; limiting self care ADL	Malignant glioma can produce alterations in sensory function limiting self care ADL
545	Nervous system disorders	Headache	Severe pain; limiting self care ADL	Malignant glioma can result in intracranial pressure resulting in severe headaches
567	Nervous system disorders	Pyramidal tract syndrome	Severe symptoms; limiting self care ADL	Malignant glioma can produce dysfunction of the pyramidal tract
571	Nervous system disorders	Seizure	Multiple seizures despite medical intervention	Malignant glioma can produce multiple seizures despite medical intervention
575	Nervous system disorders	Stroke	Severe neurologic deficit	Malignant glioma can produce stroke through its associated hypercoagulative state. Also, treatment for malignant glioma (for example bevacizumab, radiation) can result in stroke

4.3 Supportive Care Guidelines

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

- Bevacizumab or steroid administration for neurologic symptoms arising from increased edema or intracranial pressure, which are presumed mechanisms of action for M032, will be allowed, if deemed appropriate and safe in the determination of the investigator. The increase in steroid dose will be kept to a minimum as patient neurologic condition permits. In general, UAB Neuro-oncology protocol is that Avastin is given every two weeks at a dose of 10/kg mg IV. Dosage adjustments or cessation of drug

may be made should adverse events (Uncontrolled hypertension, proteinuria, bleeding, thrombosis, etc.) occur and are made on a case-by-case basis by the prescribing neuro-oncologist.

- Proton pump inhibitors or H2 antagonists for control of steroid-induced gastric irritation
- Anti-epileptic medicines for control of partial or generalized seizures
- Post-operative neurological intensive care that is routine for the neurosurgical interventions involved in the administration of M032
- 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

Management of adverse events is discussed in **Section 5**.

4.4 Duration of Therapy/Study

The therapeutic intervention in this trial involves the administration of a single infusion of M032. For this reason it is more appropriate to define the duration of the study for each patient rather than the duration of therapy. All subjects participating in the study will be considered intent to treat and will be followed through all scheduled visits without concern for the total amount of study treatment received.

In regard to the evaluations of treatment efficacy or the acceptability of other treatments for the malignant glioma, the study will continue as scheduled until one of the following criteria applies:

- Disease progression (as defined in **Section 9**)
- Patient withdrawal from the study

Scheduled post-therapy safety evaluations as indicated in the study schedule will continue for every patient regardless of the response to M032. These evaluations will stop only if one of the following criteria applies:

- Disease progression (as defined in **Section 9**) has rendered the patient not evaluable
- Patient withdrawal from the study

4.5 Halting Rules for Study

Should 2 or more Grade 3 or Grade 4 toxicities not thought to be related to tumor, prior therapy or expected to be related to HSV occur in the same dose level, enrollment will be suspended until an evaluation of these toxicities has been conducted by the trials Data Safety and Monitoring Board (DSMB). The DSMB will be the arbiter as to whether these toxicities can be attributed to M032 and if so, invoke a stopping rule, halting enrollment. If the DSMB should feel that these toxicities are not related to M032 administration, enrollment will be reinstituted as per its instructions. Should a particular DLT be evaluated by the DSMB and felt to be 1) consistent with M032 activity and 2) of acceptable duration and severity, the DSMB may decide to waive the DLT and/or enroll additional patients at this dose level due to the life-threatening nature of the underlying disease under study.

- The DSMB may interrupt study enrollment and entry at any time if medically indicated.
- If one (1) subject dies due to an event related to (caused by) the study intervention, study enrollment will be halted and enrollment may only begin after the DSMB reviews the data and the DSMB determines it is safe to continue.
- If two (2) subjects experience a Grade 4 toxicity using the NCTAE Toxicity Table, study enrollment will be halted and enrollment may only begin after the DSMB determines it is safe to continue.
- If two (2) instances of cytokine storms result in two instances of Grade 4 toxicity and/or uncontrolled corticoid steroids and acyclovir therapy are required, within the same cohort, study enrollment will be halted and enrollment may only begin after the DSMB reviews the data and the DSMB determines it is safe to continue.

- If two (2) or more grade 3 toxicities not thought to be related to tumor, prior therapy or expected to be related to HSV occurring in the same dose cohort, enrollment will be suspended until an evaluation of these toxicities has been conducted by the trial's DSMB. Enrollment may only begin after the DSMB reviews the data and the DSMB determines it is safe to continue.
- If two (2) or more Grade 4 neurologic toxicities not thought to be related to tumor, prior therapy or other treatment associated modalities occur in the same dose cohort, enrollment will be suspended until an evaluation of these toxicities has been conducted by the trial's DSMB. Enrollment may only begin after the DSMB reviews the data and the DSMB determines it is safe to continue.

5. EXPECTED ADVERSE EVENTS/DOSE MODIFICATIONS

5.1 Expected Adverse Events Associated with M032

Although M032 has not yet been used in humans, the adverse events it might produce are expected to be similar to the adverse events observed in the Phase I trials of a similar genetically-engineered herpes simplex virus, G207. G207 caused no DLT and the adverse events that were 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 following M032:

- asthenia
- amnesia
- nausea
- somnolence
- leukopenia
- peripheral edema
- fever
- pneumonia
- death
- hemiplegia
- confusion
- headache
- decreased consciousness
- anemia
- cachexia
- varicella zoster infection
- abnormal mentation
- stroke
- dysphasia
- depression
- deep vein thrombosis
- seizure
- abnormal erythrocytes
- urinary tract infection
- tumor progression
- increased liver transaminases
- pseudoprogression

Other adverse events that might be expected based on the origin of M032 and on the patients' disease include the following:

- hematoma
- encephalitis
- hepatitis
- disseminated HSV infection
- allergic response to M032
- nuchal rigidity
- photophobia
- autoimmune response to CNS tissues

Any of the above adverse events will be considered a DLT, if possibly, probably or definitely attributable to M032 and if the severity of the adverse event is Grade 3 (in some situations) or Grade 4, as defined in **Section 4.2**.

Subjects will be observed closely for evidence of any adverse events. After the delivery of M032, subjects will be observed in a Clinical Research Unit bed, a step down unit or the Neurosurgical ICU per the discretion of the surgeon. The subjects' vital signs (temperature, blood pressure, pulse, respiratory rate) and neurologic function and limited neurological exam will be monitored every hour for the first twelve hours, then every two hours for the next 12 hours, then every 4 hours for the next 24 hours. Thereafter, the frequency of monitoring will be determined by the attending physician(s) based on the medical condition of the patient.

After discharge the subject will continue to be closely followed for evidence of adverse events, with outpatient follow-up evaluations scheduled at Day 10 and Months 1, 2, 3, 4, 5, 6, 9 and 12, or more often if

medically indicated. HSV detection will be performed at additional time points. The visits at months 2, 4 and 5 will consist of sample collections and a study coordinator visit only. Due to the complicated procedure for obtaining samples remotely at visits 2, 4 and 5, if a patient is unable to travel then, and if determined feasible by the PI, an attempt can be made to have the specimens collected by the patients' referring physician and sent to UAB for analysis.

The safety evaluation schedule is outlined in detail below in Section 8. Per FDA guidelines, subjects will be followed for survival. This will be achieved with monthly phone calls to subject. Additionally, when possible, subjects will be followed for emergence of any chronic or acute conditions that might be attributed to the study agent for up to 15 years during clinical visits with their standard of care practitioner.

Most adverse events will be managed according to standard conventions. General or specific neurologic worsening observed in the first several days after M032 administration could be due to edema, hydrocephalus, hematoma, or encephalitis. Such problems occurring later may also be attributable to tumor progression. When appropriate, an MRI will be done to help determine the cause of the neurologic changes.

- Cerebral edema/hydrocephalus: This commonly occurs in tumor patients post-operatively, and usually responds to standard measures for the treatment of increased intracranial pressure.
- Hematoma: PT, PTT, and platelet count will be obtained. A small hematoma 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.
- Encephalitis: Post-operative fever is not uncommon. However, fever >102°F (with or without seizures) extending in duration >48 hours, in the presence of a waning Glascow Coma Scale and an increase in the area of hemorrhagic necrosis extending beyond the borders of the tumor on MRI are suggestive of viral encephalitis. If, in the opinion of the PI, it is safe and indicated, a cerebrospinal fluid sample will be obtained and analyzed by polymerase chain reaction (PCR) for evidence of HSV-1. Otherwise, or if CSF results are not diagnostic, a stereotactic biopsy will be considered. This biopsy will be taken to assess for presence of M032 or wild-type HSV-1, as well as for histopathologic evidence of encephalitis. This will consist of a minimum of 2-3 needle core biopsies that will undergo standard hematoxylin and eosin (H&E) staining as well as immunostaining for HSV-1, leukocyte common antigen, glial fibrillary acidic protein (GFAP), and IL-12 immunostaining if possible. High-dose antiviral therapy with intravenous acyclovir may be implemented in consultation with the Medical Monitor, Dr. John Gnann, and will be administered according to established method [37].
- Disseminated HSV Infection: Disseminated multi-organ disease attributed to HSV is exceedingly uncommon, even in the immunocompromised host. Such disease has been encountered in the newborn with disease identified as 'disseminated multiorgan involvement' and occurs in about 25% of newborns with this disease (incidence 1:10,000 deliveries).[38] In adults, disseminated disease has been encountered in stem cell transplant recipients and, very rarely, pregnant women.[39] 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 at Days 2, 10, 28 and monthly thereafter and cultured for infectious HSV. Any positive cultures will be analyzed by PCR to discriminate between wild type HSV-1 and mutant M032 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 for presumed disseminated HSV infection. 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[40]; thus, increasing levels as well as fever will both be required to therapeutically intervene. Should 2 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.

- Cytokine storm: although unlikely, this would not be entirely unexpected. Management of cytokine storm will focus on the pathology and symptoms. Anti-inflammatory agents (for example corticosteroids) can minimize the cytokine response decreasing morbidity and mortality. Should a cytokine storm or related toxicity occur, we will utilize antiviral therapy active against HSV (eg acyclovir) in combination with immunomodulatory agents to interrupt HSV replication and IL-12 production and effects, thereby reducing and/or eliminating morbidity and mortality. Close observation of the subject for 24 hours after the crisis will be implemented (vital signs q hour, respiratory and cardiovascular support as needed, fluid maintenance as needed).

5.2 Dosing Delays/Dose Modifications

Because M032 is delivered as a single dose, there will not be any intra-patient dosing delays or dose modifications.

5.3 Adverse Assessment and Management Assessment by PI/Clinician

Neurologic complications are common in patients with malignant glioma and may be a result of tumor infiltration, mass effect, or other tumor associated complications; radiation administration and its associated direct and indirect toxicities; chemotherapy administration; and other medication and condition-related toxicities. Should 2 or more neurologic toxicities that escalate to the level of a DLT and are not thought to be related to tumor, prior therapy or other treatment associated modalities occur in the same dose level, enrollment will be suspended until an evaluation of these toxicities has been conducted by the trials Data Safety and Monitoring Board (DSMB). The DSMB will be the arbiter as to whether these toxicities can be attributed to M032 and if so, invoke a stopping rule, halting enrollment. If the DSMB should feel that these toxicities are not related to M032 administration, enrollment will be reinstituted as per its instructions.

Subjects will be monitored both at UAB and by their referring physician for any treatment-related toxicities until these have resolved back to their pretreatment baseline or < Grade 2.

All unresolved toxicities will be followed regularly by the treating physician and/or research coordinator; the nurse coordinator will conduct follow-up weekly with the subject, family and/or referring physician (by phone if travel is too burdensome for the subject) until resolution of treatment-related toxicity to less than Grade 2 (or Grade 3 if that was the subject's baseline). Additionally, subjects will be monitored monthly for the first 3 months following study drug administration, and will have the nurse-coordinator contact the subject, family and/or referring physician monthly for months 4-6.

6. AGENT FORMULATION AND PROCUREMENT

All agent materials and excipient solutions will be shipped to:

Rachel Burell, Pharm.D.
Research Pharmacist
IDS Pharmacy
North Pavilion, Room 3470
1802 6th Avenue South
Birmingham, AL 35249
Phone: 205-934-7191
Fax: 205-975-6647

6.1 M032 Formulation and Storage

HSV-M032 (NSC 733972), lot # L0909005, is supplied in sterile, labeled 1.0 mL single use glass vials containing 0.5 mL of HSV-M032 suspended in the storage buffer, Dulbecco's Phosphate-buffered Saline (D-PBS), 0.4M NaCl and 10% (w/v) glycerol, pH 7.4. The NExT Program recently reported the 48-month stability assay in which several reserved vials were retitered, resulting in a final calculated titer of 3.32×10^9 plaque-forming units PFU/ml or 1.66×10^9 PFU per vial. The vials should remain frozen at -70°C or below until use.

6.2 Availability

M032 will be provided for clinical study under an Agreement between the NIH RAID program and the University of Alabama, Birmingham. For specific shipment information/handling, refer to **Appendix E**.

6.3 Excipient for Dilution of M032

For dilution of M032, a specially formulated excipient solution will be used. This excipient solution (Product Code 103382-50; Lot # 800612) has been purchased from Alanza Inc. (81 Glencameron Road, Thornhill, Ontario L3T 1N8, Canada). The formulation is Phosphate Buffered Saline and 10% (w/v) of glycerol, pH 7.0-7.4 and is supplied sterile in 50 ml aliquots in amber serum bottles with snap-cap butyl rubber stoppers.

6.4 M032 Dose Preparation

All matters related to dose preparation and syringe set up for dose administration are performed in a sterile fashion. Prior to beginning infusion, the tubing that is to connect the syringes to the intracranial catheters is primed with sterile PBS + 10% glycerol (Alanza). All of the syringes containing the proper volume of M032 will be prepared at the same time for each three (3) hour period of delivery. Syringes designated to be used beginning at three (3) hours should be kept at 4°C until dispensed to the patient.

To prepare each dose, the vial(s) containing M032 should be thawed by removing it from the controlled access -60°C freezer and rubbing the vial gently between gloved hands until the last ice crystals have melted. The vial should then be placed on ice. Care should be taken to ensure that all the liquid is at the bottom of the vial before removing the cap. 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 cap must be removed carefully to avoid spilling or contamination. The appropriate dose level of M032 will be removed from the vial and diluted in sterile diluent to the total volume. The final diluted M032 should be gently withdrawn into the syringe(s) for infusion.

Once thawed, diluted and maintained at 4°C, the dose must be completely administered to the patient within sixteen hours of preparation. The diluted virus has been shown to be stable at 4°C with no loss of activity for 9 hours.

Complete instructions for dose preparation are described in **Appendices C** and **D**.

6.5 Precautions in Handling M032

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.

6.6 Precautions in Disposal of M032

All materials that have been in contact with M032 are considered 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. Instruments should be placed in disinfectant or swabbed with disinfectant soaked gauze sponges that are then discarded appropriately. Instruments should be well cleaned and autoclaved. All disposable materials that have been in contact with the vector must be incinerated or microwaved in an institutionally approved biohazard incinerator/microwave device before disposal.

7. CORRELATIVE/SPECIAL STUDIES

To address secondary objectives of the protocol, additional correlative studies will be performed on blood/serum, conjunctival secretions, saliva samples and tumor tissue samples.

Blood Samples: Blood samples (heparinized) will be taken at Baseline, on Days 0, 2, 3, 10, 28 and at Months 3, 6, 9 and 12. One ml of each sample will be submitted to the Clinical Virology Diagnostic Laboratory for HSV culture to determine the amount of virus shedding. The balance of the blood sample will be separated by density gradient centrifugation to provide plasma (serum) and peripheral blood mononuclear cells (PBML). Plasma will be tested to permit detection and quantification of HSV antibody titer pre-and post-inoculation via ELISA. Peripheral blood mononuclear leukocytes will be quantified for subsets and other leukocyte markers by FACS analysis, expression of activation signals (intracellular lymphocyte interferon-gamma levels) and lymphocyte transformation assays will also be performed to assess aspects of the T_H1 response to treatment with M032. Plasma cytokine assays will be performed to quantify changes over time from baseline. Conjunctival, blood and saliva will be assessed for HSV shedding by standard culture methods. Any positive cultures will be further

characterized for wild-type or M032 HSV by PCR. Any remnant blood will be stored for future brain tumor research.

Tissue Samples: If available, remnant brain tissue from the biopsy will be snap-frozen in liquid nitrogen and stored for future research. Should any patient with recurrent tumor after HSV treatment be determined to benefit from additional tumor resection for diagnosis or to ameliorate intractable symptoms caused by increased mass effect and intracranial edema, the tumor will be removed *en bloc* as much as possible and sectioned in 2-4mm thick slices perpendicular to the placement of the catheters. Each section will be cut into quarters and each quarter will be labeled 1, 2, 3 or 4, processed and stored for future research as follows:

Quarter 1: Snap freeze in liquid nitrogen for immediate assessment of viral replication *in vitro.*

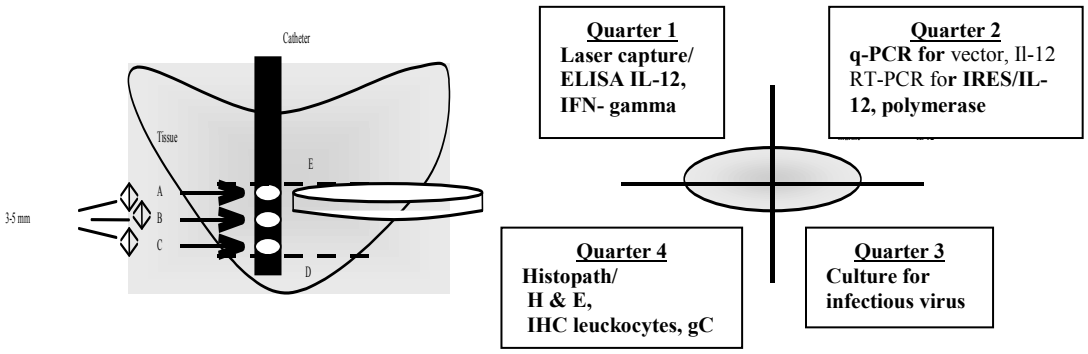
Quarter 2: Snap freeze in liquid nitrogen for RNA and DNA extraction and the following evaluations:
- Quantitative PCR (vector replication *in-vivo*; compare number of copies to vector input)
- RT-PCR to detect HSV polymerase mRNA (vector replication *in-vivo*)
- Confirmation and quantification of M032 presence by quantitative PCR of IL-12 recombinant DNA
- Confirmation and quantification of human IL-12 production by M032 by use of RT-PCR to the internal ribosomal entry site (IRES) sequence present between the p35 and p40 subunits of IL-12 in M032

Quarter 3: Snap freeze in liquid nitrogen for subsequent tissue homogenization and the following evaluations:
- Confirmation and quantification of IL-12 production by use of a p70 ELISA kit after laser capture microdissection
- Quantification of *in situ* interferon-γ production by use of Multiplex Bead and ELISA kits

Quarter 4: Processed as routine histopathology in formalin for:
- Routine histopathology (H & E, GFAP, MIB, etc.)
- Immunohistochemistry for HSV-1, glycoprotein C (gC) when possible
- Characterization of the nature of the inflammatory infiltrate by reaction with monoclonal antibodies for CD4, CD8 and macrophage markers

Subsequent 2-4mm thick Slices if available):
- Process 2 quadrants of each slice in formalin as routine histopathology; may be used for further characterization of the inflammatory infiltrate as needed
- Snap freeze 2 quadrants of each slice in liquid nitrogen; may be used for further studies, such as laser capture microdissection, RNA and DNA extraction as needed, etc.

Schematic for Processing of Tumor Tissue



8. STUDY CALENDAR

	Pre-Study (within 2 weeks prior to the administration of M032)	Day 0	Day1	Day 2	Day 3	Day 10 (± 3 days)	Day 28 (± 4 days)	Month 2 (± 12 days)	Month 3 (± 12 days)	Months 4, 5 (± 12 days)	Months 6, 9, 12 (± 12 days)	Unscheduled[20]
Informed consent	X[1]											
Inclusion/ Exclusion Criteria	X											
Demographics	X											
Pregnancy test-serum[4]	X											
Medical history	X											
Concurrent meds	X	X	X	X	X	X	X	X	X	X	X	X
Complete physical exam (including wound assessment Days 1-10)	X		X	X[17]	X[17]	X	X		X		X	X[21]
Vital signs	X	X	X[2]	X[2]	X[2]	X	X		X		X	X[21]
KPS	X				X	X	X		X		X	X[21]
CBC w/diff, plts	X			X		X	X	X	X	X	X	X
Serum chemistry[3], PT/INR, PTT	X			X		X	X	X	X	X	X	X
HIV serology	X											
EKG	X											
CXR (AP and lateral)	X											
Urinalysis with micro	X											
Adverse event evaluation[11, 12]		X	X	X	X	X	X	X	X	X	X	X
MRI[5]	X[13]	X			X		X		X		X	X[21]
Neurologic exam	X	X	X[6]	X[6]	X[6]	X	X		X		X	X[21]
HSV Ab titer[7]	X					X	X		X		X	X
HSV detection (saliva, conjunctival secretions, blood)[8]	X			X[18]	X[18] blood only	X	X	X	X	X	X	X
IL-12 detection (serum)[9]	X			X[18]	X[18]	X	X		X		X	X
Blood sample for LTA, Elispot	X			X[18]	X[18]	X	X		X		X	X
IFN gamma assay	X			X[18]	X[18]	X	X		X		X	X
Blood to be stored for future immune studies	X	X[15]		X[18]	X[18]	X	X		X		X	X
Head CT		X[10]										

	Pre-Study (within 2 weeks prior to the administration of M032)	Day 0	Day1	Day 2	Day 3	Day 10 (± 3 days)	Day 28 (± 4 days)	Month 2 (± 12 days)	Month 3 (± 12 days)	Months 4, 5 (± 12 days)	Months 6, 9, 12 (± 12 days)	Unscheduled[20]
Biopsy, Catheter placement		X										
Prophylactic AED	X	X	X	X	X	X	X[16]					
M032 Administration			X									
Rickham/Ommaya reservoir aspiration[19]	X	X	X	X	X	X	X		X		X	X[21]

1. Will be obtained prior to study screening procedures.
2. Frequency post initiation of infusion: vital signs will be every hour for 12 hours post initiation, every 2 hours for the next 12 hours, then every 4 hours for the next 24 hours.
3. Includes sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride.
4. Only women of child-bearing potential. If necessary, can be repeated during the course of the study.
5. All MRI scans will be performed as prescribed by the Principal Investigator.
6. Neurological exam includes assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness. This will be evaluated every hour for 12 hours post initiation, every 2 hours for the next 12 hours, then every 4 hours for the next 24 hours.
7. By ELISA; neutralizing antibody assays may also be performed.
8. Samples will be evaluated by PCR and culture; quantitative PCR may be performed on positive samples.
9. IL-12 detection (serum) by ELISA.
10. After catheter placement but before start of infusion; may also take place on Day 1.
11. All AEs will be assessed throughout the course of the study, but specific CRFs will only be required at the time points delineated in the above schedule.
12. All unresolved toxicities be followed regularly by the treating physician and/or research coordinator; the nurse coordinator will conduct follow-up weekly with the subject, family and/or referring physician (by phone if travel is too burdensome for the subject) until resolution of treatment-related toxicity to less than Grade 2 (or Grade 3 if that was the subject's baseline).
13. MRI's that have been obtained within 30 days of pre-study visit may be used at the discretion of the PI.
14. Sample will be stored for future brain tumor research.
15. To be obtained post-op.
16. Prophylactic AED's may be discontinued at the Day 28 visit.
17. Wound examination only.
18. Acceptable up to 2 days after
19. Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12.
20. If a participant is to miss a regularly scheduled research follow-up visit, an unscheduled visit can be conducted to capture research data at an otherwise standard of care clinic visit.
21. Collected only if done during an otherwise non-research visit (i.e., unscheduled visit).

8.1 Study Procedures

Screening Phase: (within 2 weeks prior to Treatment Phase)

- Obtain informed consent before any study-related procedures are performed
- Review Inclusion/Exclusion Criteria for eligibility

- Collect the following information:
  - Demographics
  - Medical history
  - Medications currently being taken
- Obtain vital signs (pulse, blood pressure, respiratory rate, temperature, height and weight)
- Perform a complete physical examination
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness
- Complete the Karnofsky Performance Scale (KPS)

- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HIV Serology
  - Pregnancy test (if applicable)
  - HSV antibody titer
  - HSV detection
  - IL-12 detection
  - LTA Elispot
  - IFN gamma assay
  - Immune studies - to be stored for future research
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection
- Collect urine sample for urinalysis and microscopic examination
- Obtain:
  - ECG
  - Chest x-ray
  - MRI - MRI's that have been obtained within 30 days of pre-study visit may be used at the discretion of the PI.
- All patients will be required to be placed on an anticonvulsant at the time of screening; those patients already on an anti-convulsant (anti-epileptic drug/AED) will have a second anti-convulsant added per the discretion of the investigator. This anti-convulsant will be continued for a minimum of 28 days unless the patient develops a reaction to the medication. If this develops, all attempts will be made to implement an alternative agent.
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Day 0 (biopsy and catheter placement)

- Admit to the hospital for biopsy surgery and if eligible, catheter placement
- Review medications
- AE monitoring
- Obtain vital signs (pulse, blood pressure, respiratory rate, temperature and weight) prior to surgery
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness
- Obtain a stereotactic MRI to determine site for the biopsy and catheter placement
- Perform the biopsy and, if eligibility criteria are met, place and label up to 4 catheters

- Prime each catheter with 0.4 ml sterile saline solution and cap the catheters
- Admit to the Neurosurgery Intensive Care Unit for routine post-biopsy care and observation
- Obtain CT scan to ensure the catheters are in the proper position. (May be completed on Day1 prior to drug administration
- Collect blood for the following:
  - Immune studies - to be stored for future research – collect post-op
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Day 1 (drug administration):

- If subject's condition is stable, transfer to a Clinical Research Unit inpatient bed for drug infusion and monitoring
- Review medications
- Perform a complete physical exam including wound assessment
- If not completed on Day 0, obtain a CT scan to ensure the catheters did not move and are still in the proper place
- Obtain pathology report and verify diagnosis

- Administration of study drug which will take approximately 6 hours (may last up to 12 hours)
- Study Drug Administration
  - Acquisition of study drug and diluent
  - Store syringes in refrigerator (4° C) until needed
  - Flush infusion tubing with diluent provided by pharmacy
  - Connect syringe with study drug to infusion tubing
  - Prime with 4.6 ml of the diluted virus preparation making sure that any excess study drug is disposed according to Biosafety Level 2 procedure
  - Connect tubing to catheter
  - Place each syringe in syringe pump
  - Repeat for each active catheter
  - Program pump(s) to flush saline solution at a rate of 0.4 ml per catheter for 35 minutes
  - Re-program pump(s) to deliver study drug as follows:
    - Four (4) active catheters – 0.1ml/hour
    - Three (3) active catheters – 0.13 ml/hour
    - Two (2) active catheters – 0.2 ml/hour
    - One (1) active catheter – 0.4ml/hour
  - Begin infusion
  - After the 3 hour study drug infusion is complete (study drug syringe will not be empty) and prior to disconnecting first infusion tubing from catheter, flush second infusion tubing with diluent supplied by pharmacy
  - Obtain second syringes from refrigerator
  - Connect syringe to infusion tubing and prime with 4.6 ml of the diluted virus preparation
  - Disconnect first tubing from catheter and connect second tubing
  - Place syringe in syringe pump
  - Repeat for each active catheter
  - Program pump(s) to deliver study drug at
    - Four (4) active catheters – 0.1ml/hour
    - Three (3) active catheters – 0.13 ml/hour
    - Two (2) active catheters – 0.2 ml/hour
    - One (1) active catheter – 0.4ml/hour

    - Begin infusion **If there is any problems with the catheters during the infusion, the PI and/or Co-PI are to be notified as well as the Study Coordinator**
- Obtain vital signs (pulse, blood pressure, respiratory rate and temperature) according to the following schedule:
    - Before initiation of study drug
    - Every hour for 12 hours post initiation
    - Every 2 hours for the next 12 hours
    - Every 4 hours for the next 24 hours
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness:
    - Before initiation of study drug
    - Every hour for 12 hours post initiation
    - Every 2 hours for the next 12 hours
    - Every 4 hours for the next 24 hours
- AE monitoring
- After infusion is completed (study drug syringe will not be empty), the catheters will be disconnected from the infusion tubing and capped off. The catheters will remain in place for 6-18 hours and then removed.
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Day 2:

- Continuation of vital signs and neurological examinations (see Day 1 for schedule)
- Wound examination
- Review of medications
- Collect blood for the following:
    - CBC with diff and platelets
    - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
    - PT/INR, PTT
    - HSV detection
    - IL-12 detection
    - LTA Elispot
    - IFN gamma assay
    - Immune studies - to be stored for future research
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection
- AE monitoring
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Day 3:

- Continuation of vital signs and neurological examinations (see Day 1 for schedule)
- Wound examination
- Review of medications
- Complete the Karnofsky Performance Scale (KPS)
- Obtain MRI scan prior to discharge

- Collect blood for the following:
  - HSV detection
  - IL-12 detection
  - LTA Elispot
  - IFN gamma assay
  - Immune studies - to be stored for future research
- AE monitoring
- Discharge if subject is stable enough to leave the hospital
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Day 10 ± 3 days:

- Review medications
- AE monitoring
- Obtain vital signs (pulse, blood pressure, respiratory rate, temperature and weight)
- Perform a complete physical exam
- Wound examination
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness
- Complete the Karnofsky Performance Scale (KPS)
- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HSV antibody titer
  - HSV detection
  - IL-12 detection
  - LTA Elispot
  - IFN gamma assay
  - HSV detection
  - Immune studies - to be stored for future research
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Day 28 ± 4 days:

- Review medications
- AE monitoring
- Obtain vital signs (pulse, blood pressure, respiratory rate, temperature and weight)
- Perform a complete physical exam
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness
- Complete the Karnofsky Performance Scale (KPS)

- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HSV antibody titer
  - HSV detection
  - IL-12 detection
  - LTA Elispot
  - IFN gamma assay
  - Immune studies - to be stored for future research
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection
- Obtain MRI scan
- Discontinue prophylactic AED medication (if applicable)
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Month 2 (study coordinator visit):

- Review medications
- AE monitoring
- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HSV detection
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection

Month 3:

- Review medications
- AE monitoring
- Obtain vital signs (pulse, blood pressure, respiratory rate, temperature and weight)
- Perform a complete physical exam
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness
- Complete the Karnofsky Performance Scale (KPS)
- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HSV antibody titer
  - HSV detection
  - IL-12 detection
  - LTA Elispot
  - IFN gamma assay
  - Immune studies - to be stored for future research

- Collect samples of conjunctival (eye) secretions and saliva for HSV detection
- Obtain MRI scan
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Months 4 and 5 (study coordinator visit):

- Review medications
- AE monitoring
- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HSV detection
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection

Months 6 and 9:

- Review medications
- AE monitoring
- Obtain vital signs (pulse, blood pressure, respiratory rate, temperature and weight)
- Perform a complete physical exam
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness
- Complete the Karnofsky Performance Scale (KPS)
- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HSV antibody titer
  - HSV detection
  - IL-12 detection
  - LTA Elispot
  - IFN gamma assay
  - Immune studies - to be stored for future research
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection
- Obtain MRI scan
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Month 12 (end of study):

- Review medications
- AE monitoring
- Obtain vital signs (pulse, blood pressure, respiratory rate, temperature and weight)

- Perform a complete physical exam
- Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousness
- Complete the Karnofsky Performance Scale (KPS)
- Collect blood for the following:
  - CBC with diff and platelets
  - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
  - PT/INR, PTT
  - HSV antibody titer
  - HSV detection
  - IL-12 detection
  - LTA Elispot
  - IFN gamma assay
  - Immune studies - to be stored for future research
- Collect samples of conjunctival (eye) secretions and saliva for HSV detection
- Obtain MRI scan
- Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12

Unscheduled Visit:

- The following data will be collected at a standard of care follow-up visit only if it has been collected for standard of care purposes (that is: these procedures will not be conducted for research purposes only):
  - Collect vital signs (pulse, blood pressure, respiratory rate, temperature and weight), if done as SOC
  - Collect a complete physical exam data, if done as SOC
  - Perform a neurological (nervous system) examination to include assessment of cranial nerve abnormalities, gait abnormalities (when appropriate), sensory abnormalities, muscle strength, and level of consciousnesss, if done as SOC
  - Collect the Karnofsky Performance Scale (KPS), if done as SOC
  - Obtain MRI scan, if done as SOC

- The following procedures will be performed for research purposes only:
  - Review medications
  - AE monitoring
  - Collect samples of conjunctival (eye) secretions and saliva for HSV detection
  - Should a patient have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon's discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12
  - Collect blood for the following:
    - CBC with diff and platelets
    - Serum Chemistry (sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, total protein, uric acid, GGT, AST/ALT, CK, LDH, alkaline phosphatase, total bilirubin, cholesterol, triglyceride)
    - PT/INR, PTT

- HSV antibody titer
- HSV detection
- IL-12 detection
- LTA Elispot
- IFN gamma assay
- Immune studies - to be stored for future research

9. MEASUREMENT OF EFFECT

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria.

9.1 Definitions

Although the Response Evaluation Criteria in Solid Tumors (RECIST) Committee has proposed new international criteria for the evaluation of response and progression of solid tumor, unidimensional measurements alone have not yet been sufficiently validated for the evaluation of malignant gliomas. The MacDonald criteria will be used for evaluation of treatment responses which is more appropriate for patients who have failed surgery, radiation and chemotherapy prior therapy than recently described RANO criteria which is more appropriate for newly diagnosed patients with high-grade, malignant brain tumors [39].

9.1.1 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$10 mm with MRI scan. All intracranial malignant glioma is therefore measurable disease. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

9.1.2 Non-measurable Disease

This type of lesion is not applicable in this study.

9.1.3 Target Lesions

All intracranial lesions that are likely manifestations of the patient's malignancy will be considered target lesions.

9.1.4 Non-target Lesions

This type of lesion is not applicable in this study.

9.2 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers, or digitized method. All screening evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Because all of the patients enrolled in this study will have received prior radiation therapy, tumor lesions that are situated in a previously irradiated area will be considered measurable.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is the only means of assessing the antitumor effect of this treatment for this disease.

9.3 Response Criteria

The following criteria are based upon the volume changes that correspond to the definitions proposed by the MacDonald criteria. For the purposes of this study, the objective response of each patient's disease to M032 will be evaluated by the comparison of a baseline MRI scan to follow-up MRI. For those patients receiving intratumoral inoculation, the screening MRI will serve as the baseline MRI, and follow-up scans will be done at study Day 3, Day 28, Month 3, Month 6, Month 9 and Month 12. To prevent the introduction of observer bias, a software package which determines tumor areas and volumetrics via assessment of pixel intensity will be utilized to compare pre-and post-treatment images.

9.3.1 Evaluation of Target Lesions

"Response" is defined as follows:

Complete Response (CR) - Disappearance of all treated enhancing tumor on MRI scan, off steroids, and neurologically stable or improved.

Partial Response (PR) - greater than 50% reduction in the treated enhancing tumor on MRI scan, stable or reduced steroid dose, and neurologically stable or improved.

Progressive Disease (PD) - greater than 25 % increase in the treated enhancing tumor on MRI scan, stable or increased steroid dose, and neurologically stable or worse.

Stable Disease (SD) - all other situations

Some patients on this trial may be taking dexamethasone and/or bevacizumab. Those who have decreased the dose of one of these medications to one lower than that utilized at the time of the previous MRI AND this dose decrease has occurred within two weeks of the MRI shall not be determined to have progressed even if the MRI meets the criteria for PD above, but rather that MRI shall be considered non-evaluable for response. Similarly, should an increase in steroid dosage or bevacizumab dosage occur within two weeks of an MRI compared to the prior MRI, the patient will not be eligible for consideration of a CR or PR but instead that MRI will be considered non-evaluable.

Note that since this is considered an immunotherapy trial, the appearance of lesion(s) does not necessarily indicate PD.

9.3.2 Evaluation of Non-target Lesions

Because all intracranial lesions will be considered target lesions, this assessment is not applicable to this study.

9.3.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see **section 9.3.1**).

Note: In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is often recommended that the residual lesion be investigated (biopsy) before confirming the complete response status. Because this disease would require a brain biopsy in this situation, with potential increased risk to the patient, patients with an ambiguous complete response status will only undergo histological confirmation if deemed necessary for additional treatment interventions. If tumor is not the predominant feature of biopsy specimen, e.g., if only necrosis is present, or inflammation or gliosis are prominent, then progression will not be deemed to have occurred. [40]

9.3.4 Pseudoprogression

Pseudoprogression is a well-known entity in oncolytic viral therapy of glioma. Currently, the immunotherapy Response Assessment in Neuro-Oncology (iRANO) group is proposing criteria for determination of pseudoprogression in immunotherapy trials which are not yet published. [40] We will utilize the following criteria to address this phenomenon in this trial : If tumor appears to enlarge or demonstrates increased enhancement by MRI consistent with PD, patients will undergo repeat imaging and clinical assessments at 3 months to determine whether the changes demonstrate true progression or pseudoprogression. Note that the investigator may obtain an MRI prior to this 3 month interval as indicated. Should significant neurologic deterioration occur that cannot be ascribed to tumor or pseudoprogression related events such as seizures or medication changes (e.g., steroid tapers) the patient will be considered to have progressed. During the interval, the patient may be watched or treated with bevacizumab and/or dexamethasone at the discretion of the investigators. If the patient improves with bevacizumab (to be used

preferentially at a suggested dose of 10mg/kg IV, every two weeks, for three months; previous tumor progression on bevacizumab does not preclude its use herein) 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 suffered 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 PD as defined above.

9.4 Confirmatory Measurement/Duration of Response

9.4.1 Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by the next scheduled MRI after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 12 weeks.

9.4.2 Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

9.4.3 Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

9.5 Progression-Free Survival

Because this study is a non-randomized phase 1 trial that will enroll relatively few patients, progression-free survival will be reported as a secondary endpoint.

10. REGULATORY AND REPORTING REQUIREMENTS

Adverse events (AE) will use the descriptions and grading scales found in the revised NCI Common Toxicity Criteria for Adverse Events (CTCAE). This study will utilize the CTCAE version 4.0 for adverse event reporting. All appropriate treatment areas will have access to a copy of the CTCAE version 4.0. A table showing the expected adverse events associated with M032 and the related IMT terms can be found in **Appendix A**.

10.1 Adverse Event Reporting (AE; formerly known as Adverse Drug Reaction)

10.1.1 Adverse Event

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) which can be found in Section 10.1.2. 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".

An AE is considered unexpected if it is not listed in **Section 5.1**.

10.1.2 Serious Adverse Event Expedited Reporting Guidelines – Phase 1 studies with investigational agents:

A serious adverse event (SAE) is defined as:

- Unexpected AE
  - For grade 1, no expedited reporting required
  - For grade 2, 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. If found to be possibly, probably or definitely related to study drug, an expedited report will be submitted within 7 working days.
  - For grade 3, 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. If found to be possibly, probably or definitely related to study drug, an expedited report will be submitted by phone within 24 hours with expedited report to follow within 7 working days.
  - For grade 4-5, regardless of relationship to study drug, a report will be submitted by phone within 24 hours with expedited report to follow within 7 working days.
- AE whose grade has increased to grade 3 or 4

- Adverse Event and IND safety reports will be filed in a timely manner with institutional authorities (IRB, IBC) as well as with the FDA and NIH/ORDA.
- 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 seven days. Additionally, unexpected Grade 2 or Grade 3 toxicities will require a written expedited report within seven

  days and Grade 3 unexpected adverse events will also require a fax, email, or phone call to the UAB IRB, the Neuro-Oncology CTMC, CCC PRC, FDA, and OBA within 24 hours.
- For events that are related, unexpected and serious, reporting will be reported to the FDA within 7 days of knowledge of the event.
- A list of agent-specific expected adverse events can be found in Appendix A.
- Any adverse event requiring an expedited report will also be reported immediately to the Medical Monitor and the investigator's Institutional Review Board (IRB).

UNEXPECTED EVENT		**EXPECTED EVENT**	
GRADES 2 – 3 **Attribution of Possible, Probable or Definite**	**GRADES 4 and 5** **Regardless of Attribution**	**GRADES 1 - 3**	**GRADES 4 and 5** **Regardless of Attribution**
Grade 2 - Expedited report within 7 working days. **Grade 3** - Report by phone to IDB within 24 hrs. Expedited report to follow within 7 working days.	Report by phone to IDB within 24 hrs. Expedited	Adverse Event Expedited	Report by phone to IDB within 24 hrs. Expedited report to follow within 7 working days.

(**Grade 1** – Adverse Event Expedited Reporting NOT required.)	report to follow within 7 working days. This includes deaths within 30 days of the last dose of treatment with an investigational agent.	Reporting NOT required.	This includes deaths within 30 days of the last dose of treatment with an investigational agent.

10.1.3 AE and SAE Summary Table

10.1.4 Forms

We will utilize MedWatch for adverse event reporting. A copy of this form is provided in **Appendix F**.

10.1.5 Secondary Malignancies

Investigators are required to report secondary malignancies occurring on or following treatment on NCI sponsored protocols using the form noted above. **Exception**: Cases of secondary AML/MDS are to be reported using the NCI/CTEP Secondary AML/MDS Report Form.

10.2 Data Reporting

This study will be under the supervision of an external Data and Safety Monitoring Committee without conflicts of interest who will be appointed and chartered to oversee the review of all adverse events and efficacy determinations. The reviews will be completed quarterly and in real time. The committee will also oversee the clinical trial data analysis and publication of study results. The DSMB will be comprised of a medical infectious disease expert, a neurosurgeon familiar with oncolytic virus therapy for brain tumors, a neuro-oncologist familiar with oncolytic virus therapy for patients with brain tumors and an expert in the use of current molecular biology techniques for the detection and quantification of Herpes Simplex Virus in clinical specimens. The Medical Monitor is a medical infectious disease expert. Any member who resigns or otherwise leaves service on the DSMB will be replaced by an expert with equivalent experience and expertise and without any potential conflicts. The Data and Safety Monitoring Board for this Phase I study will utilize the appended NCI-approved Data and Safety Monitoring Plan (**Appendix G**).

10.3 CTEP Multicenter Guidelines:

Not applicable.

10.4 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA):

Not applicable.

11. STATISTICAL CONSIDERATIONS

We will implement the CRM design (the dosing range is described previously in this protocol) in a manner similar to that of Geletneky et al. [43] We will define the primary criterion for dose modification as the relative frequency of a DLT at a given dose level. A dose will be considered as tolerable if the probability of toxicity is $\leq$33%. The Recommended Phase 2 Dose (RP2D) will be the dose level with the highest posterior probability of having a toxicity of $\leq$33%. The Optimal Biologic Dose (OBD) will be the RP2D that is obtained by enrolling the fewest number of evaluable patients possible. The posterior probability for toxicity will be determined using a logistic model, where the toxicities will be parameterized as probabilities in the model. When a patient enters the study, the dose level will be determined by calculating the posterior probability for each dose level to be tolerable. The highest dose level that is tolerable will be allocated. Regarding the dose escalation scheme, a conventional three-at-one [44] design will be used as long as no DLT is observed. After the first DLT is observed, one additional patient will be enrolled at the same dose before the dose will be determined as the RP2D using the logistic model. The highest dose with a median probability of at least 33% will be applied as the current RP2D to the subsequent patient, following the CRM [45]. If the RP2D is calculated as dose level -1 (i.e. 1 X 10^4 PFU), the trial will end with no safe dose established.

11.1 Study Design/Endpoints

This study is an open-label, dose escalating phase 1 study of the safety of intracranial administration of

M032, a genetically engineered HSV-1 expressing IL-12. For this reason, the primary endpoints of this study is to determine the safety and tolerability of a single 12 hour stereotactic intracerebral infusion of escalating doses of M032 virus, and to determine the maximally tolerated dose (MTD) of M032. The dose escalation scheme and the definition of the MTD are found in **Section 4.2**. Because M032 is administered as a single dose (although that single dose may be delivered via different catheters), intra-patient dose escalations will not be feasible.

Demographic Analysis: Demographic and baseline characteristics will be summarized for each cohort using the statistics of number, mean, median and range for continuous variables and for discrete factors, values will be tabulated.

Safety Analysis: Descriptive statistics will be used in the reporting of adverse events. Adverse events will be tabulated and frequencies of events will be determined. All events with a toxicity of Grade 3 or above (except as discussed in section 4.2) will be tabulated by event, as well as tabulations for all events (where toxicity is defined by the CTCAE, version 4.0). Laboratory analyses (chemistries, hematology, urinalysis, serological/immunological analyses, 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 by univariate and multivariable analysis as possible confounders in the treatment response relationship. Concurrent medications will also be listed. Effects of previous treatments for cancer will also be examined by univariate and multivariable analysis, and any potential related side effects will be analyzed and discussed.

11.2 Sample Size/Accrual Rate

Because the number of patients enrolled at each dose level depends on observed toxicities, it is not possible to state a definite sample size that will be accrued. The dose escalation plan does allow for a minimum of 4 additional patients to be enrolled (if the first two patients enrolled experience dose-limiting toxicity as do patients at each of two CRM-defined de-escalated dose levels) and a maximum of 20additional patients to be enrolled and treated. It is expected that the actual enrollment will fall well between these two extremes.

It is expected that this study will accrue approximately 10-12 patients per year.

11.3 Stratification Factors

This study will not stratify patients according to any baseline factors.

11.4 Analysis of Secondary Endpoints

11.4.1 Delineation of the local and systemic immune response to M032 administration.

- Virus reactivation and shedding will be detected by PCR and culture of serial serum and saliva samples.
- Immunogenicity of M032 will be evaluated by the use of ELISA to detect HSV antibody titers.
- Each of these binomial responses will be summarized by frequencies for each cohort and overall.

11.4.2 Gather preliminary information about the potential benefit of M032 in the treatment of patients with recurrent malignant gliomas.

- The percentage of patients experiencing complete response, partial response, stable disease and progressive disease will be reported by cohort and overall based on follow-up radiographic imaging.
- Changes in clinical disease status and steroid administration will be considered when reviewing changes in tumor volumetric size.
- All changes in tumor volume will also be analyzed with consideration of any other anti-tumor cancer therapies (either prior to M032 administration or following M032 failure) and the timeframes in which they were administered.

- Quality of life response: A Karnofsky Performance Status score will be recorded pre-treatment and then measured serially post-treatment for each patient. Time to KPS <60 will be measured for each patient by the Kaplan-Meier analytical method.

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APPENDIX A

Expected Adverse Events Associated with M032 and Related International Medical Terminology (IMT) Terms

Category	Adverse Event	IMT Preferred Term
Allergy/Immunology	Allergic reaction/hypersensitivity	Hypersensitivity NOS
Blood/Bone Marrow	Leukocytes	Leucopenia NOS
Blood/Bone Marrow	Hemoglobin	Haemoglobin decreased
Blood/Bone Marrow	Hemolysis	Hemolysis NOS
Cardiovascular (General)	Edema	Oedema NOS
Cardiovascular (General)	Thrombosis/embolism	Thrombosis NOS
Constitutional Symptoms	Fatigue	Fatigue
Constitutional Symptoms	Fever	Pyrexia
Constitutional Symptoms	Weight loss	Weight decreased
Gastrointestinal	Nausea	Nausea
Hemorrhage	Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia	Haemorrhage NOS
Hepatic	SGPT (ALT)	Alanine aminotransferase increased
Infection/Febrile Neutropenia	Infection without neutropenia	Infection NOS
Neurology	Pyramidal tract dysfunction	Upper motor neurone lesion
Neurology	Speech impairment	Speech disorder NEC
Neurology	Memory loss	Amnesia NEC
Neurology	Confusion	Confusion
Neurology	Mood alteration—depression	Depression NEC
Neurology	Personality/Behavioral	Personality change
Neurology	Neurology—Other (Specify, Somnolence)	Not Available
Neurology	Depressed level of consciousness	Depressed level of consciousness
Neurology	Seizure(s)	Convulsions NOS
Neurology	Neurology—Other (Specify, Tumor Progression)	Not Available
Pain	Headache	Headache NOS

Note: The full list of IMT terms is available on the CTEP home page (http://ctep.info.nih.gov/CtepInformatics/IMT.htm).

APPENDIX B

Performance Status Criteria

Karnofsky Performance Scale	
Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX C

INSTRUCTION FOR M032 DOSE PREPARATION

Preparation of M032 dilutions will take place in the hospital pharmacy.
M032 (NSC 733972) 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 M032 DOSE FOR SUBJECT ADMINISTRATION

1. 70% Isopropanol solution
2. MetriSpray, Cavicide or equivalent disinfectant.
3. 5 cc Becton-Dickson disposable syringes, Product code (total of 4 syringes needed)
4. Tuberculin syringe and 18-23 G needle
5. Sterile Vector Diluent (10% Glycerol in Phosphate Buffered Saline, pH 7.0-7.4, USP, preservative-free for injection, 50 cc bottles, Alanza, Inc.)) cooled to 4°C.
6. Sterile Gloves
7. Alcohol Swabs/Wipes
8. Kim-wipes

EQUIPMENT NEEDED FOR PREPARATION OF M032 DOSE FOR SUBJECT ADMINISTRATION

1. Laminar Flow Biological Safety Cabinet (LFBSC)
2. Rack for vector M032 Stock Vials
3. Refrigerator
4. Wet ice or coolant

METHOD FOR PREPARATION OF M032 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 15 minutes prior to using.
2. Cool virus diluent (sterile preservative-free 10% Glycerol in Phosphate Buffered Saline, pH 7.0-7.4, USP) to approximately 4°C in a refrigerator or on ice.
3. Thaw the appropriate number of vector stock M032 vial(s) according to Table 1 in **Appendix D Dose Preparation Guide** below, by rubbing gently between gloved hands. Continue until the last ice crystals have melted and then place vials on ice.
4. Note the time that the contents have completely thawed. Do not allow the vials to warm.
5. Wipe stock M032 vials dry with Kim-Wipes. Gently mix contents of stock M032 vials by swirling.
6. Wipe stock M032 vials with alcohol swab and place in a vial rack in the LFBSC to dry.
7. Dilute M032 according to Table 1. Dose Preparation Guide
   - 7.1 Draw up the required amount of sterile preservative-free Phosphate-buffered saline + 10% glycerol (Alanza), USP for injection (vector diluent)
   - 7.2 Remove the required volume of stock M032 from the glass vials. Add stock M032 to the receptacle/vehicle containing the required diluent
   - 7.3 Gently invert end over end at least 5 times to mix contents

APPENDIX C
INSTRUCTION FOR M032 DOSE PREPARATION (CONT)

8. Label administration syringe(s) appropriately and load 6-10 ml diluted M032 into each syringe. Prepare all syringes for each of the three hour infusions. Store syringes that will be used for the 3 hour exchanges at 4 °C until dispensed to the patient.

    8.1 Intra-tumor infusion via catheters: Use a 10 cc syringe containing 6- 10 cc of diluted M032. The syringe will be fitted with a luer lok connection to non-compressible tubing connected to each catheter so luer-lok syringes are preferred.

9. Place syringe(s) in sterile syringe holder. Transport the required number of syringes to the patient. There should be 1 syringe for each active catheter to be used. The remainder of the loaded syringes may remain in the Research Pharmacy at 4°C until dispensed to the patient at 3 hours or, if a 4° C refrigerator is available, in the CRU.

10. All remaining biological material must be chemically disinfected or autoclaved. All instruments coming in contact with M032 must be autoclaved. All other items coming in contact with M032 must be incinerated or autoclaved.

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

APPENDIX D
INSTRUCTION FOR M032 DOSE PREPARATION
FOR GMP LOT 0909005 ONLY
6 hr Infusion Only

Summary:

The following protocol for preparation of different doses of M032 for infusion is provided for informational purposes only and describes the methods used in preparing doses at 1 log intervals, beginning at the starting dose of 1 x 10^5 PFU delivered in the total volume of 2.4cc. The Continual Reassessment Method (CRM) will estimate the exact dose to be administered to each subsequent subject in 2.4cc. This calculation will be made once each subject has met the 24 day interval and all relevant events have been recorded. A subject- and dose-specific protocol will be prepared and provided to the Trial PIs, the Research Nurse (Study Coordinator) and the Research Pharmacist within a 24 hour period to ensure adequate time to review and approve, before that next subject is enrolled in the study.

Stock Virus:	3.32E+09 per cc		0.5 cc/vial		1.66E+09 per vial		
Infusion Rate/Catheter:	0.1 cc/hr		**Number of Catheters:**		4		
Total Rate:	0.40 cc/hr		**Infusion Time:**		6 hrs		
Total Infusion Volume:	2.4 cc		**Infusion Dead Space:**		4.5 cc		
			Volume	Concentration			
Intermediate Virus Dilution:	Stock Virus		0.5 cc/vial				
	Diluent		3.4 cc				
	[Final]		3.9	**4.26E+08 pfu/cc**			
		2.4 cc =		1.02E+09 PFU	Total		
Desired Dose and Concentration							
Dose (PFU)	Concentration						
1.00E+05	4.17E+04 per cc	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)			
Intermediate Virus Dilution		1	9	4.26E+07			
4.26E+07 Virus Dilution		1	9	4.26E+06			
4.26E+06 Virus Dilution		1	9	4.26E+05			
4.26E+05 Virus Dilution		8	72	4.26E+04	Fill Eight 10cc syringes with ~8 cc each		
		2.4 cc =		**1.02E+05**	PFU, final dose		
1.00E+06	4.17E+05 per cc	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)			
Intermediate Virus Dilution		1	9	4.26E+07			
4.26E+07 Virus Dilution		1	9	4.26E+06			
4.26E+06 Virus Dilution		8	72	4.26E+05	Fill Eight 10cc syringes with ~8 cc each		
		2.4 cc =		**1.02E+06**	PFU, final dose		
1.00E+07	4.17E+06 per cc	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)			
Intermediate Virus Dilution		1	9	4.26E+07			
4.26E+07 Virus Dilution		8	72	4.26E+06	Fill Eight 10cc syringes with ~8 cc each		
		2.4 cc =		**1.02E+07**	PFU, final dose		
1.00E+08	4.17E+07 per cc	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)			
Prepare an intermediate Virus Dilution using 2 Vials (1.0 cc) diluted in			**6.8 cc Diluent = 7.8 cc**				
Intermediate Virus Dilution		6	54	4.26E+07	Fill Eight 10cc syringes with ~7 cc each		
		2.4 cc =		**1.02E+08**	PFU, final dose		

This Dose will involve Diluting 14 vials of M032 (7 ml) in the defined diluent volume to produce the required concentration in 2.4 ml

Dose	Concentration				
1.00E+09	4.17E+08 per cc	Virus, cc	Diluent, cc		
Stock Virus (14 vials)		7	48	4.23E+08	Fill Eight 10cc syringes with ~6 cc each
		2.4 cc =		**1.01E+09**	PFU, final dose

APPENDIX D
INSTRUCTION FOR M032 DOSE PREPARATION (CONT)
FOR GMP LOT 0909005 ONLY
6 hr Infusion Only

Dose Preparation Guide for Dose Level One:

Required Volume and Rate of Infusion:	0.1 cc/hr	3 hr	4 syringes	1.2 cc	0.3 cc/syringe
	Two 3hr sets			2.4 cc	total infused volume
	Excess for dead volume				4.5cc/syringe
	Need Eight 10cc syringes with 8 cc each =			~80 cc	

Virus: 3.32E+09 per ml — 0.5 cc/vial — 1.66E+09 per vial

	Volume	Concentration
Intermediate Virus Dilution: Virus	0.5 cc/vial	
Diluent	3.4 cc	
[Final]	3.9	4.26E+08 pfu/cc
	2.4 cc =	1.02E+09 PFU

Desired Dose and Concentration

Dose (PFU)	Concentration	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)	
1.00E+05	4.17E+04 per cc				
Intermediate Virus Dilution		1	9	4.26E+07	
4.26E+07	Virus Dilution	1	9	4.26E+06	
4.26E+06	Virus Dilution	1	9	4.26E+05	
4.26E+05	Virus Dilution	8	72	4.26E+04	Fill Eight 10cc syringes with ~8 cc each
		2.4 cc =		**1.02E+05**	PFU, final dose

Note: The virus is stable for over 9 hrs at 4 degrees C, once diluted. The virus dose can be prepared and refrigerated until used, if used within 9 hours.

Note: A larger volume than needed for infusion is required so that the 4 m infusion tubing can be flushed with fresh virus preparation (dead volume is ~4.5 cc).

Dose Preparation Guide for Dose Level 2:

Required Volume and Rate of Infusion:	0.1cc/hr	3 hr	4 syringes	1.2 cc	0.3 cc/syringe
	Two 3hr sets			2.4 cc	total infused volume
	Excess for dead volume				4.5cc/syringe
	Need 8 syringes x 10cc each =			~80cc	50cc/dose

Virus: 3.32E+09 per cc — 0.5 cc/vial — 1.66E+09 per vial

	Volume	Concentration
Intermediate Virus Dilution: Virus	0.5 cc/vial	
Diluent	3.4 cc	
[Final]	3.9	4.26E+08 pfu/cc
	2.4 cc =	1.02E+09 PFU

Desired Dose and Concentration

Dose (PFU)	Concentration	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)	
1.00E+06	4.17E+05 per cc				
Intermediate Virus Dilution		1	9	4.26E+07	
4.26E+07	Virus Dilution	1	9	4.26E+06	
4.26E+06	Virus Dilution	8	72	4.26E+05	Fill Eight 10cc syringes with ~8cc each
		2.4 cc =		**1.02E+06**	PFU, final dose

Note: The virus is stable for over 6 hrs at 4 degrees C, once diluted. The virus dose can be prepared and refrigerated until used, if used within 9 hours.

Note: A larger volume than needed for infusion is required so that the 4 m infusion tubing can be flushed with fresh virus preparation (dead volume is ~4cc).

APPENDIX D
INSTRUCTION FOR M032 DOSE PREPARATION (CONT)
FOR GMP LOT 0909005 ONLY
6 hr Infusion Only

Dose Preparation Guide for Dose Level 3:

Required Volume and Rate of Infusion:		0.1cc/hr	3 hr	4 syringes	1.2 mL	0.3 cc/syringe
		Two 3hr sets			2.4 cc	total infused volume
		Excess for dead volume				4.5cc/syringe
		Need 8 syringes x 10cc each =			~80cc	50cc/dose

Virus:	3.32E+09 per cc		0.5 cc/vial	1.66E+09 per vial	
			Volume	Concentration	
Intermediate Virus Dilution:		Virus	0.5 cc/vial		
		Diluent	3.4 cc		
		[Final]	3.9	4.26E+08 pfu/cc	
		2.4 Total		1.02E+09 PFU	

Desired Dose and Concentration

Dose (PFU)	Concentration				
1.00E+07	4.17E+06 per cc	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)	
Intermediate Virus Dilution		1	9	4.26E+07	
4.26E+07 Virus Dilution		8	72	4.26E+06	Fill Eight 10cc syringes with ~8cc each
		2.4 cc =		**1.02E+07**	PFU, final dose

Note: The virus is stable for over 6 hrs at 4 degrees C, once diluted. The virus dose can be prepared and refrigerated until used, if used within 9 hours.
Note: A larger volume than needed for infusion is required so that the 4 m infusion tubing can be flushed with fresh virus preparation (dead volume is ~4cc).

Dose Preparation Guide for Dose Level 4:

Required Volume and Rate of Infusion:		0.1cc/hr	3 hr	4 syringes	1.2 mL	0.3 cc/syringe
		Two 3hr sets			2.4 cc	total infused volume
		Excess for dead volume				4.5cc/syringe
		Need Eight 10cc syringes x 8 cc each =			~80cc	

Virus:	3.32E+09 per cc		0.5 cc/vial	1.66E+09 per vial	
			Volume	Concentration	
Intermediate Virus Dilution:		Virus	0.5 cc/vial		
		# of vials	2		
		Diluent	6.8 cc		
		[Final]	7.8	4.26E+08 pfu/cc	
		2.4 cc =		1.02E+09 PFU	

Desired Dose and Concentration

Dose (PFU)	Concentration				
1.00E+08	4.17E+07 per cc	Virus (cc)	Diluent (cc)	Concentration (PFU/cc)	
Intermediate Virus Dilution		6	54	4.26E+07	Fill Eight 10cc syringes with ~7cc each
		2.4 cc =		**1.02E+08**	PFU, final dose

Note: The virus is stable for over 6 hrs at 4 degrees C, once diluted. The virus dose can be prepared and refrigerated until used, if used within 9 hours.
Note: A larger volume than needed for infusion is required so that the 4 m infusion tubing can be flushed with fresh virus preparation (dead volume is ~4cc).

APPENDIX D
INSTRUCTION FOR M032 DOSE PREPARATION (CONT)
FOR GMP LOT 0909005 ONLY
6 hr Infusion Only

Dose Preparation Guide for Dose Level 5:

Required Volume and Rate of Infusion:	0.1cc/hr	3 hr	4 syringes	1.2 cc	0.3 cc/syringe
	Two 3hr sets			2.4 cc	total infused volume
	Excess for dead volume				4.5 cc/syringe
	Need 8 syringes x 6cc each =			~48cc	55 cc total

STOCK VIRUS

Virus:	3.32E+09 per cc	0.5 cc/vial	1.66E+09	PFU per vial

This dilution will involve Diluting 14 vials of M032 (7 cc) in the defined diluent volume to produce the required concentration in 2.4 cc

Dose	Concentration	Stock Virus, cc	Diluent, cc		
1.00E+09	4.17E+08 per cc	7	48	4.23E+08	Fill Eight 10cc syringes with ~6 cc each (Note Volume Change)
		2.4 cc =		**1.01E+09**	PFU, final dose

Note: The virus is stable for over 6 hrs at 4 degrees C, once diluted. The virus dose can be prepared and refrigerated until used, if used within 9 hours.

Note: A larger volume than needed for infusion is required so that the 4 m infusion tubing can be flushed with fresh virus preparation (dead volume is ~4.5 cc).

APPENDIX E
M032 SHIPMENT INFORMATION/HANDLING

M032 (NSC 733972) Lot L0909005 will be supplied by the NIH RAID program. M032 shipment requests or questions regarding M032 and investigational drug supply should be addressed to:

Anthony Welch
Program Director
Biological Resources Branch, DTP
Division of Cancer Treatment and Diagnosis
NCI_Frederick
Building 1052, Room 249
Frederick, MD 21702
Phone: 301-846-5691
Fax: 301-846-5429
Email: welcha@mail.nih.gov

M032 is formulated in Dulbecco's phosphate buffered saline (D-PBS), 0.4M NaCl and 10% (w/v) glycerol at a concentration of 5.8e9 pfu/ml. The virus product for the study is supplied in 1.0 ml, single use glass vials with a fill volume of 0.50 mls.

M032 will be shipped on dry ice to the Research Pharmacy, at the following address:

Rachel Burell, Pharm.D.,
Senior Pharmacist
UAB Hospital Investigational Drug Service Pharmacy
North Pavilion, Room 3470
1802 6th Avenue South
Birmingham, AL 35249
Phone: 205-934-7191

Upon shipment, contents will be checked against the shipping paperwork supplied by the manufacturer and condition on arrival documented. Receipt will be verified by faxing completed paperwork to the manufacturer. Virus will then be logged into the M032 Study Drug Inventory Log.

M032 will be stored frozen at –75 ± 15 °C. Immediately prior to use, virus should be thawed, diluted, and prepared according to the instructions provided in Appendix C. All vials of M032 must be signed out on the Study Drug Inventory Log for inventory control.

Precautions consistent with institutional standard operating procedures (SOPs) for the handling of viruses (Biosafety Level 2) should be maintained during the preparation and administration of M032. Virus preparation should be performed in a laminar flow biological safety cabinet using disposable materials. Study personnel involved in the preparation, administration, and disposal of M032 should wear appropriate safety gown, gloves and glasses (refer to Appendix C).

Unused vials of M032 should be returned to the NIH RAID program at the end of the study as instructed. The clinical site will maintain accurate documentation of drug use and the destruction of partially used vials. The monitor will check these records as part of the review of drug accountability.

APPENDIX F
Serious Adverse Event Reporting Form

We will utilize MedWatch to report any AEs or SAEs, using Form FDA3500A. A copy of the form can be found on the following pages.

U.S. Department of Health and Human Services

MEDWATCH

The FDA Safety Information and Adverse Event Reporting Program

For VOLUNTARY reporting of adverse events, product problems and product use errors

Page 1 of ____

Form Approved: OMB No. 0910-0291, Expires: 12/31/2011
See OMB statement on reverse.

FDA USE ONLY
Triage unit sequence #

PLEASE TYPE OR USE BLACK INK

A. PATIENT INFORMATION

1. Patient Identifier	2. Age at Time of Event or Date of Birth:	3. Sex	4. Weight
In confidence		☐ Female ☐ Male	____ lb or ____ kg

B. ADVERSE EVENT, PRODUCT PROBLEM OR ERROR

Check all that apply:

1. ☐ **Adverse Event** ☐ **Product Problem** *(e.g., defects/malfunctions)*
☐ **Product Use Error** ☐ **Problem with Different Manufacturer of Same Medicine**

2. **Outcomes Attributed to Adverse Event** *(Check all that apply)*
☐ Death: _____ *(mm/dd/yyyy)* ☐ Disability or Permanent Damage
☐ Life-threatening ☐ Congenital Anomaly/Birth Defect
☐ Hospitalization - initial or prolonged ☐ Other Serious (Important Medical Events)
☐ Required Intervention to Prevent Permanent Impairment/Damage (Devices)

3. Date of Event *(mm/dd/yyyy)*	4. Date of this Report *(mm/dd/yyyy)*

5. **Describe Event, Problem or Product Use Error**

6. **Relevant Tests/Laboratory Data, Including Dates**

7. **Other Relevant History, Including Preexisting Medical Conditions** *(e.g., allergies, race, pregnancy, smoking and alcohol use, liver/kidney problems, etc.)*

C. PRODUCT AVAILABILITY

Product Available for Evaluation? *(Do not send product to FDA)*
☐ Yes ☐ No ☐ Returned to Manufacturer on: _____ *(mm/dd/yyyy)*

D. SUSPECT PRODUCT(S)

1. **Name, Strength, Manufacturer** *(from product label)*
#1 Name:
Strength:
Manufacturer:

#2 Name:
Strength:
Manufacturer:

2. Dose or Amount	Frequency	Route
#1		
#2		

3. **Dates of Use** *(If unknown, give duration) from/to (or best estimate)*
#1
#2

4. **Diagnosis or Reason for Use** *(Indication)*
#1
#2

6. Lot #	7. Expiration Date
#1	#1
#2	#2

5. **Event Abated After Use Stopped or Dose Reduced?**
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

8. **Event Reappeared After Reintroduction?**
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

9. **NDC # or Unique ID**

E. SUSPECT MEDICAL DEVICE

1. **Brand Name**

2. **Common Device Name**

3. **Manufacturer Name, City and State**

4. Model #	Lot #	5. Operator of Device
Catalog #	Expiration Date *(mm/dd/yyyy)*	☐ Health Professional ☐ Lay User/Patient ☐ Other:
Serial #	Other #	

6. If Implanted, Give Date *(mm/dd/yyyy)*	7. If Explanted, Give Date *(mm/dd/yyyy)*

8. **Is this a Single-use Device that was Reprocessed and Reused on a Patient?**
☐ Yes ☐ No

9. **If Yes to Item No. 8, Enter Name and Address of Reprocessor**

F. OTHER (CONCOMITANT) MEDICAL PRODUCTS

Product names and therapy dates *(exclude treatment of event)*

G. REPORTER *(See confidentiality section on back)*

1. **Name and Address**
Name:
Address:
City: State: ZIP:

Phone #	E-mail

2. Health Professional?	3. Occupation	4. Also Reported to:
☐ Yes ☐ No		☐ Manufacturer ☐ User Facility ☐ Distributor/Importer

5. **If you do NOT want your identity disclosed to the manufacturer, place an "X" in this box:** ☐

FORM FDA 3500 (1/09) Submission of a report does not constitute an admission that medical personnel or the product caused or contributed to the event.

ADVICE ABOUT VOLUNTARY REPORTING

Detailed instructions available at: http://www.fda.gov/medwatch/report/consumer/instruct.htm

Report adverse events, product problems or product use errors with:

- Medications *(drugs or biologics)*
- Medical devices *(including in-vitro diagnostics)*
- Combination products *(medication & medical devices)*
- Human cells, tissues, and cellular and tissue-based products
- Special nutritional products *(dietary supplements, medical foods, infant formulas)*
- Cosmetics

Report product problems - quality, performance or safety concerns such as:

- Suspected counterfeit product
- Suspected contamination
- Questionable stability
- Defective components
- Poor packaging or labeling
- Therapeutic failures (product didn't work)

-Fold Here-

Report SERIOUS adverse events. An event is serious when the patient outcome is:

- Death
- Life-threatening
- Hospitalization - initial or prolonged
- Disability or permanent damage
- Congenital anomaly/birth defect
- Required intervention to prevent permanent impairment or damage (devices)
- Other serious (important medical events)

Report even if:

- You're not certain the product caused the event
- You don't have all the details

How to report:

- Just fill in the sections that apply to your report
- Use section D for all products except medical devices
- Attach additional pages if needed
- Use a separate form for each patient
- Report either to FDA or the manufacturer *(or both)*

Other methods of reporting:

- 1-800-FDA-0178 - To FAX report
- 1-800-FDA-1088 - To report by phone
- www.fda.gov/medwatch/report.htm - To report online

If your report involves a serious adverse event with a device and it occurred in a facility outside a doctor's office, that facility may be legally required to report to FDA and/or the manufacturer. Please notify the person in that facility who would handle such reporting.

-Fold Here-

If your report involves a serious adverse event with a vaccine, call 1-800-822-7967 to report.

Confidentiality: The patient's identity is held in strict confidence by FDA and protected to the fullest extent of the law. FDA will not disclose the reporter's identity in response to a request from the public, pursuant to the Freedom of Information Act. The reporter's identity, including the identity of a self-reporter, may be shared with the manufacturer unless requested otherwise.

The public reporting burden for this collection of information has been estimated to average 36 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
1350 Piccard Drive, Room 400
Rockville, MD 20850

Please DO NOT RETURN this form to this address.

OMB statement:
"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number."

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

FORM FDA 3500 (1/09) (Back) **Please Use Address Provided Below -- Fold in Thirds, Tape and Mail**

DEPARTMENT OF
HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

Official Business
Penalty for Private Use $300

NO POSTAGE NECESSARY IF MAILED IN THE UNITED STATES OR APO/FPO

BUSINESS REPLY MAIL
FIRST CLASS MAIL PERMIT NO. 946 ROCKVILLE MD

POSTAGE WILL BE PAID BY FOOD AND DRUG ADMINISTRATION

MEDWATCH
The FDA Safety Information and Adverse Event Reporting Program
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20852-9787

Page 3 of AE reporting form

U.S. Department of Health and Human Services

MEDWATCH

The FDA Safety Information and Adverse Event Reporting Program

(CONTINUATION PAGE)

For VOLUNTARY reporting of adverse events and product problems

Page 3 of ____

B.5. **Describe Event or Problem** *(continued)*
B.6. **Relevant Tests/Laboratory Data, Including Dates** *(continued)*
B.7. **Other Relevant History, Including Preexisting Medical Conditions** *(e.g., allergies, race, pregnancy, smoking and alcohol use, hepatic/renal dysfunction, etc.) (continued)*
F. **Concomitant Medical Products and Therapy Dates** *(Exclude treatment of event) (continued)*

General Instructions for Completing the MedWatch Form FDA 3500

For use by health professionals and consumers for **VOLUNTARY** reporting of adverse events, product use errors and product quality problems with:

- Drugs
- Biologics (including blood components, blood derivatives, allergenics, human cells, tissues, and cellular and tissue-based products (HCT/Ps)
- Medical devices (including *in-vitro* diagnostics)
- Combination products (e.g. drug-device, biologic-device)
- Special nutritional products (dietary supplements, infant formulas, medical foods)
- Cosmetics

Adverse events involving **vaccines** should be reported to the Vaccine Adverse Event Reporting System (VAERS), http://vaers.hhs.gov/pdf/vaers_form.pdf Adverse events involving **investigational (study) drugs, such as those relating to Investigational New Drug (IND) applications**, should be reported as required in the study protocol and sent to the address and contact person listed in the study protocol. They should generally not be submitted to FDA MedWatch as voluntary reports.

Note for consumers: If possible, please take the 3500 form to your health professional (e.g., doctor or pharmacist) so that information based on your medical record that can help in the evaluation of your report will be provided. If, for whatever reason, you do not wish to have your health professional fill out the form, you are welcome to do so yourself.

GENERAL INSTRUCTIONS

- Please make sure that all entries are either typed, printed in a font no smaller than 8 point, or written using black ink.
- Please complete all sections that apply to your report.
- Dates should be entered as mm/dd/yyyy (e.g., June 3, 2005 = 06/03/2005). If exact dates are unknown, please provide the best estimate (see block **B3**).
- For narrative entries, if the fields do not provide adequate space, attach additional pages as needed.
- If attaching additional pages, please do the following:
  - Identify all attached pages as Page __ of __
  - Indicate the appropriate section and block number next to the narrative continuation.
  - Include the phrase continued at the end of each field that has additional information continued on to another page.
- **Section D,** Suspect product(s), should be used to report on special nutritional products and cosmetics as well as drugs or biologics, including human cells, tissues, and cellular and tissue-based products (HCT/Ps).
- If your report involves a serious adverse event with a device and it occurred in a facility other than a doctor's office, that facility may be legally required to report to FDA and/or the manufacturer. Please notify the person in that facility who would handle such reporting.

SECTION A: PATIENT INFORMATION

Complete a separate form for each patient, unless the report involves a medical device where multiple patients were adversely affected through the use of the same device. In that case, please indicate the number of patients in block **B5** (Describe event or problem) and complete Section A and blocks **B2**, **B5**, **B6**, **B7**, and **F** for each patient. Enter the corresponding patient identifier in block **A1** for each patient involved in the event.

Parent-child/fetus report(s) are those cases in which either a fetus/breast-feeding infant or the mother, or both have an adverse event that is possibly associated with a product administered to the mother during pregnancy. Several general principles are used for filing these reports:

- If there has been no event affecting the child/fetus, report only on the parent.
- For those cases describing fetal death, miscarriage or abortion, report the parent as the patient in the report.
- When only the child/fetus has an adverse reaction/ event (other than fetal death, miscarriage or abortion), the information provided in **Section A** applies to the child/fetus. However, the information in **Section D** would apply to the parent who was the source of exposure to the product.
- When a newborn baby is found to have a birth defect/congenital anomaly that the initial reporter considers possibly associated with a product administered to the mother during pregnancy, the patient is the newborn baby.
- If both the parent and the child/fetus have adverse events, separate reports should be submitted for each patient.

A1: Patient Identifier

Please provide the patient's initials or some other type of identifier that will allow you, the reporter, to readily locate the case if you are contacted for more information. Do not use the patient's name or social security number.

The patient's identity is held in strict confidence by FDA and protected to the fullest extent of the law. FDA will not disclose the reporter's identity in response to a request from the public, pursuant to the Freedom of Information Act.

If no patient was involved (such as may be the case with a product problem), enter none.

A2: Age at Time of Event or Date of Birth

Provide the most precise information available. Enter the patient's birth date, if known, or the patient's age at the time of event onset. For age, indicate time units used (e.g., years, months, days):

- If the patient is 3 years or older, use years (e.g., 4 years).
- If the patient is less than 3 years old, use month (e.g., 24 months).
- If the patient is less than 1 month old, use days (e.g., 5 days).
- Provide the best estimate if exact age is unknown.

A3: Sex

Enter the patient's gender. If the adverse event is a congenital anomaly/birth defect, report the sex of the child.

A4: Weight

Indicate whether the weight is in pounds (lb) or kilograms (kg). Make a best estimate if exact weight is unknown.

SECTION B: ADVERSE EVENT, PRODUCT PROBLEM, PRODUCT USE ERROR

B1: Adverse Event, Product Problem, Product Use Error, or Problem with Different Manufacturer of Same Medicine.

Choose the appropriate box(es). If a product problem may have caused or contributed to the adverse event, check both boxes.

Adverse event: Any incident where the use of a medication (drug or biologic, including HCT/P), at any dose, a medical device (including *in-vitro* diagnostics) or a special nutritional product (e.g., dietary supplement, infant formula or medical food) is suspected to have resulted in an adverse outcome in a patient.

To report, it is not necessary to be certain of a cause/ effect relationship between the adverse event and the use of the medical product(s) in question. Suspicion of an association is sufficient reason to report. Submission of a report does not constitute an admission that medical personnel or the product caused or contributed to the event.

Please limit your submissions to those events that are serious. An event is classified as serious when the patient outcome is:

- Death
- Life-threatening
- Hospitalization (initial or prolonged)
- Disability or Permanent Damage
- Congenital Anomaly/Birth Defect
- Required Medical or Surgical Intervention to Prevent Permanent Impairment or Damage (Devices)
- Other Serious (Important Medical Events)

Please see instructions for block **B2** for further information on each of these criteria.

Product problem (e.g., defects/malfunctions): Any report regarding the quality, performance, or safety of any medication, medical device or special nutritional product. In addition, please select this category when reporting device malfunctions that could lead to a death or serious injury if the malfunction were to recur. Product problems include, but are not limited to, such concerns as:

- Suspected counterfeit product
- Suspected contamination
- Questionable stability
- Defective components
- Therapeutic failures (product didn't work)
- Product confusion (caused by name, labeling, design or packaging)
- Suspected superpotent or subpotent medication
- Labeling problems caused by printing errors/ omissions

Product Use Error:

Medication Use Error: Any report of a medication error regardless of patient involvement or outcome. Also report circumstances or events that have the capacity to cause error (e.g., similar product appearance, similar packaging and labeling, sound-alike/look-alike names, etc.).

Medication errors can and do originate in all stages of the medication use system, which includes selecting and procuring drugs, prescribing, preparing and dispensing, administering and monitoring. A medication error is defined as "any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the health care professional, patient, or consumer. Such events may be related to professional practice, health care products, procedures, and systems, including prescribing, order communication, product labeling, packaging, nomenclature, compounding, dispensing, distribution, administration, education, monitoring and use."

Medical Device Use Error: Health care professionals, patients, and consumers can unintentionally cause harm to patients or to themselves when using medical devices. These problems can often arise due to problems with the design of the medical device or the manner in which the device is used. Often, use errors are caught and prevented before they can do harm (close call). Report use errors regardless of patient involvement or outcome. Also report circumstances or events that could cause use errors. Medical device use errors usually occur for one or more of the following reasons:

- Users expect devices to operate differently than they do.
- Product use is inconsistent with use's expectations or intuition.
- Product use requires physical, perceptual, or cognitive abilities that exceed those of the user.
- Devices are used in ways not anticipated by the manufacturer.
- Product labeling or packaging is confusing or inadequate.
- The environment adversely affects or influences device use.

Problem with Different Manufacturer of Same Medicine: Any incident, to include, but not be limited to, differences in noted therapeutic response, suspected to have resulted from a switch, or change, from one manufacturer to another manufacturer of the **same** medicine or drug product. This could be changes from a brand name drug product to a generic manufacturer's same product, or from a generic manufacturer's product to the same

(continued on next page)

SECTION B: ADVERSE EVENT, PRODUCT PROBLEM, PRODUCT USE ERROR *(continued)*

product as supplied by a different generic manufacturer, or from a generic manufacturer's product to a brand name manufacturer of the same product. In order to fully evaluate the incident, please include in **Section B5**, if available, specific information relative to the switch between different manufacturers of the same medicine, to include, but not be limited to, the names of the manufacturers, length of treatment on each manufacturer's product, product strength, and any relevant clinical data.

B2: Outcomes Attributed to Adverse Event: Indicate all that apply to the reported event:

Death: Check ony if you suspect that the death was an outcome of the adverse event, and include the date if known.

Do not check if:

- The patient died while using a medical product, but there was no suspected association between the death and the use of the product
- A fetus is aborted because of a congenital anomaly (birth defect), or is miscarried

Life-threatening: Check if suspected that:

- The patient was at substantial risk of dying at the time of the adverse event, or
- Use or continued use of the device or other medical product might have resulted in the death of the patient

Hospitalization (initial or prolonged): Check if admission to the hospital or prolongation of hospitalization was a result of the adverse event.

Do not check if:

- A patient in the hospital received a medical product and subsequently developed an otherwise nonserious adverse event, unless the adverse event prolonged the hospital stay

Do check if:

- A patient is admitted to the hospital for one or more days, even if released on the same day
- An emergency room visit results in admission to the hospital. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious (medically important event)

Disability or Permanent Damage: Check if the adverse event resulted in a substantial disruption of a person's ability to conduct normal life functions. Such would be the case if the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life.

Congenital Anomaly/Birth Defect: Check if you suspect that exposure to a medical product prior to conception or during pregnancy may have resulted in an adverse outcome in the child.

Required Intervention to Prevent Permanent Impairment or Damage (Devices): Check if you believe that medical or surgical intervention was necessary to preclude permanent impairment of a body function, or prevent permanent damage to a body structure, either situation suspected to be due to the use of a medical product.

Other Serious (Important Medical Events): Check when the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes. Examples include allergic brochospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias (blood disorders) or seizures/convulsions that do not result in hospitalization. The development of drug dependence or drug abuse would also be examples of important medical events.

B3: Date of Event

Provide the actual or best estimate of the date of first onset of the adverse event. If day is unknown, month and year are acceptable. If day and month are unknown, year is acceptable.

- When a newborn baby is found to have a congenital anomaly, the event onset date is the date of birth of the child.
- When a fetus is aborted because of a congenital anomaly, or is miscarried, the event onset date is the date pregnancy is terminated.
- If information is available as to time during pregnancy when exposure occurred, indicate that information in narrative block **B5**.

B4: Date of this Report

The date the report is filled out.

B5: Describe Event, Problem or Product Use Error

For an **adverse event:**

Describe the event in detail, including a description of what happened and a summary of all relevant clinical information (medical status prior to the event; signs and/or symptoms; differential diagnosis for the event in question; clinical course; treatment; outcome, etc.). If available and if relevant, include synopses of any office visit notes or the hospital discharge summary. To save time and space (and if permitted by your institution), please attach copies of these records with any confidential information deleted. **Do not identify any patient, physician, or institution by name. The reporter's identity should be provided in full in Section G.**

(continued on next page)

SECTION B: ADVERSE EVENT, PRODUCT PROBLEM, PRODUCT USE ERROR *(continued)*

Information as to any environmental conditions that may have influenced the event should be included, particularly when (but not exclusive to) reporting about a device.

- Results of relevant tests and laboratory data should be entered in block **B6**. (See instructions for **B6**.)
- Preexisting medical conditions and other relevant history belong in block **B7**. Be as complete as possible, including time courses for preexisting diagnoses (see instructions for **B7**).

If it is determined that reuse of a medical device labeled for single use may have caused or contributed to an adverse patient outcome, please report in block **B5** the facts of the incident and the perceived contribution of reuse to the occurrence.

For a product problem: Describe the problem (quality, performance, or safety concern) in sufficient detail so that the circumstances surrounding the defect or malfunction of the medical product can be understood.

- If available, the results of any evaluation of a malfunctioning device and, if known, any relevant maintenance/service information should be included in this section.
- For a medication or special nutritional product problem, please indicate if you have retained a sample that would be available to FDA.

For a product use error: Describe the sequence of events leading up to the error in sufficient detail so that the circumstances surrounding the error can be understood.

- **For Medication Use Errors:** Include a description of the error, type of staff involved, work environment in which the error occurred, indicate causes or contributing factors to the error, location of the error, names of the products involved (including the trade (proprietary) and established (proper) name), manufacturer, dosage form, strength, concentration, and type and size of container.

- **For Medical Device Use Errors:** Report circumstances or events that could cause use errors. Medical device use errors usually occur for one or more of the following reasons:

  - Users expect devices to operate differently than they do.
  - Product use is inconsistent with user's expectations or intuition.
  - Product use requires physical, perceptual, or cognitive abilities that exceed those of the user.
  - Devices are used in ways not anticipated by the manufacturer.
  - Product labeling or packaging is confusing or inadequate.
  - The environment adversely affects or influences device use.

For a problem with a different manufacturer of the same medicine:

Please include specific information relative to the switch between different manufacturers of the same medicine, to include, but not be limited to, the names of the manufacturers, length of treatment on each manufacturer's product, product strength, and any relevant clinical data.

B6: Relevant Tests/Laboratory Data, Including Dates

Please provide all appropriate information, including relevant negative test and laboratory findings, in order to most completely convey how the medical work-up/ assessment led to strong consideration of medical product-induced disease as etiology for clinical status, as other differential diagnostic considerations were being eliminated.

Please include:

- Any relevant baseline laboratory data prior to the administration or use of the medical product
- All laboratory data used in diagnosing the event
- Any available laboratory data/engineering analyses (for devices) that provide further information on the course of the event

If available, please include:

- Any pre- and post-event medication levels and dates (if applicable)
- Synopses of any relevant autopsy, pathology, engineering, or lab reports

If preferred, copies of any reports may be submitted as attachments, with all confidential information deleted. **Do not identify any patient, physician or institution by name.** The initial reporter's identity should be provided in full in **Section G.**

B7: Other Relevant History, Including Preexisting Medical Conditions

Knowledge of other risk factors can help in the evaluation of a reported adverse event. If available, provide information on:

- **Other known conditions in the patient, e.g.,**
  - Hypertension (high blood pressure)
  - Diabetes mellitus
  - Liver or kidney problems

- **Significant history**
  - Race
  - Allergies
  - Pregnancy history
  - Smoking and alcohol use, drug abuse
  - Setting

SECTION C: PRODUCT AVAILABILITY

Product available for evaluation? (Do not send the product to FDA.)
To evaluate a reported problem with a medical product, it is often critical to be able to examine the product. Please indicate whether the product is available for evaluation. Also indicate if the product was returned to the manufacturer and, if so, the date of the return.

SECTION D: SUSPECT PRODUCT(S)

For adverse event reporting:

A suspect product is one that you suspect is associated with the adverse event. In **Section F** enter other concomitant medical products (drugs, biologics including human cells, tissues, and cellular and tissue-based products (HCT/Ps), medical devices, etc.) that the patient was using at the time of the event but which you do not think were involved in the event.

Up to two (2) suspect products may be reported on one form (#1=first suspect product, #2=second suspect product). Attach an additional form if there were more than two suspect products associated with the reported adverse event.

For product quality problem reporting:

A suspect product is the product that is the subject of the report. A separate form should be submitted for each individual product problem report.

Identification of the labeler/distributor and pharmaceutical manufacturer and labeled strength of the product is important for prescription or non-prescription products.

This section may also be used to report on special nutritional products (e.g., dietary supplements, infant formula or medical foods), cosmetics, human cells, tissues, or cellular and tissue-based products (HCT/Ps) or other products regulated by FDA.

If reporting on a special nutritional or drug product quality problem, please attach labeling/packaging if available.

If reporting on a special nutritional product only, please provide directions for use as listed on the product labeling.

D1: Name, Strength, Manufacturer

Use the trade/brand name. If the trade/brand name is not known or if there is no trade/brand name, use the generic product name and the name of the manufacturer or labeler. These names are usually found on the product packaging or labeling. Strength is the amount in each tablet or capsule, the concentration of an injectable, etc. (such as "10mg", "100 units/cc", etc.).

For human cells, tissues, and cellular and tissue-based products (HCT/Ps), please provide the common name of the HCT/P. You can also indicate if the HCT/P has a proprietary or trade name. Examples: Achilles tendon, Iliac crest bone or Islet cells.

D2: Dose or Amount, Frequency, Route

Describe how the product was used by the patient (e.g., 500 mg QID orally or 10 mg every other day IV). For reports involving overdoses, the amount of product used in the overdose should be listed, not the prescribed amount. (See **APPENDIX** for list of **Routes of Administration** on the next page.)

D3: Dates of Use

Provide the date administration was started (or best estimate) and the date stopped (or best estimate). If no dates are known, an estimated duration is acceptable (e.g., 2 years) or if therapy was less than one day, then duration is appropriate (e.g., 1 dose or 1 hour for an IV).

For human cells, tissues, and cellular and tissue-based products, provide the date of transplant and if applicable, the date of explanation.

D4: Diagnosis or Reason for Use (Indication)

Provide the reason or indication for which the product was prescribed or used in this particular patient.

D5: Event Abated After Use Stopped or Dose Reduced

If available, this information is particularly useful in the evaluation of a suspected adverse event. In addition to checking the appropriate box, please provide supporting lab tests and dates, if available, in block **B6**.

D6: Lot #

If known, include the lot number(s) with all product quality problem reports, or any adverse event report with a biologic, or medication.

D7: Expiration Date

Please include if available.

(continued on next page)

SECTION D: SUSPECT PRODUCT(S) *(continued)*

D8: Event Reappeared After Reintroduction

This information is particularly useful in the evaluation of a suspected adverse event. In addition to checking the appropriate box, please provide a description of what happened when the drug was stopped and then restarted in block **B5**, and any supporting lab tests and dates in block **B6**.

D9: NDC # or Unique ID

The national drug code (NDC #) is requested only when reporting a drug product problem. Zeros and dashes should be included as they appear on the label. NDC # can be found on the original product label and/or packaging, but is usually not found on dispensed pharmacy prescriptions.

If the product has a unique or distinct identification code, please provide this here. This is applicable to human cells, tissues, and cellular and tissue-based products (HCT/Ps).

Appendix - Routes of Administration

Auricular (otic) 001
Buccal 002
Cutaneous 003
Dental 004
Endocervical 005
Endosinusial 006
Endotracheal 007
Epidural 008
Extra-amniotic 009
Hemodialysis 010
Intra corpus cavernosum 011
Intra-amniotic 012
Intra-arterial 013
Intra-articular 014
Intra-uterine 015
Intracardiac 016
Intracavernous 017
Intracerebral 018
Intracervical 019
Intracisternal 020
Intracorneal 021
Intracoronary 022
Intradermal 023
Intradiscal (intraspinal) 024
Intrahepatic 025
Intralesional 026
Intralymphatic 027
Intramedullar (bone marrow) 028
Intrameningeal 029
Intramuscular 030
Intraocular 031
Intrapericardial 032
Intraperitoneal 033
Intrapleural 034
Intrasynovial 035
Intratumor 036
Intrathecal 037
Intrathoracic 038
Intratracheal 039
Intravenous bolus 040
Intravenous drip 041
Intravenous (not otherwise specified) 042
Intravesical 043
Iontophoresis 044
Occlusive dressing technique 045
Ophthalmic 046
Oral 047
Oropharingeal 048
Other 049
Parenteral 050
Periarticular 051
Perineural 052
Rectal 053
Respiratory (inhalation) 054
Retrobulbar 055
Subconjunctival 056
Subcutaneous 057
Subdermal 058
Sublingual 059
Topical 060
Transdermal 061
Transmammary 062
Transplacental 063
Unknown 064
Urethral 065
Vaginal 066

SECTION E: SUSPECT MEDICAL DEVICE

The suspect medical device is 1) the device that may have caused or contributed to the adverse event or 2) the device that malfunctioned.

In **Section F**, report other concomitant medical products (drugs, biologics including HCT/Ps, medical devices, etc.) that the patient was using at the time of the event but which you do not think were involved in the event.

If more than one suspect medical device was involved in the event, complete all of **Section E** for the first device and attach a separate completed **Section E** for each additional device.

If the suspect medical device is a single-use device that has been reprocessed, then the reprocessor is now the device manufacturer.

E1: Brand Name

The trade or proprietary name of the suspect medical device as used in product labeling or in the catalog (e.g., Flo-Easy Catheter, Reliable Heart Pacemaker, etc.). This information may 1) be on a label attached to a durable device, 2) be on a package of a disposable device, or 3) appear in labeling materials of an implantable device. Reprocessed single-use devices may bear the Original Equipment Manufacturer (OEM) brand name. If the suspect device is a reprocessed single-use device, enter "NA".

E2: Common Device Name

The generic or common name of the suspect medical device or a generally descriptive name (e.g., urological catheter, heart pacemaker, patient restraint, etc.). Please do not use broad generic terms such as "catheter", "valve", "screw", etc.

E3: Manufacturer Name, City and State

If available, list the full name, city and state of the manufacturer of the suspected medical device. If the answer of block **E8** is "yes", then enter the name, city and state of the reprocessor.

E4: Model #, Catalog #, Serial #, Lot #, Expiration Date, Other #

If available, provide any or all identification numbers associated with the suspect medical device exactly as they appear on the device or device labeling. This includes spaces, hyphens, etc.

Model #:

The exact model number found on the device label or accompanying packaging.

Catalog #:

The exact number as it appears in the manufacturer's catalog, device labeling, or accompanying packaging.

Serial #:

This number can be found on the device label or accompanying packaging; it is assigned by the manufacturer, and should be specific to each device.

Lot #:

This number can be found on the label or packaging material.

Expiration Date (mm/dd/yyyy):

If available, this date can often be found on the device itself or printed on the accompanying packaging.

Other #:

Any other applicable identification number (e.g., component number, product number, part bar-coded product ID, etc.)

E5: Operator of Device

Indicate the type (not the name) of person operating or using the suspect medical device on the patient at the time of the event as follows:

- Health professional = physician, nurse, respiratory therapist, etc.
- Lay user/patient = person being treated, parent/ spouse/friend of the patient
- Other = nurses aide, orderly, etc.

E6: If Implanted, Give Gate (mm/dd/yyyy)

For medical devices that are implanted in the patient, provide the implant date or your best estimate. If day is unknown, month and year are acceptable. If month and day are unknown, year is acceptable.

E7: If Explanted, Give Date (mm/dd/yyyy)

If an implanted device was removed from the patient, provide the explantation date or your best estimate. If day is unknown, month and year are acceptable. If month and day are unknown, year is acceptable.

E8: Is this a Single-use Device that was returned before Reprocessed and Reused on a Patient?

Indicate "Yes" or "No".

E9: If Yes to Item No. 8, Enter Name and Address of Reprocessor

Enter the name and address of the reprocessor of the single-use device. Anyone who reprocesses single-use devices for reuse in humans is the manufacturer of the reprocessed device.

SECTION F: OTHER (CONCOMITANT) MEDICAL PRODUCTS

Product names and therapy dates (exclude treatment of event)
Information on the use of concomitant medical products can frequently provide insight into previously unknown interactions between products, or provide an alternative explanation for the observed adverse event. Please list and provide product names and therapy dates for any other medical products (drugs, biologics including HCT/Ps, medical devices, etc.) that the patient was using at the time of the event. Do not include products used to treat the event.

SECTION G: REPORTER

FDA recognizes that confidentiality is an important concern in the context of adverse event reporting. The patient's identity is held in strict confidence by FDA and protected to the fullest extent of the law. However, to allow for timely follow-up in serious cases, the reporter's identity may be shared with the manufacturer unless specifically requested otherwise in block G5. FDA will not disclose the reporter's identity in response to a request from the public, pursuant to the Freedom of Information Act.

G1: Name, Address, Phone #, E-mail

Please provide the name, mailing address, phone number and E-mail address of the person who can be contacted to provide information on the event if follow-up is necessary. While optional, providing the fax number would be most helpful, if available. This person will also receive an acknowledgment letter from FDA on receipt of the report.

G2: Health Professional?

Please indicate whether you are a health professional (e.g., physician, pharmacist, nurse, etc.) or not.

G3: Occupation:

Please indicate your occupation (particularly type of health professional), and include specialty, if appropriate.

G4: Also Reported to:

Please indicate whether you have also notified or submitted a copy of this report to the manufacturer and/ or distributor of the product, or, in the case of medical device reports only, to the user facility (institution) in which the event occurred. This information helps to track duplicate reports in the FDA database.

G5: Release of reporter's Identity to the manufacturer

In the case of a serious adverse event, FDA may provide name, address and phone number of the reporter denoted in block **G1** to the manufacturer of the suspect product. If you do not want your identity released to the manufacturer, please put an X in this box.

APPENDIX G
DATA AND SAFETY MONITORING BOARD

The Independent Data and Safety Monitoring Board will utilize the DSMB plan that has been formulated by the UAB Comprehensive Cancer Center and is NCI-approved (Section 4.5, page 21).

Medical Monitor:

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Chairman: Steven S. Rosenfeld, M.D., Ph.D.Mayo Clinic
Jacksonville,. FL
(In process of moving from Cleveland Clinic – new position, contact and address information incomplete at this time.)

Richard L. Hodinka, PhD
Emeritus Professor, Department of Pathology and Laboratory Medicine
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INFORMED CONSENT

TITLE OF RESEARCH:	A Phase 1 Study of M032 (NSC733972), a Genetically Engineered HSV-1 Expressing IL-12, in Patients with Recurrent/Progressive Glioblastoma Multiforme, Anaplastic Astrocytoma, or Gliosarcoma
IRB PROTOCOL #:	UAB: IRB-131004002 Vanderbilt: 131589
PRINCIPAL INVESTIGATOR:	James M. Markert, M.D.
CO-INVESTIGATOR:	L. Burt Nabors, M.D. Barton L. Guthrie, M.D. Xiaosi Han, MD
SPONSOR:	NCI Grant

Purpose of the Research

This study is being conducted by Dr. James M. Markert at the University of Alabama at Birmingham. The purpose of this study is to establish a safe dose range based on side effects of the study treatment being tested, in this case, M032. All the patients who take part in this study will receive the same type of experimental treatment, although some people will receive higher doses than other people and the method of administration may vary. There is no "placebo" in this study. The total length of time you will be followed for this study is 1 year. When possible, safety data will be collected for up to 15 years from your clinical visits with your standard of care practitioner.

Varying doses of M032 will be administered in this study. There will be no more than 6 subjects in each dose group. The dose you receive will be determined by the number of subjects given the drug before you. We will only increase the dose you receive if the dose given to those who were treated before you was determined to be safe. Anywhere from 8 to 24 patients are expected to take part and be treated in the study; the final number will depend on the safety results.

Explanation of Procedures

You are being asked to participate in a research study because you have a malignant brain tumor that has not responded to standard treatment and have elected to undergo a tumor biopsy as part of your standard medical care. This document will tell you about the purpose, risks, and benefits of this study. You should provide your consent only after you have received all the necessary information and have had enough time to decide whether you wish to participate. Please feel free to ask any questions before you agree to take part in this study.

This study is being conducted to determine how safe and how well-tolerated the experimental study drug, "M032" is when administered into the brain where the tumor is located. "M032" is a genetically modified herpes simplex virus or "HSV" (the virus that usually causes cold sores and rarely, a severe infection of the brain). It has been known that viruses may kill tumor cells. When tumor cells are mixed with certain viruses in the laboratory, the tumor cells die. The (HSV) virus has been modified so that tumor cells may be killed when infected by M032. The changes made to the virus (HSV) should help prevent the (M032) virus from infecting normal brain tissue. Extensive testing in both mice and monkeys, paid for by the Federal Government, has demonstrated that M032 is not able to cause disease

when injected directly into the brain. M032 may also help kill tumor cells by making a protein that should help your body's own immune system to do a better job of fighting off the tumor.

Twelve Owl monkeys with normal brains were injected with M032 at a dose (100 million active viral particles) that is equivalent to a human dose of 5 billion active viral particles (150 times the highest dose planned in this clinical trial). Owl monkeys were selected because there were known to be extremely sensitive to HSV infection, similar to a newborn human baby. In toxicity studies with these monkeys, one monkey began losing weight, became lethargic but did not show signs of viral encephalitis (infection in the brain). The researchers killed the animal in order to study its brain and all organ systems at the microscopic and molecular levels and saw inflammation in the nervous system but were not able to confirm that the cause for its deteriorating condition was due to an HSV infection.

Summary of Study Design:

This study is divided into the following sections, also called phases: the Screening Phase, the Treatment Phase and the Follow-up Phase. Before you can participate in the study, certain tests, exams and procedures will be performed to make sure that you can be in this study. This is called the Screening Phase. If you qualify for the study you will then enter the Treatment Phase which is the phase where you receive the study drug and then enter the follow-up phase.

During this study a total of 13 blood samples and one (1) urine sample will also be collected and tested to evaluate the general state of your health, look at your immunity, including HIV status, and test for the presence of the herpes virus. HIV status is checked because patients with an immune deficiency are not eligible to participate in this research study. Special research blood tests to monitor the body's ability to send signals to the immune system to target the tumor will also be done. The blood samples will be obtained by inserting a needle into a vein in your arm (venipuncture). The total amount of blood that will be drawn over the course of this study will be approximately 2 cups.

Samples conjunctival (eye) secretions and saliva will be collected a total of 11 times and tested for presence of the herpes virus. Saliva will be collected by placing one sterile, cotton tipped swab into the mouth and conjunctival (eye) fluid will be collected by gently touching separate sterile cotton tipped swabs to the corner of each eye.

This study will include having a routine physical examination performed approximately 8 times. Nervous system exams and vital signs assessments will be performed approximately 34 times during this study.
You will also have an electrocardiogram (ECG) to check the electrical activity of your heart and a chest x-ray.

You will be placed on an anticonvulsant (anti-seizure drug) at the screening visit. If you are already taking an anticonvulsant, you will have a second one added. The medications will be taken for a minimum of 28 days.

A total of up to 8 MRI (magnetic resonance imaging) scans will be performed on you throughout the study period to monitor your tumor. If there are signs or symptoms that the tumor has become larger, additional MRIs may be performed if your doctor feels they are necessary for your routine care. It is also possible that your doctor may need to take a biopsy sample of the brain, as part of your routine care, to determine the cause of any increase in signs or symptoms you may be having. If this is the case, your doctor will discuss this with you at the time and explain details of the procedure to you.

One head CT scan (a type of imaging procedure using x-rays) will be performed before administration of the “M032” to make sure the catheters are in the proper place.

During part of the MRI and CT procedures, a needle will be placed into your vein (intravenous line or "IV") and dye will be injected into your vein. This dye helps to give a better picture of the brain tumor and surrounding brain. If you have a known history of allergic reactions to the intravenous MRI contrast material used for these assessments or if you have a pacemaker, ferro-magnetic aneurysm clips, metal infusion pump, metal or shrapnel fragments in your body or certain types of stents, you will not be able to be in this study.

You will have one surgery to insert between one and four small tubes (catheters) into your tumor and the next day you will receive all of the assigned dose of the study drug injected directly through these tubes into your tumor over a 6 hour period (if needed may extend to up to 12 hours). It is important to understand that, if you decide to take part in this trial, your particular clinical situation has determined that this would be the most appropriate portion of the trial for you. Your doctor will explain to you precisely why this trial may be appropriate to your situation, if you decide to take part in this study.

You will be admitted to the hospital on the day of the surgical procedure. You will also be assigned to receive a pre-determined dose of M032 and a MRI of the head to identify the exact location of the tumor. You will also be given medication into a vein (intravenous) to help you relax and feel sleepy before the surgical procedure. The surgical procedure is called stereotactic surgery because the catheters that will be used to deliver the drug need to be inserted precisely into the tumor based on the MRI scan and using a special frame explained below. You will receive medication to minimize any pain (local anesthesia) before the surgery. This surgery involves using a special removable frame (stereotactic frame) that can be temporarily connected to the outside of your skull with small screws. This frame is used to guide the tubing used for injecting M032 in a fixed and precise position so that the study drug can be administered precisely into the tumor. For your doctor to be able to do this stereotactic surgery, you will have a stereotactic frame temporarily connected to your skull with small screws. Importantly, if the tumor has grown significantly since the preceding MRI such that it’s maximum diameter is more than 5.5 cm, the frame will be removed and you will not be allowed to receive drug or participate in the study to minimize the chance of you having a complication from the virus

Before the study drug is injected, your doctor will take a small sample of tissue (biopsy) to check for tumor cells and verify the diagnosis of your type of brain tumor (glioma). A thin, long needle connected to the stereotactic frame will be guided to the tumor. This procedure is routinely performed in subjects for whom a brain biopsy is needed. If the diagnosis is confirmed, then the surgical procedure will proceed to prepare for infusion of the drug.

If the biopsy does not verify that tumor cells are present or your doctor determines that the area containing the tumor cannot be injected, M032 will not be administered.

Some of your tumor sample may be sent to The Translational Genomics Research Institute (TGen) for additional testing and research. You will not receive any results from this testing.

Should you have an access device (eg., Rickham or Ommaya reservoir) that accesses either the more central portion of the tumor/cyst, or the CSF space, fluid will be aspirated as possible (expected, 2-10cc) from the catheter for laboratory studies. These specimens will be obtained at either the pre-op clinic visit or the day of surgery, as well as up to daily during the inpatient hospitalization (per the surgeon’s discretion), and again at post op visit day 10, Day 28, and Months 3,6,9 and 12.

Information for Women of Childbearing Potential, Nursing Mothers, and/or Men Capable of Fathering a Child

All women will also have a serum pregnancy test and the test must be negative within 14 days of starting study treatment to be allowed to enter this study. Additional serum pregnancy tests will be performed during the study if needed. Because it is unknown if M032 affects mother's milk or the developing fetus, women who are pregnant or breast-feeding will not be allowed to participate in the study. In addition, men and women must have been using an effective method of birth control before receiving the study drug. You must also agree to continue to use "barrier" birth control (condoms) during the study and for six (6) months following the administration of the study drug. Should you become pregnant while participating in this study, you should inform your treating physician immediately. For two weeks after receiving M032, you should avoid intimate contact with pregnant women, infants and young children and individuals with decreased immunity (ability to fight infection). Subjects should also refrain from donating blood during the trial.

Treatment Plan:

If you voluntarily decide to participate in this study, you will be hospitalized for a total of approximately 4 days. On the first day, you will have the surgical procedure described above to place up to 4 small tubes called “catheters” into various parts of the tumor. These catheters are slightly larger than the lead of a pencil and will be left in place and the surgical wound will be closed with sutures. Following the surgery, you will be monitored closely in the Neuro-intensive Care Unit overnight. If your condition is stable, your doctor may transfer you to a Clinical Research Unit bed and you will have a CT scan to make sure the catheters are in the correct place and have not shifted. If the surgeon determines that the catheters are properly placed in your tumor, a small syringe will be attached to each catheter and all of the drug, M032, will be slowly pumped into the brain tumor - this is designated as Day 1. This infusion will take about 6 hours (if needed may extend to up to 12 hours). After the infusion is completed, the catheters will be removed at your bedside and you will be closely monitored by your physicians and nurses for the next day or two. Your temperature, blood pressure, breathing and heart rate ("vital signs") and nervous system checks will be performed frequently after surgery and during the M032 administration to monitor your progress. The nervous system checks may include all or some of the following: walking, measures of alertness, muscle strength and any changes in movement.

You will be discharged when your doctor feels you are stable enough to leave the hospital. Total time in the hospital will likely be 3-4 days.

You will return to clinic for 8 follow-up visits (Day 10, Day 28, and Months 3, 6, 9, and 12) after your surgery. At months 2, 4, and 5, you will be asked to come to UAB and have blood drawn and conjunctival and saliva samples collected and to meet with the study coordinator. The study coordinator will ask you questions about your current medications and any changes in your health since your last visit. You will not see the doctor at these 3 visits.

Study Procedures:

Screening Phase: (approximately 2 weeks prior to Treatment Phase)

- Informed consent will be signed before any study-related procedures are performed.
- You will be asked questions about your medical history, medications you are taking, and how you feel.

- You will have a complete physical examination and have your pulse, blood pressure, respiratory rate, temperature, height and weight checked.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- Samples of blood, conjunctival (eye) secretions, saliva, and urine will be collected at this time for testing. This includes a pregnancy test for women capable of bearing children.
- An ECG, chest x-ray and MRI will be performed. MRI's that have been obtained within 30 days of pre-study visit may be used at the discretion of the study doctor.
- You will be started on anticonvulsant medication. If you are already taking an anticonvulsant medication you may have a second one added per the discretion of the study doctor.
- Rickham/Ommaya reservoir fluid aspiration (if applicable).

Day 0 (biopsy and catheter placement)

- You will be admitted to the hospital for biopsy surgery and if eligible, catheter placement.
- You will be asked questions about medications you are currently taking and any changes to your health since last visit.
- Vital signs (pulse, blood pressure, respiratory rate and temperature) will be obtained prior to surgery.
- A MRI will be performed to determine site for the biopsy and catheter placement.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- After the surgery, you will be admitted to the Neurosurgery Intensive Care Unit where your vital signs will be monitored frequently and frequent neurological exams will be performed.
- A CT scan will be performed to make sure the catheters did not move and are still in the proper place (may be completed on Day 1).
- Rickham/Ommaya reservoir fluid aspiration (if applicable).

Day 1 (drug administration):

- If your condition is stable, your doctor may transfer you to a Clinical Research Unit bed for drug infusion and monitoring.
- A complete physical exam will be performed and your wound will be examined.
- If not done on Day 0, a CT scan will be performed to make sure the catheters did not move and are still in the proper place.
- Administration of study drug which will take approximately 6 hours (may last up to 12 hours).
- Your vital signs will be monitored frequently and frequent neurological exams will be performed before, during and after drug administration.
- You will be monitored for any signs of adverse events or reactions to the drug.
- After completion of the study drug infusion, the catheters will be disconnected from the infusion tubing and capped. They will be removed after 6-18 hours.
- A blood sample will be collected.
- Rickham/Ommaya reservoir fluid aspiration (if applicable).

Day 2:

- Nervous system exams and vital signs assessments will be performed.
- Wound examination
- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing. (Acceptable up to 2 days after visit)
- Rickham/Ommaya reservoir fluid aspiration (if applicable; per the surgeon's discretion).

Day 3:

- Nervous system exams and vital signs assessments will be performed.
- Wound examination
- A MRI scan will be performed on you to monitor your tumor.
- A blood sample will be collected. (Acceptable up to 2 days after visit)
- You will be discharged if your doctor feels you are stable enough to leave the hospital.
- Rickham/Ommaya reservoir fluid aspiration (if applicable; per the surgeon's discretion).

Day 10 ± 3 days:

- You will be asked questions about medications you are currently taking.
- You will have a complete physical examination and have your pulse, blood pressure, respiratory rate, and temperature checked.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- You will be asked about any changes to your health since your last visit.
- Rickham/Ommaya reservoir fluid aspiration (if applicable).

Day 28 ± 4 days:

- You will be asked questions about medications you are currently taking.
- You will have a complete physical examination and have your pulse, blood pressure, respiratory rate, temperature and weight checked.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- A MRI scan will be performed on you to monitor your tumor.
- You will be asked about any changes to your health since your last visit.
- Rickham/Ommaya reservoir fluid aspiration (if applicable)**.**

Month 2:

- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- You will be asked about medications you are taking and about any changes to your health since your last visit.

Month 3:

- You will be asked questions about medications you are currently taking.
- You will have a complete physical examination and have your pulse, blood pressure, respiratory rate, temperature and weight checked.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- A MRI scan will be performed on you to monitor your tumor.
- You will be asked about any changes to your health since your last visit.

- Rickham/Ommaya reservoir fluid aspiration (if applicable).

Month 4:

- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- You will be asked about medications you are taking and about any changes to your health since your last visit.

Month 5:

- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- You will be asked about medications you are taking and about any changes to your health since your last visit.

Month 6:

- You will be asked questions about medications you are currently taking.
- You will have a complete physical examination and have your pulse, blood pressure, respiratory rate, temperature and weight checked.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- A MRI scan will be performed on you to monitor your tumor.
- You will be asked about any changes to your health since your last visit.
- Rickham/Ommaya reservoir fluid aspiration (if applicable)**.**

Month 9:

- You will be asked questions about medications you are currently taking.
- You will have a complete physical examination and have your pulse, blood pressure, respiratory rate, temperature and weight checked.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- A MRI scan will be performed on you to monitor your tumor.
- You will be asked about any changes to your health since your last visit.
- Rickham/Ommaya reservoir fluid aspiration (if applicable)**.**

Month 12:

- You will be asked questions about medications you are currently taking.
- You will have a complete physical examination and have your pulse, blood pressure, respiratory rate, temperature and weight checked.
- You will have a neurological (nervous system) examination to check your general nervous system function and muscle strength.
- Samples of blood, conjunctival (eye) secretions and saliva will be collected at this time for testing.
- A MRI scan will be performed on you to monitor your tumor.
- You will be asked about any changes to your health since your last visit.

- Rickham/Ommaya reservoir fluid aspiration (if applicable)**.**

Unscheduled Visit:

- You will be asked questions about medications you are currently taking.
- Samples of blood, conjunctival (eye) secretions and saliva may be collected at this time for testing.
- You will be asked about any changes to your health since your last visit.
- Rickham/Ommaya reservoir fluid aspiration (if applicable)**.**

Risks and Discomforts

Blood Draws:

There may be some temporary pain, bruising, bleeding or rarely, infection at the site where blood samples are drawn from your arm. Although rare, some individuals may become faint during blood drawing procedures. These complications are rarely severe.

MRI Scans:

Magnetic Resonance Imaging (MRI) scans are a painless imaging procedure and are very safe for most people. Some discomfort may be experienced since you must lie flat and remain as still as possible in a long plastic cylinder for approximately 30-45 minutes. Some people also experience anxiety due to fear of being in close spaces. You will be closely observed at all times and can be assisted, if necessary by the hospital staff performing the procedure. You may be moved out of the machine at your request or, if you are experiencing severe anxiety, you may be given anti-anxiety medication prescribed by your doctor to make you less anxious. If you would like, ear plugs are available to you to decrease the knocking noise you hear that is made by the machine. Pillows will be placed under your knees to make you comfortable and you will be covered with a sheet or blanket to keep you warm, if needed.

A small portion of people may develop brief reactions during administration of the dye used in MRI testing, including nausea, headaches, hot flashes and heart palpitations (heart skipping a beat). A smaller group of subjects may also be allergic to the dye and may develop a rash, itching, hives, breathing difficulties and in extreme cases, death. You will be closely monitored throughout the procedure and if an allergic reaction develops, you will be treated promptly. Subjects, who are at risk for injury from MRI such as former welders or those with pace makers, aneurysm clips, (metal clips on the wall of a large artery), metal infusion pumps, or metal and/or shrapnel fragments, will not be entered into the study. Also, there is a risk of "nephrogenic systemic fibrosis" (NSF) which is a fibrosis (scarring and contraction) of skin, eye, joints and internal organs due to the contrast agent used for MRIs scans. While uncommon, this can be debilitating. Your kidney function will be determined to be within an acceptable range before you will be given a contrast agent so that any risk of developing nephrogenic systemic fibrosis due to poor kidney function will be minimized.

CT Scan and Chest x-ray:

The CT scan procedure is a special x-ray procedure that involves lying flat and as still as possible under a scanning machine and typically lasts about 15 minutes. Some subjects may experience an uncomfortable warm flushing sensation when the dye is injected. The dye could cause problems with kidney function or an allergic reaction resulting in a rash, itching, hives, breathing difficulties and in extreme cases, death. You will be closely monitored throughout the procedure and if an allergic reaction develops, you will be treated promptly. The amount of radiation you receive from the CT scan is approximately 3 "rem". For

comparison, the standard limit for occupational radiation exposure is 5 rem/year and the amount from natural background radiation in Alabama is about 1 rem/year.

A chest X-ray is a common diagnostic procedure that requires a small dose of radiation. Although radiation is cumulative over a lifetime, small doses from a chest X-ray should not affect your health.

Surgery/Catheter Placement and removal:

The surgical risks of the stereotactic procedure you will have depend on your condition before the surgery and the location and size of your tumor. Risks known to be associated with brain surgery, involving catheter placement include:

- Hemorrhage (bleeding)
- Deterioration of nervous system functions such as:
  - weakness in the arm and or leg
  - loss of sensation over parts of your body
  - partial or complete loss of function related to communication, such as speech and comprehension
  - other functions related to intellectual capacity, such as memory
- Infection and death
- Catheter removal requires placement of a new suture to close the skin and prevent infection and this is usually done while you are not under anesthesia. This may cause some mild pain or discomfort. Pain medicine will be available to minimize your discomfort during this portion of the procedure should you wish it.

The relative risks of these procedures, considering your condition, will be discussed with you by your doctor. You will be assessed closely post-surgery for any signs that the surgery may have any of these events.

Brain tumor/tissue biopsy and Infusion of M032:

Risks of brain tumor/tissue biopsy include:

- Bleeding
- Infection
- Possible deterioration of nervous system function
- Inadvertent inoculation of virus into the ventricles or other non-tumor area

Stereotactic frame placement:

In order for your doctor to be able to do the surgery, you will have to have a special removable frame temporarily attached to your skull with small screws. Although you will also receive medication to minimize any pain (local anesthesia and IV tranquilizer), you may feel some pain right when the frame is first attached to your head, and these sites may be sore/tender for several days after the treatment. There is a very small risk of a local infection from the site where the screws are placed.

Risks of Herpes Simplex Virus/M032:

Herpes simplex virus is the virus that usually causes cold sores and rarely, a severe brain infection. It can also infect other tissues such as skin and the mucous membranes of the mouth, eyes and urinary tract. This research study will involve the injection of a modified herpes simplex virus into the brain. Based on all of the laboratory studies done in mice and monkeys, the modification should allow the M032 virus to infect and kill tumor cells but not normal brain tissue. This cannot be assured and the purpose of this

study is to demonstrate that high doses of the virus can be given without any toxic effects. The potential risks of M032 include, but are not limited to:

- Inflammation of the liver (hepatitis) that could cause death (very rarely)
- Wide-spread viral infection with effects ranging from flu-like symptoms to more severe reactions
- Allergic reaction to the virus causing symptoms ranging from itching and hives to severe cases, difficulty breathing.
- Infection or inflammation of the brain (encephalitis) or spinal fluid space (meningitis), which may cause high fevers, confusion, weakness, problems with alertness or thinking, loss of consciousness, neurologic difficulties, seizures and even death.

The virus in this study has been modified to prevent the development of infection of normal brain cells. However, if you should develop an infection of the brain, you will be treated with the standard medical therapy that is very effective in treating and eliminating this kind of infection. This therapy to destroy the virus uses medications to fight the virus (anti-viral drugs).

Should this anti-viral therapy be needed, it would require that you be hospitalized and receive daily intravenous infusion of the anti-viral drug acyclovir or similar drug for 14-21 days. With early treatment, anti-viral drugs like acyclovir and others are likely to halt progression of herpes simplex viral infection. Your doctor may need to perform tests to help determine if there is such an infection. These might include a biopsy of brain tissue or testing of the fluid surrounding the spinal cord and the brain called cerebral spinal fluid (CSF).

Your doctor will discuss these procedures with you if it becomes necessary to perform them. The risks of a brain biopsy include the possibility of bleeding, infection and in rare cases, deterioration in nervous system functioning. If a sample of CSF is needed, a small needle will be placed in the small of the back into the space around the spinal cord (lumbar puncture) and a sample of fluid removed. This can result in headache and in rare cases, worsening of nervous system functioning.

There is also a risk that the tumor itself may swell as a result of virus injection, causing headache, lethargy (sleepiness and tiredness), nausea, vomiting, seizures, neurological deficits or even death. Should tumor swelling develop, you will receive treatment with steroids (drugs that decrease inflammation and swelling) for as long as it is necessary. If nervous system deficits persist despite steroid treatment, there is a chance that your doctor would recommend surgery to remove some or all of the swollen tumor and ease the pressure on the surrounding brain. You will be monitored closely throughout the trial for signs and symptoms of infection so that you can be treated promptly.

The majority of people in the United States have already been exposed to herpes simplex virus and may have antibodies against the virus. If you do not have antibodies against the virus, it is possible that you will develop them after receiving M032. These antibodies help fight infection from the herpes simplex virus and are not harmful.

Risk of Interleukin-12 (IL-12) gene therapy in the brain:

The risk of interleukin-12 (IL-12) gene therapy in the brain is unknown, but could potentially include permanent damage to neurologic function due to inflammation or even the development of an autoimmune response in which the body's infection fighting cells perceive normal brain as infection and attack it. This could potentially produce problems similar to Multiple Sclerosis (MS).

Other, unknown effects could also occur as a result of interleukin-12 (IL-12) gene therapy in the brain, including but not limited to stroke, bleeding, dangerously low blood pressure, damage to liver or kidney function, or even death.

Risk of accessing Ommaya or Rickham reservoir (only if present)

The risk of accessing an Ommaya or Rickham reservoir includes pain and bleeding at the site of the needle puncture. The procedure will be done under sterile conditions, but there is a remote risk of infection. Finally, there is a very small risk of causing bleeding at the catheter tip which could be asymptomatic but could potentially result in neurologic deficit.

Unknown Risks

M032 administration into tumor is new and it is possible that despite our extensive efforts, unforeseen problems may occur including the possibility of unknown and possible disabling effects or death.

Benefits

There may be no direct benefit to you from this study. While it is also possible that this experimental treatment may kill some of your tumor cells, there may still be no beneficial effect on the course of your illness. Because of your participation in this study, we may learn more about potential ways to treat brain tumors. This information may prove useful in the future treatment of patients with brain tumors.

Alternatives

You are being offered the opportunity to participate in this study after your tumor recurred, despite appropriate standard therapies for your disease. Other therapy options have been explained to you, including:

- Gliadel ®, a wafer that releases a chemotherapy agent that is implanted into the area of the brain tumor
- Avastin, a tumor-starving (or anti-angiogenic) therapy that blocks a protein called vascular endothelial growth factor, or VEGF.
- Radiation therapy to the brain
- Additional surgery, to remove tumor
- Other Chemotherapy

There are no other standard treatments that have been shown to have significant effects in patients with your disease. A variety of experimental studies for the treatment of brain tumors are conducted in medical centers around the world, but the benefit of their approaches is yet unknown. In addition, you may decline any further treatment for your disease.

If at any time after receiving M032, there are signs or symptoms indicating growth of the tumor, your doctor will again discuss alternative therapies that may be of benefit to you. If you are treated with alternative therapies after receiving M032, you will still be permitted to continue on the study and be monitored for effects of M032.

Confidentiality

Your medical information will be maintained and processed on a computer for the purposes of quality assurance and safety monitoring. This medical information will be kept confidential, to the extent allowed by law, by the study doctor, staff, and authorized institutional personnel and will not be available outside of the UAB-Comprehensive Cancer Center unless there is required disclosure by law. However, your doctor, the Federal funding agent, the U.S. Food and Drug Administration (FDA), National Institutes of Health (NIH) and UAB's Institutional Review Board (IRB) will be able to inspect your medical records and have access to confidential information that identifies you by name. The results of

the study, including laboratory tests and x-rays may be published for scientific purposes, however your identity will not be revealed.

Information relating to this study, including your name, medical record number, date of birth and social security number may be shared with the billing offices of UAB and UAB Health System-affiliated entities so that claims may be submitted for payment to your insurance company for standard care, non-study related clinical services and procedures provided to you during the course of this study. A copy of this consent form will be placed in your chart as part of your permanent medical record.

If you are eligible for Medicare, your participation in this research study will be noted in your medical record.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov (NCT02062827). This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

You will be tested for HIV and for hepatitis. You will be told of your tests results and be offered counseling before and after the test. Positive HIV and hepatitis test results must be report to the local health authorities.

Voluntary Participation and Withdrawal

Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in the study. If you decide not to be in the study, you will not lose any benefits you are otherwise owed. You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your relationship with this institution. However, you should return to see the study doctor for safety reasons so you can be taken off the study drug and referred for follow-up care.

If you end the study early, you will be asked to have blood tests and the same physical and nervous system exams you previously had. It is also possible your doctor will request a MRI at this time.

It is also possible that your doctor may withdraw you from the study without your consent if he/she thinks this is in your best interest, or if you are not following the procedures required by the study. If you withdraw after the catheter is placed for the first injection of M032, the catheter will be removed and you will receive routine follow-up care. Your doctor will discuss any alternatives with you in these cases.

New Findings

Any significant new findings that develop during the course of the study, which may affect your willingness to continue in the research, will be provided to you by Dr. James M. Markert or his staff.

Cost of Participation

There will be no cost to you for the tests and procedures associated with this research study. All study related medications, examinations and medical treatment will be provided at no cost during the twelve-month study period. This includes charges for the surgical placement of the catheters, injections of M032, chest x-ray, EKG, MRIs, CT scan, all clinic visits which are part of the study, and research tests performed on blood, saliva, conjunctival secretions and tumor tissue samples.

You and/or your insurance company **will be billed for standard of care charges** which include the costs of the surgical biopsy of your tumor, your NICU one night stay and all costs associated with these.

If you are eligible for Medicare or other health care reimbursement, certain costs (referred to as "routine costs") may be billed to Medicare or your health care (insurance) provider if they are not paid for by the sponsor. "Routine costs" include the charges for the medical care and items that you would receive even if you were not in this study. If the routine costs are billed to Medicare or your provider, it will be noted on the claim that the services were provided in the course of your participation in a clinical trial.

If you are in Medicare Advantage (Medicare managed care plan), you should contact someone at your plan before you start a clinical trial. They can provide more information about additional costs you could incur from participating in clinical trials.

Payment for Participation in Research

No compensation is available for taking part in this research study.

Payment for Research-Related Injuries

The University of Alabama at Birmingham (UAB) has not made any provision for monetary compensation in the event of injury resulting from this research study. In the event of injury, treatment will be provided, but is not provided free of charge. These costs will be billed to you or your insurance company in the usual manner.

Questions

You are invited to ask as many questions regarding this research project as you feel are necessary. If you have any questions about the research or a research related injury, Dr. Markert or Dr. Nabors will be glad to answer them.

Dr. Markert may be reached by calling 205-934-3411 (pager # 5562).
Dr. Markert's mailing address is:

University of Alabama at Birmingham
510 20th Street South, 1046 FOT
Birmingham, AL 35294-3410

Dr. Nabors may also be reached by calling 205-934-3411(pager # 7394).
Dr. Nabors' mailing address is:

University of Alabama at Birmingham
510 20th Street South, 1020 FOT
Birmingham, AL 35294-3410

If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the UAB Office of the IRB, (OIRB) at (205) 934-3789 or toll free at 1-855-860-3789. Regular hours for the OIRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday. You may also call this number in the event the research staff cannot be reached or you wish to talk to someone else.

Legal Rights

You are not waiving any of your legal rights by signing this consent form.

Storage of Specimens for future use

Since the purpose of this research is to evaluate the potential for the development of M032 for potential use in participants with your diagnosis (malignant glioma), it is possible that a commercial product may result from this research study. Blood and any tissue samples obtained from you as part of this research study could be part of the development of such a product. There are no plans to share any profits with you or your heirs that may result from such a commercial product.

Remnant (what is left over after clinical tests completed) blood and brain tumor tissue samples collected during this study may be stored for future use. Specimens will be identified by your subject #, date of birth, and initials. The use of these specimens will be restricted to support assessment of M032 and treatments for glioma. No commercial development of assays or cell-lines will be undertaken with these specimens. If at any time, you withdraw your consent from participation in this study, the samples will be destroyed.

Initial your choice below:

_____ I agree to allow my samples (blood and brain tumor tissue) to be kept and used for future research on brain tumors.

_____ I **do not** agree to allow my samples (blood and brain tumor tissue) to be kept and used for future research.

SIGNATURES

Your signature below indicates that you agree to participate in this study. You will receive a copy of this informed consent.

I understand that I will be enrolled in this Phase I Clinical Trial of M032 injected into my tumor which requires one surgical procedure and approximately 4 days of hospitalization.

_____

Signature of Participant Date

_____

Signature of Person Obtaining Consent Date

Reviewed by:

_____

Signature of Principal Investigator Reviewing Consent Document Date

University of Alabama at Birmingham

AUTHORIZATION FOR USE/DISCLOSURE OF PROTECTED HEALTH INFORMATION (PHI) FOR RESEARCH

Participant Name: _____

Research Protocol: A Phase 1 Study of a Genetically Engineered HSV-1 Expressing IL-12, in Patients with Recurrent/Progressive Glioblastoma Multiforme, Anaplastic Astrocytoma, or Gliosarcoma

UAB IRB Protocol Number: IRB-131004002

Principal Investigator: James M. Markert, MD

Sponsor: **NCI Grant**

What is the purpose of this form? You are being asked to sign this form so that UAB may use and release your protected health information for research. Participation in research is voluntary. If you choose to participate in the research, you must sign this form so that your protected health information may be used for the research.

Why do the researchers want my protected health information? The researchers want to use your protected health information as part of the research protocol listed above and as described to you in the informed consent.

What protected health information do the researchers want to use? All medical information, including but not limited to information and/or records of any diagnosis or treatment of disease or condition, which may include sexually transmitted diseases (e.g., HIV, etc.) or communicable diseases, drug/alcohol dependency, etc.; all personal identifiers, including but not limited to your name, social security number, medical record number, date of birth, dates of service, etc.; any past, present, and future history, examinations, laboratory results, imaging studies and reports and treatments of whatever kind, including but not limited to drug/alcohol treatment, psychiatric/psychological treatment; financial/billing information, including but not limited to copies of your medical bills, and any other information related to or collected for use in the research protocol, regardless of whether the information was collected for research or non-research (e.g., treatment) purposes.

Who will disclose, use and/or receive my protected health information? All Individuals/entities listed in the informed consent documents, including but not limited to, the physicians, nurses and staff and others performing services related to the research (whether at UAB or elsewhere); other operating units of UAB, HSF, UAB Highlands, Children's of Alabama, Eye Foundation Hospital, and the Jefferson County Department of Health, as necessary for their operations; the IRB and its staff; the sponsor of the research and its employees and agents, including any CRO; and any outside regulatory agencies, such as the Food and Drug Administration, providing oversight or performing other legal and/or regulatory functions for which access to participant information is required; including The Translational Genomics Research Institute (TGen).

How will my protected health information be protected once it is given to others? Your protected health information that is given to the study sponsor will remain private to the extent possible, even though the study sponsor is not required to follow the federal privacy laws. However, once your information is given to other organizations that are not required to follow federal privacy laws, we cannot assure that the information will remain protected.

How long will this Authorization last? Your authorization for the uses and disclosures described in this Authorization does not have an expiration date.

Can I cancel this Authorization? You may cancel this Authorization at any time by notifying the Principal Investigator, in writing, referencing the research protocol and IRB Protocol Number. If you cancel this Authorization, the study doctor and staff will not use any new health information for research. However, researchers may continue to use the protected health information that was provided before you cancelled your authorization.

Can I see my protected health information? You have a right to request to see your protected health information. However, to ensure the scientific integrity of the research, you will not be able to review the research information until after the research protocol has been completed.

Signature of participant: _____ Date: _____

or participant's legally authorized representative: _____ Date: _____

Printed Name of participant's representative: _____

Relationship to the participant: _____