NU Study Number: NU16C04

PROTOCOL TITLE: A Phase I Study Of Neural Stem Cell Based Virotherapy In Combination With Standard Radiation And Chemotherapy For Malignant Glioma

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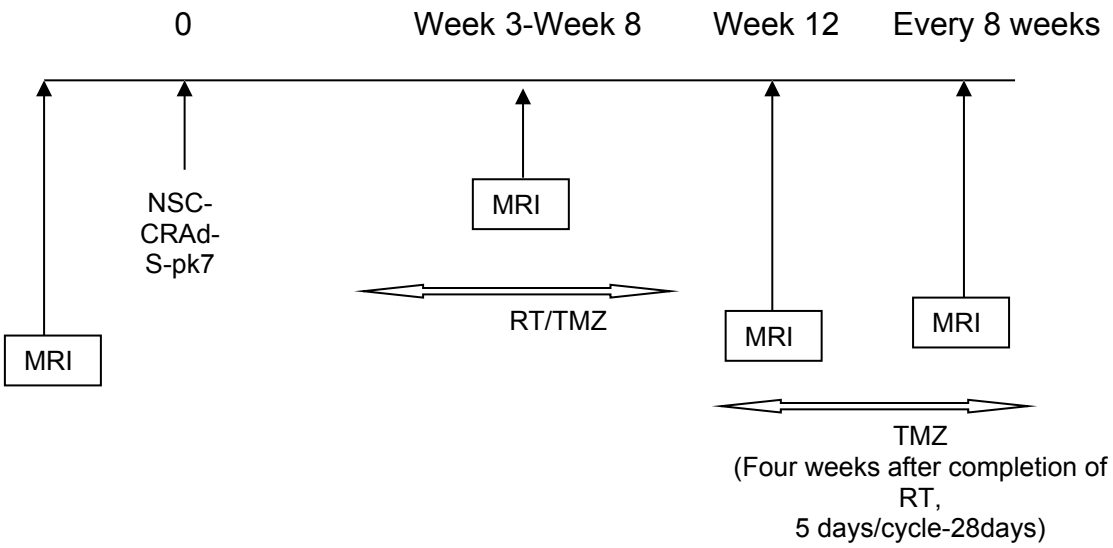
TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse Event
AVO	Acidic Vesicular Organelles
CD	Cytosine Deaminase
CE	Carboxylesterase
CR	Complete Response
CRAd	Conditionally replicative Adenovirus
CRAd-S-pk7	Crad-Survivin-pk7
DLT	Dose Limiting Toxicity
DMC	Data Monitoring Committee
FDA	Food and Drug Administration
GBM	Glioblastoma
GFAP	Glial fibrillary acidic protein
GTR	Gross total resection
hTR	Human telomerase RNA
hTERT	Human telomerase reverse transcriptase
ICP	Increased intracranial pressure
KPS	Karnofsky Performance Status
LC3	Light-chain 3
MR	Minor Response
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stem Cell
MTD	Maximum Tolerated Dose
NSC	Neural Stem Cell
OS	Overall Survival
OV	Oncolytic Virus
P	Progression
PFS	Progression Free Survival
PFU	Plaque Forming Units
PR	Partial Remission
PRb	Retinoblastoma protein
RT	Radiotherapy
TMZ	Temozolomide
TRAIL	Tumor necrosis factor–related apoptosis-inducing ligand
TSP	Tumor Specific Promoters
VP	Viral Particle

STUDY SCHEMA



Sample Schedule

Week 1	
Day 0	Intratumoral injection of NSC-CRAd-S-pk7
Days 6-7 (± 3 days)	Simulation for RT
Week 2-7	
Days 1-7 (±3 days)	RT (Days 1-5 only)
	TMZ (75 mg/m^2)
Week 12	
5 Days on/23 Days off	TMZ (150-200 mg/m^2)

STUDY SUMMARY

Protocol Title:
A Phase I study of neural stem cell based virotherapy in combination with standard radiation and chemotherapy for patients with malignant glioma
Brief Protocol Title for the Lay Public (if applicable):

Viral stem cell based therapy for malignant glioma
Study Phase:
Phase I
Participating Sites:
Northwestern University
Rationale for this Study:
Malignant gliomas have a very poor prognosis with median survival measured in months rather than years. It is a disease in great need of novel therapeutic approaches. Based on the encouraging results of our preclinical studies described below, which demonstrate improved efficacy without added toxicity, the paradigm of NSC-CRAd-S-pk7 in combination with radiation and chemotherapy is well-suited for evaluation in malignant gliomas. The standard-of-care allows application of virotherapy as neoadjuvant therapy and assessment of the cooperative effects with radiation/chemotherapy without altering the standard treatment.
Objectives:
The primary objectives are to evaluate the safety of the combined therapy and determine the dose-limiting toxicity and maximum tolerated dose (MTD) for a future Phase II study. Secondary objectives are assessment of tumor response, overall survival, progression-free survival, overall survival and quality of life. An exploratory objective will be to evaluate cytokine profile and blood immune response to NSC-CRAd-S-pk7 and to determine whether survival rate correlates with extent of immune response.
Study Design:
Open label, Phase I, dose-escalation gene therapy clinical trial with two arms: **Arm A** – Unresectable disease, receive stereotactic intratumoral injection of NSC-CRAd-S-pk7 immediately following stereotactic biopsy confirmation of clinical diagnosis **Arm B** – Resectable or partially resectable, receive injection of NSC-CRAd-S-pk7 into remaining tumor or tumor bed after resection. Dose escalation will be performed independently for each arm and 3-6 patients will be enrolled per dose in each arm. Standard radiotherapy and chemotherapy will begin after NSC-CRAd-S-pk7 injection.
Endpoints:
The primary endpoint of this dose-escalation study is to determine the maximum tolerated dose (MTD) of NSC-CRAd-S-pk7 when administered with standard radiation and chemotherapy in patients with malignant glioma. The secondary endpoints include objective tumor response, overall survival, progression-free survival and quality of life. The exploratory endpoints include cytokine profile and blood immune response and their correlation with overall survival. In addition, RCR testing will be performed to determine long-term gene expression, and if present, correlation with overall survival.
Sample Size:

If all three dose levels are administered, the minimum number of patients enrolled will be 9 per arm and the maximum number of patients will be 18 per arm.

Estimated Duration of the Study

Based on projections from current clinical practice, it is anticipated that the accrual will take 12-18 months to complete.

Summary of Subject Eligibility Criteria:

Inclusion Criteria

- Patients must have presumed malignant glioma based on clinical and radiologic evaluation (pathologic confirmation of malignant glioma must be made at the time of stereotactic biopsy or resection prior to NSC-CRAd-S-pk7 injection; if this is not possible, the injection will not be performed and the subject will no longer be eligible for the study).
- Tumor must be accessible for injection and must not be located in the brainstem, or contained within the ventricular system.
- Planning to undergo standard radiation/chemotherapy
- 18 years of age or older.
- Performance status must be KPS ≥ 70
- SGOT (AST) < 3x upper limit of normal
- Serum creatinine < 2mg/dl
- Platelets > 100,000/mm^3 and WBC > 3000/mm^3

Exclusion Criteria

- Prior or ongoing liver disease including known cirrhosis, hepatitis B or C infection but not to exclude patients with a distant history of resolved hepatitis A infection.
- Immunosuppressive drugs (with exception of corticosteroid).
- Known HIV+ patients.
- Acute infections (viral, bacterial or fungal infections requiring therapy).
- Pregnant or breast-feeding patients.
- Evidence of metastatic disease or other malignancy (except squamous or basal cell skin cancers).
- Prior radiation therapy to the brain or prior treatment for brain tumor
- Other serious co-morbid illness or compromised organ function.

Investigational Product Dosage and Administration:

Table 1 – Dose Escalation Schema

Cohort	Number NSCs Injected	Total Viral Dose Viral particles (vp)/patient
1	50 million ($5x10^7$)	6.25×10^{10}
2	100 million ($1x10^8$)	1.25×10^{11}
3	150 million ($1.5x10^8$)	1.875×10^{11}

Clinical Observations and Tests to be Performed:

- On day 0, stereotactic biopsy or resection of the tumor
- History and physical will be performed weekly, 2-4 weeks after completion of radiation and at least every 3 months for the first year.
- Quality of life questionnaire (FACT-Br version 4) once during week 2-3, 2-4 weeks after completion of radiation and every 3 months for the first year or until disease progression.
- CBC, platelets, creatinine, AST, ALT, and bilirubin will be monitored bi-weekly during administration (weeks 2, 4, and 6).
- Blood for immune research studies* (10 to 40 cc) may be collected in week 2-3 after the NSC-CRAd injection and 2-4 weeks after completion of radiation therapy
- MRI of the brain approximately 28 days after study product injection, 2-4 weeks after completion of radiation, and then every 2-3 months for the first year or until disease progression.
- If surgery is performed, tumor samples will be examined in conjunction with the pathologist and samples retained for future studies if consent given by the patient.
- Evaluation once a year for 5 years. Evaluations will include: Physical and neurological examination, MRI, and CBC with differential count.

Statistical Considerations:

Statistical analysis will be descriptive only, with no hypothesis testing. All the endpoint data will be collected and described in summary tables for both two arms at each dose. In general, descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be calculated for categorical and ordinal variables at each category. All study data will be presented in by-subject data listings. Statistical analyses will be performed using SAS9.4 (SAS Institute Inc., Cary, NC).

The maximum tolerated dose (MTD) will be determined according to the dose escalation algorithm. If >2 patients do not complete the initial RT/TMZ phase or rule if > 2 patients experience life threatening or fatal neurologic toxicity, the trial will be stopped to analyze, in conjunction with the DMC, whether this regimen is tolerable.

Demographic (age, sex, height and weight) and baseline disease characteristics (including baseline KPS), will be summarized for both arms as well as the entire group. Kaplan-Meier curves and median survival estimates with confidence limits will be given for both overall survival and progression-free survival. The association of extent of immune response with overall survival will be determined by Cox's proportional hazards model.

Safety parameters will be analyzed descriptively, and presented in standard frequency tables (adverse events), shift tables (Laboratory parameters) and summaries of change from baseline over time (vital signs, physical examination, ECG and laboratory parameters).

Sponsor/Licensee:

Northwestern University/Maciej S. Lesniak, MD

1.0 INTRODUCTION – BACKGROUND & RATIONALE

1.1 Disease Background

Brain tumors are a relatively common malignancy for which standard treatments do not provide satisfactory median or long-term results. There are an estimated 17,000 new cases and 13,100 deaths from primary nervous system tumors in 2007, the majority of which are malignant gliomas (American Cancer Society). Slower growing, less aggressive gliomas are referred to as low grade or benign, whereas higher grade, malignant gliomas include malignant astrocytomas (36% of gliomas) and glioblastoma multiforme (GBM; 47% of gliomas and 21.7% of all primary brain tumors) (Levin et al, 2001).Therapy, including surgery, radiation and chemotherapy, is essentially palliative. Although neoplasms located within the frontal, temporal or occipital poles of the brain are amenable to “gross total resection” (GTR), these operations provide little control to the microscopic foci of tumor outside the main mass. Many malignant gliomas located within the corpus callosum, basal ganglia, brainstem and areas of eloquent cortex (speech, motor or visual

areas) are not resectable. For both GTR and non-resectable masses, radiation therapy is the major therapy provided after diagnosis. Its efficacy is limited by the low radiation sensitivity of tumor cells and the inclusion of normal brain tissue within radiation fields (Levin et al, 2001). With conventional radiation therapy, the median survival time for patients with anaplastic astrocytoma is 36 months, and the 3-year survival rate is approximately 55%. (Prados et al, 1999). In contrast, the median survival time for patients with GBM is 10 months and the 3-year survival rate is only 6% (Leibel et al, 1994).

Temozolomide (TMZ), an oral alkylating agent, was approved for refractory anaplastic astrocytoma in the United States and also for GBM in Europe. The drug has been shown to be modestly active as single agent in glioblastoma and moderately active against anaplastic astrocytoma (Yung et al, 1999). Up front therapy of malignant gliomas with TMZ concomitant with radiation followed by adjuvant TMZ was shown to be well tolerated with the most significant toxicity being grade 3-4 neutropenia and thrombocytopenia in less than 10% of patients (Stupp et al, 2002). In the last decade, TMZ was approved for use with radiation based on results of a randomized Phase III trial comparing this regimen to radiation alone which demonstrated a significant improvement in median survival from 12 to 15 months (Stupp et al, 2005).

1.2 Intervention Background & Overview

Malignant gliomas have a very poor prognosis with median survival measured in months rather than years. It is a disease in great need of novel therapeutic approaches. Based on the encouraging results of our preclinical studies described in the sections that follow, which demonstrate improved efficacy without added toxicity, the paradigm of NSC-CRAd-S-pk7 in combination with radiation and chemotherapy is well-suited for evaluation in malignant gliomas. The standard-of-care allows application of virotherapy as neoadjuvant therapy and assessment of the cooperative effects with radiation/chemotherapy without altering the standard treatment.

1.3 Rationale for the Current Study

1.3.1 Conditionally Replicative Adenoviruses (CRAds)

CRAds replicate under certain conditions that are usually found in cancer cells and absent in normal healthy cells. These viruses may:

1. Have inessential regions of their genomes deleted
2. Transduce only cancer cells and spare normal cells because of the over-expression of receptors on the cell surface of gliomas
3. Have a tumor specific promoter driving an essential gene in the Ad genome (for example E1A early promoter) (Lamfers, 2002; Heise, 1997; Fueyo, 2000; Suzuki, 2001; Miller, 1998; van Beusechem, 2002; Kohno, 2004, Parr, 1997; Shinoura, 2000; Vandier, 2000).

The human adenovirus E1B protein interacts with the tumor suppressor p53 and blocks its transcriptional activity. Deletion of this region in Ad 5 genome prevents this interaction and this principle has been used to create a CRAd that replicates in glioma cells that lack p53 protein. Deletion of this 55kDa protein in Ad 5 led to the creation of the adenovirus dl1520, also known as ONYX 015 or CI-1042. This virus replicated efficiently in gliomas that are attenuated for p53 but not in p53 wild-type tumors or normal human cells that have normal levels of p53 (McCormick, 2000). Initial studies with ONYX 015 showed significant tumor cell killing and reduction in tumor mass in preclinical experiments both *in vivo* and *in vitro* (Heise, 1999). In clinical studies, ONYX has been used in a Phase I clinical trial (Chiocca, 2004). Safety and efficacy being the objective of this study, they were met satisfactorily in all 24 patients enrolled, 17 of which were grade IV glioma (GBM) patients. The virus was well tolerated even at its highest dose of 10^{10} plaque forming units (PFU). Ten out of 24 patients had some adverse effects but were eventually determined to be not related to the virus treatment. However, only about 50% gliomas are p53 negative, making this virus ineffective in the remaining 50% that are p53 positive, reducing the scope of its application.

Another more successful conditionally replicative approach was that undertaken by Fueyo and collaborators (Fueyo, 2000). In this approach, a 24-bp deletion in the Ad 5 E1A CR2 domain

inhibited the binding of E1A protein to the Retinoblastoma protein (pRb). Since the pRb protein negatively regulates cell growth by releasing E2F, a failure to release this inhibition therefore stops cell growth. This E1A mutant Ad, known as Ad5-Δ24 replicates only in tumor cells and not in normal brain cells. Both *in vitro* and *in vivo* studies with this tumor have shown potent cytolytic activities, particularly *in vivo* where a single low dose local injection inhibited tumor grown in a mouse graft model of glioma. Building up on the success of these preclinical studies, a modified virus Ad5-Δ24RGD was created that has transductional targeting. Addition of this modification increased the cytotoxic effect of this virus on glioma cell lines and *in vivo* experiments showed 9 out of 10 animals showing complete tumor regression (Lamfers, 2002). A still further modification of this virus was the ICOVIR series: ICOVIR-2 and the later generation ICOVIR-5. ICOVIR-5 has, in addition to the modifications in Ad5-Δ24RGD, an E2F responsive element in lieu of the E1A promoter that further confines the replication of this Ad in tumor cells (Alonso, 2007). *In vitro*, ICOVIR-5 showed an increased anti-glioma effect and increased replication in glioma cell lines; however *in vivo*, there was no significant increase over the second generation Ad5-Δ24RGD virus in controlling tumor growth and long term survival was lower for ICOVIR-5 than Ad5-Δ24RGD (46.5d vs. 71.0d) in a mouse xenograft model of glioma.

Brain tumor specific promoters (TSP), like nestin and glial fibrillary acidic protein (GFAP) have been previously used in non-replicating adenoviruses to test the hypothesis of tumor specificity and glioma targeting. These TSP can be used to drive the E1A gene of Ad 5, which would then replicate selectively in glioma cells only where the levels of these proteins are high and not in normal brain tissues. The midkine promoter is one such protein over-expressed in malignant gliomas and a CRAd driven by this promoter showed strong virolytic effects in glioma cells but not in midkine negative normal brain cells. In animal experiments, Ad-MK ablated tumor xenografts in midkine expressing cells (Kohono, 2004). The loss of pRb in tumor cells potentially leads to an excess of free E2F in tumor cells. This hypothesis was elegantly tested by Parr et al., in a rat glioma model. Using a βgal reporter system, an Ad.E2F1.βgal adenoviral vector specifically targeted tumor cells in an *in vivo* model while sparing normal brain tissues when compared to a similar construct driven by the CMV promoter or wild type Ad 5. The human telomerase RNA (hTR) and the human telomerase reverse transcriptase (hTERT) is active in a vast majority of cancer cells including gliomas; however, their use in CRAds for glioma specific treatment has been proposed but not yet achieved.

Survivin is also one such tumor specific promoter that has recently come into prominence (Das, 2002; Kajiwara, 2003; Kelinschmidt-Demasters, 2003; Ulasov, 2007). It has been used in *in vitro* and *in vivo* studies in glioma models and has shown promise in specificity in targeting gliomas (Zhu, 2005; Van Houdt, 2006; Ulasov, 2007). Specifically a CRAd with its E1A driven by the survivin promoter and its knob modified with a pK7 moiety (CRAd-S-pK7) has been shown to have increased targeting of glioma cell lines and cause smaller tumors in an *in vivo* mouse xenograft model (Ulasov, 2007; Nandi, 2008).

1.3.2 CRAd-Survivin-pk7

Our group has created the oncolytic vector CRAd-Survivin-pk7 (CRAd-S-pk7) for the treatment of malignant gliomas. CRAd-S-pk7 is a chimeric vector containing a pk7 fiber modification and a survivin promoter driving E1A replication. CRAd-S-pk7 vector effectively replicates in many glioma cell lines like U-87MG, U-251MG, A172, Kings-1, No. 10 and U-118MG and leads to tumor oncolysis in these cell lines with minimal viral replication and toxicity in normal human brain. Intratumoral injection of CRAd-S-pk7 in U-87MG glioma xenograft inhibited tumor growth by more than 300% with 67% of the mice surviving long term (>120 days). Analysis of xenograft tumor tissue indicated increased adenoviral infectivity, decreased mitotic activity, and enhanced tumor apoptosis (Ulasov, 2007). These results suggest that a combination of transductional approach (pk7 modification) and a transcriptional approach (incorporation of the survivin promoter) results in both enhanced replication and enhanced cell lysis; thus revealing a promising potential vector for clinical use in glioma gene therapy.

1.3.3 Neural Stem Cells (NSCs)

While the use of various adenoviral vectors has proven safe for intracranial injection in several preclinical trials, a significant therapeutic effect is yet to be seen in patients. The reason behind this shortcoming is largely a result of the distribution limitations involved with local delivery of virolytic agents by themselves. Due to the complex biological nature of high grade gliomas, local injection of adenoviral vectors fails to reach scattered infiltrative tumor cells within the brain parenchyma (Chiocca*.,* 2004) The therapeutic effect of intracerebral injections of CRAds is only seen in the vicinity of the injection site, however, a broader vector distribution is necessary to impact all tumor cells. To remedy this and prevent possible tumor recurrence, stem cells have been explored as vehicles for gene therapy in brain tumors given that they possess an intrinsic tropism for pathologies [Aboody *et al.,* 2000; Aboody *et al.,* 2008].

The initiative behind exploiting stem cells as delivery vehicles for CRAds is based on the previous success in delivering other cancer toxic agents and transgenes to tumors in the brain. Aboody *et al*. (2008) showed that NSCs can selectively deliver therapeutic genes to intracranial tumor sites including prodrug activating enzymes [cytosine deaminase (CD), carboxylesterase (CE), thymidine kinase], interleukins (IL-2, IL-4, IL-12, IL-23), interferon-β, apoptosis-promoting genes (tumor necrosis factor-related apoptosis-inducing ligand) and metalloproteinases (PEX). The well-established delivery potential of stem cells for gene therapy in the brain has prompted researchers to investigate their application in virotherapy as well.

Given their ability to localize to tumor sites, stem cell mediated CRAd delivery offers a more specific and thorough therapeutic effect than local delivery of CRAds alone. A benefit to using a cell-based delivery approach of an adenovirus is that a stem cell is capable of responding to diverse pathological signals released by tumor tissue (Zhenggang*,* 2004; Khalid*,* 2005; Aboody*,* 2008). Stem cell specificity and tropism is likely mediated by several cell surface receptors as well as secreted cytokines and growth factors. Additionally, extracellular matrix proteins have been also been associated with the tumor-tropism of stem cells (Aboody*,* 2008). While the exact mechanism of their tumor affinity has yet to be fully elucidated, NSCs and mesenchymal stem cells (MSCs) are currently being examined as viable vehicles for targeting and delivering CRAds to disseminated tumor cells.

Aside from the ethical and technical difficulties associated with isolating NSCs, their endogeneity to the CNS renders them a vastly explored vehicle for vector delivery in the brain. Aboody (2008) revealed that NSCs have the ability to invade tumor foci and track single insidious tumor cells infiltrating into normal tissue away from the primary tumor mass. Experiments evaluating the delivery potential of NSCs revealed that these cells possess an inherent tropism and unique capacity to target invading glioma stem cells *in vitro* as well as *in vivo* (Zhenggang*.,* 2004; Khalid*,* 2005; Tyler*,* 2008).

In terms of delivering an oncolytic adenovirus, Tyler et al. (2008) demonstrated that loading NSCs with CRAds does not significantly compromise their homing abilities. NSC permissiveness for Ad-vectors is due to the fact that NSCs express many Ad- target surface receptors including: avb3 and avb5 integrins, adhesion proteins targeted by vectors possessing RGD motifs, CAR, CD46, and syndecan and perlecan, two heparan sulfate proteoglycans that bind to vectors containing a poly-L-lysine (pk7) modification. A luciferase assay analyzing the transduction of NSCs with various recombinant Ad vectors revealed that the pk-7 modified vector, AdWT-pk7, showed the greatest transduction capacity followed by the AdWT.

In addition to providing a carrier function, it is also imperative that NSCs allow for adequate CRAd genome amplification to achieve optimal infectivity and sustained tumor toxicity upon reaching distant glioma cells. Tyler et al. (2008) proposed that tumor specific promoters (TSP), which control transcription and translation of the viral E1A replication gene, are vital to this process. Qualitative RT-PCR revealed that two tumor specific promoters, survivin and chemokine receptor

CXCR4, allow for robust transcriptional activity in most glioma cell lines, while exhibiting relatively modest activity in normal cell lines. As such, the authors compared CRAd-S-pk7 and CRAd-CXCR4-5/3, two oncolytic vectors possessing these promoters, by evaluating their activity in NSC mediated delivery to gliomas. The results indicate relatively attenuated replicative cytotoxicity in NSCs, but sufficient replicative cytotoxicity in U87MG tumor cells. In particular, CRAd-S-pk7 displayed limited toxicity to the NSC carrier, superb levels of NSC transduction, potent cytotoxicity to glioma cells, and could be delivered to U87MG cells *in vitro.*

When comparing the effectiveness of delivering NSC loaded with CRAd-S-pk7 versus CRAd-S-pk7 alone, results show that vector distribution to distant tumor cells is drastically enhanced when mediated by a stem cell carrier. qPCR of laser-captured brain tissue sections from mice receiving injections of NCS-loaded-CRAd-S-pk7 showed greater E1A gene distribution than those of mice injected with CRAd-S-pk7 alone (Tyler*,* 2008). In addition, an *in vivo* efficacy study investigating the ability of NCS-loaded-CRAd-S-pk7 to reduce U87 tumor growth in athymic mice revealed an overall reduction in tumor volume when compared to mice receiving intratumoral injections of CRAd-S-pk7 alone.

Most recently, our group has shown that NSCs loaded with CRAd-Survivin-pk7 effectively migrate and deliver a therapeutic payload to malignant gliomas (Ahmed, 2011). First, we determined the optimal loading/infecting dose that would result in maximal production of therapeutic virus by NSCs. The maximum amount of viral progeny released from the carrier cells was attained after loading with the dose of 50 IU per NSC. Next, we evaluated the therapeutic potential of oncolytic virus loaded NSCs in an orthotopic glioma model. Five days following tumor implantation, animals were randomly divided into separate groups and treated with CRAd loaded NSCs (2.5×10^7 IU total/mouse = loading dose 50 IU/cell of oncolytic virus loaded into 5×10^5 of carrier cells), an equal dose of CRAd-S-pk7 alone (2.5×10^7 IU total/mice), or control. As a control, we injected equal volumes of PBS and the same number of NSCs without the therapeutic virus intratumorally. Mice treated with NSCs alone displayed similar survival to the PBS treated control group. About 50% of the animals from the group treated with NSCs loaded with CRAd-S-pk7 virus (50 IU/cell loading dose) survived beyond 100 days, whereas none of the animals in the group treated with CRAd-S-pk7 virus alone survived beyond 75 days. We also observed a significant improvement in median survival from 63 days to 93 days (~50%) between these two groups (p=0.0007). These results indicate that CRAd-S-pk7-loaded NSCs effectively deliver oncolytic adenovirus to malignant glioma *in vivo,* resulting in a significant inhibition of tumor growth with a simultaneous increase in the median survival of treated animals as compared to CRAd-S-pk7 treatment alone.

Finally, to confirm the feasibility of our approach for human applications, we used an NSC line, HB1.F3.CD, which has been accepted for use in clinical trials (BB-IND 14041, BB-IND 16265) by the US Food and Drug Administration (FDA) (Ahmed, 2013). HB1.F3.CD cells were loaded with an oncolytic adenovirus, CRAd-Survivin-pk7, and mice bearing various human-derived GBMs were assessed with regard to NSC migration, viral replication, and therapeutic efficacy. Antiglioma activity of oncolytic virus (OV)-loaded HB1.F3.CD cells was effective against clinically relevant human-derived glioma models as well as a glioma stem cell-enriched xenograft model. Median survival was prolonged by 34% to 50% compared with mice treated with OV alone (GBM43FL model median survival = 19.5 days, OV alone vs NSC + OV, hazard ratio of survival = 2.26, 95% confidence interval [CI] = 1.21 to 12.23, P = .02; GBM12 model median survival = 43.5 days, OV alone vs NSC + OV, hazard ratio of survival = 2.53, 95% CI = 1.21 to 10.38, P = .02). OV-loaded HB1.F3.CD cells were shown to effectively migrate to the contralateral hemisphere and hand off the therapeutic payload of OV to targeted glioma cells. *In vivo* distribution and migratory kinetics of the OV-loaded HB1.F3.CD cells were successfully monitored in real time by magnetic resonance imaging (MRI). OV-loaded NSCs retained their differentiation fate and were non-tumorigenic *in vivo*. In conclusion, HB1.F3.CD NSCs loaded with CRAd-Survivin-pk7 overcome major limitations of OV *in vivo* and warrant translation in a phase I human clinical trial for patients with malignant glioma.

1.3.4 Preclinical Studies of CRAd-Survivin-pk7 in Combination with Radiation and Chemotherapy

In a recent study, Nandi et al., showed that survivin is a radiation inducible promoter and when exposed to 2Gy of radiation, increases transgene expression by 10-fold in glioma cell lines (Nandi, 2008). Radiation therapy has also shown to increase CD133+ cells in glioma cell cultures implicating their increased proliferative capacity following radiation. Upon infection of CD133+ cells with CRAd-S-pk7 from glioma cell lines and primary tumor samples, the authors found significantly higher toxicity in cells and tumor tissue infected by the vector compared to wild type virus. Addition of 2Gy of radiation was shown to further enhance this virolytic effect of CRAd-S-pk7 with increased viral replication in virus infected radiated cells. *In vivo*, tumors formed from CD133+ enriched cells treated with CRAd-S-pk7 showed most significant tumor growth inhibition compared to untreated group. Of note, the combination of CRAd-S-pk7 treatment with radiation therapy led to a 100-fold increase in viral replication compared to CRAd-S-pk7 treatment alone. This great synergistic effect seen in combination treatment of CRAd-S-pk7 with radiation is also evident when it is combined with chemotherapeutic agent TMZ. TMZ is known to induce glioma cell death via autophagy. Ulasov et al., have shown that combination of adenoviral virotherapy and TMZ chemotherapy eradicates malignant glioma via autophagic and apoptotic cell death *in vivo* (Ulasov, 2009). This combination therapy revealed an increased expression of pro-apoptotic proteins p53 and BAX, and a decreased expression of anti-apoptotic protein BCL-2 implicating apoptosis as one of the cytotoxic mechanisms of combination therapy. At the same time treatment with TMZ followed by CRAd-S-pk7 led to a significant increase of acidic vesicular organelles (AVO), characteristic feature of autophagy, and microtubule-associated protein 1 light-chain 3 (LC3), a protein playing crucial role in autophagosomes formation, in comparison to TMZ or CRAd-S-pk7 treatment alone, suggesting enhanced induction of autophagy in combination therapy. I*n vivo* evaluation in mice with U87MG intracranial glioma xenografts with combination therapy revealed that the effect of combination therapy is additive, and 90% of all mice survived long term. Immunohistochemical analysis of the tumor treated with combination therapy showed an increase in the expression of both LC3 and cleaved Caspase-3 over time, proving that both autophagy and apoptosis are responsible for cell death *in vivo*.

Finally, we examined the timing of NSC based virotherapy with regard to optimal therapeutic efficacy when combined with radiation and chemotherapy (Tobias, 2013). We showed that CRAd-S-pk7-loaded HB1.F3-CD cells retain their tumor-tropic properties and capacity to function as in situ viral manufacturers in the presence of ionizing radiation (XRT) and TMZ. Furthermore, we established a logical experimental model that aims to recapitulate the complex clinical scenario for the treatment of GBM and tests the compatibility of NSCs loaded with OV. We reported that applying OV-loaded NSCs together with XRT and TMZ can increase the median survival of glioma bearing mice by approximately 46%. Most importantly, the timing and order of therapeutic implementation impact therapeutic outcome. When OV-loaded NSCs are delivered prior to rather than after XRT and TMZ treatment, the median survival of mice bearing patient-derived GBM43 glioma xenografts was extended by 30%. Together, data from this report support the testing of CRAd-S-pk7-loaded HB1.F3-CD cells in the newly diagnosed clinical setting and argue in favor of a multimodality approach for the treatment of patients with malignant glioma.

1.4 Exploratory Studies

An exploratory objective will be to evaluate cytokine profile and blood immune response to NSC-CRAd-S-pk7 and to determine whether survival rate correlates with extent of immune response.

2.0 OBJECTIVES & ENDPOINTS

2.1 Primary Objective & Endpoint

The primary objectives are to evaluate the safety of the combined therapy and determine the dose limiting toxicity and maximum tolerated dose (MTD) for a future Phase II study.

2.2 Secondary Objectives & Endpoints

Secondary objectives are assessment of tumor response, progression-free survival (PFS), overall survival (OS), and quality of life for patients undergoing NSC-CRAd-S-pk7 injection.

2.3 Exploratory Objectives & Endpoints

An exploratory objective will be to evaluate cytokine profile and blood immune response to NSC-CRAd-S-pk7 and to determine whether survival rate correlates with extent of immune response.

3.0 PATIENT ELIGIBILITY

The target population for this study is patients with newly diagnosed malignant glioma. This will be a single-center trial conducted at Northwestern Memorial Hospital and Northwestern Medicine Neurological Surgery clinic of Northwestern University.

Up to 36 subjects will be included in this trial. Approximately 6 potentially eligible patients are seen per month, and it is anticipated that at least 1 per month will be accrued. Potential patients are under the direct care of the Principal Investigator (PI) at Northwestern University, Dr. Maciej Lesniak at (312) 695-6200.

Eligibility will be evaluated by the study team according to the following criteria. Eligibility waivers are not permitted. Subjects must meet all of the inclusion and none of the exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered. Please refer to Section 11 for complete instructions regarding registration procedures.

3.1 Inclusion Criteria

3.1.1 Patients must have a presumed newly identified malignant glioma based on clinical and radiologic evaluation. Pathologic confirmation of malignant glioma must be made at the time of stereotactic biopsy or resection on frozen section by a neuropathologist prior to NSC-CRAd-S-pk7 injection; if this is not possible, the injection will not be performed and the subject will no longer be eligible for the study.

3.1.2 Patients must be planning to undergo standard radiation/chemotherapy.

3.1.3 The tumor must be accessible for injection.

3.1.4 Patients must be age ≥ 18 years.

3.1.5 Patients must exhibit a Karnofsky performance status of $\geq$ 70.

3.1.6 Patients must have adequate organ and bone marrow function within 28 days prior to registration, as defined below:

- SGOT (AST) < 3x upper limit of normal
- Serum creatinine < 2mg/dl
- Platelets > 100,000/mm^3 and WBC > 3000/mm^3

3.1.7 Be able to undergo a brain magnetic resonance imaging scan.

3.1.8 Females of child-bearing potential (FOCBP) and males must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 90 days following completion of therapy. Should a female patient become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

NOTE: A FOCBP is *any woman* (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:

- *Has not* undergone a hysterectomy or bilateral oophorectomy
- *Has had* menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)

3.1.9 FOCBP must have a negative pregnancy test within 7-10 days prior to receiving study drug.

3.1.10 Patients must have the ability to understand and the willingness to sign a written informed consent prior to registration on study.

3.2 Exclusion Criteria

3.2.1 Tumor which invades the ventricular system.
3.2.2 Have had prior radiation therapy to the brain or prior treatment for their brain tumor (except prior biopsy or subtotal resection).
3.2.3 Planning to undergo additional treatment for the brain tumor other than standard of care
3.2.4 Prior or ongoing liver disease including known cirrhosis, active hepatitis B or C infection; not to exclude patients with a distant history of resolved hepatitis A infection.
3.2.5 Known known human immunodeficiency virus (HIV) infection.
3.2.6 Evidence of metastatic disease or other malignancy (except squamous or basal cell skin cancers).
3.2.7 Patients receiving any other investigational agents.
3.2.8 Currently taking immunosuppressive drugs within 28 days of study product injection (with exception of corticosteroids).
3.2.9 Patients who have a history of allergic reactions attributed to compounds of similar chemical or biologic composition to temozolomide are not eligible.
3.2.10 Patients who have an uncontrolled intercurrent illness including, but not limited to any of the following, are not eligible:

- Acute hypertension requiring control with intravenous medication
- Ongoing or active infection requiring systemic treatment
- Symptomatic congestive heart failure
- Unstable angina pectoris
- Unstable cardiac arrhythmia
- Psychiatric illness/social situations that would limit compliance with study requirements
- Any other illness or condition that the treating investigator feels would interfere with study compliance or would compromise the patient's safety or study endpoints

3.2.11 Female patients who are pregnant or nursing are not eligible.

4.0 TREATMENT PLAN

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Reference is made to Section 10.0 – Study Calendar.

4.1 Overview

This study is an open label, Phase I, dose-escalation gene therapy clinical trial with two arms:

1. **Arm A** – Unresectable disease, will receive stereotactic intratumoral injection of NSC-CRAd-S-pk7 immediately following stereotactic biopsy confirmation of clinical diagnosis
2. **Arm B** – Resectable or partially resectable disease, will receive injection of NSC-CRAd-S-pk7 into remaining tumor or tumor bed after resection.

Pathologic confirmation of a malignant glioma must be made at the time of surgery prior to injection. If this is not possible, the injection will not be performed and the patient will no longer be eligible for the study. In the event that the final pathology reading reveals anything other than a malignant glioma, the patient will be removed from the protocol but will continue to be followed for toxicity. Patients having not received any study treatment will need to be replaced and will not be counted as one of the minimum number required without DLT for advancement to the next dose level but will be counted if a DLT is experienced.

Standard radiotherapy and chemotherapy will begin 10-14 days after NSC-CRAd-S-pk7 injection. Long-term follow-up of general health status and any side effects may continue for life. This will be done by telephone contact or letter with the patient or their primary care physician. Medical records will also be reviewed to assess for any potential side effects and for general health status.

An attempt will be made to perform a complete autopsy on any patient who dies, if consent is given by the legally authorized representative. In addition to standard pathological examinations, tissues will be taken for additional studies such as analysis of CRAd-S-pk7 presence and expression.

4.2 Phase I Dose Escalation Scheme

Dose escalation will be performed independently for each arm and three to six patients will be enrolled per dose in each arm. A standard "3+3" dose escalation design will be utilized. Accrual will be staggered by two weeks between the first and second patients in a dose level. If one of three patients suffers a dose limiting toxicity (DLT), then accrual of the next three patients will also be staggered by two weeks each. However, staggering will not be required for accrual of additional patients to define the MTD at the next lowest dose level or at level 3 if no DLT is experienced in first three patients. **Table 1** below presents a summary of the dose escalation schema.

Table 1 – Dose Escalation Schema			
Cohort	**Number NSCs Injected**	**Total Viral Dose (vp/patient)**	**Number of Subjects**
1	50 million (5×10^7)	6.25×10^{10}	3-6
2	100 million (1×10^8)	1.25×10^{11}	3-6
3	150 million (1.5×10^8)	1.875×10^{11}	3-6

Three dose levels as described above will be tested. The two arms will be dose-escalated independently. Once the MTD is determined or after six subjects are evaluated and found to tolerate dose level 3 (the highest dose), the Phase I study will end and the results will be reviewed. The appropriate dose for a future Phase II study will be the highest administered or maximum tolerated dose from the Phase I study. Dose escalation decision rules are found in **Table 2** below.

Table 2 – Dose Escalation and Stopping Rules	
Number of Patients with DLT At a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter 3 patients at the next dose level
≥2	Dose escalation will be stopped. Three additional patients may be entered, after discussion with the sponsor, at the next lowest dose level if only three patients were treated previously at that dose.

1 out of 3	Enter three more patients at this dose level. If none of these three additional patients experience a DLT, then dose escalation occurs, unless this is the highest dose, in which case dose escalation is stopped and the highest dose is declared the MTD. If 1 or more of these additional 3 patients have a DLT, then three additional patients may be entered, after discussion with the sponsor, at the next lowest does level if only three patients were treated previously at that dose.
Highest dose level without ≥ DLT	This will be recommended Phase II dose.

NOTE: Whichever dose level is declared the MTD must have 6 total patients treated at that level. For example, if 3 patients are treated at level 2 and 0 patients experience DLT, escalation would then proceed to level 3. However, if ≥ 2 patients at level 3 experience DLT, enrollment to level 2 would need to be re-opened to enroll an additional 3 patients at that level (with 0 or 1 DLT observed in 6 total patients) in order to declare level 2 the MTD.

4.2.1 Definitions of Dose-limiting Toxicities

- Subjects will be evaluated for dose-limiting toxicities (DLT period) from the time of signing informed consent through completion of concomitant radiation and temozolomide.
- DLTs will be evaluated using CTCAE v 4.03 criteria
- The DLT will be defined as any of the following:
  - Toxicity attributed to study product
  - Any grade 3 toxicity including hematologic and non-hematologic toxicities
  - Any grade 3 toxicity related to the intervention requiring interruption or delay in radiation therapy for more than seven days except CNS hemorrhage*

    * Grade 3 (asymptomatic) radiologic evidence of CNS hemorrhage (commonly seen after biopsy) is not a DLT. Grade 4 (symptomatic) CNS hemorrhage is a DLT. Assessment of neurological toxicity relates to the relative changes from the pre-treatment neurological status. Other anticipated toxic phenomena that are not related directly to viral transduction of the brain tumor or neurological complications which can be explained by the presence of the mass lesion and are compatible with the natural history of malignant brain tumors may require cessation of treatment in individual patients. Such occurrences would not be considered DLT unless they occur with a greater frequency than expected in this patient population.
- A safety analysis will take place 14 days after the first and second subjects receive treatment, and before moving to the next higher viral dose level. The DMC will assess the safety of administering NSC-CRAd-S-pk7 in future patients either at the same dose or a modified dose. No patients will receive treatment until the DMC provides their recommendation.
- The maximally tolerated dose is defined as the dose level immediately below the dose where the DLT was observed.

4.3 Toxicity Management and Dose Delays or Modifications

Any patient who receives an injection of NSC-CRAd-S-pk7 will be evaluable for toxicity endpoints. In the event that the final pathology reading reveals anything other than a malignant glioma, the patient will be removed from the protocol but will continue to be followed for toxicity. Such a patient will not be counted as one of the minimum number required without DLT for advancement to the next dose level but will be counted if a DLT is experienced. Each patient will be assessed for the development of toxicity according to the timeframe referenced in the Schedule of Events table. Treatment all toxicity events, based on CTCAE v4.03 criteria, will be managed according to institutional guidelines.

There will be no dose delays or modifications as this is a single-dose therapy.

4.4 Study Product Administration

Arm A – Unresectable Patients

The patient will be brought to the operating room following an MRI. Biopsy coordinates will then be selected. Biopsy coordinates will consist of one to three sites; however, the neurosurgeon may elect to select as many as needed to obtain diagnosis, as is done with standard neurosurgical practice. Target infusion coordinates will consist of one to three sites within areas of tumor selected by the neurosurgeon. These can be the same or different from the biopsy coordinates. The scalp will be prepped and draped in the usual sterile fashion. After the application of local anesthesia, an incision is made and the skull is exposed. A single burr hole is drilled and, after opening the dura mater and underlying pia, the brain is penetrated with a standard stereotactic needle down to the biopsy coordinates. After frozen tissue confirmation of the presence of malignant glioma, a syringe is filled with up to 1 ml of the appropriate dose of NSC-CRAd-S-pk7. This syringe is attached to a fresh, disposable stereotactic needle. Slowly, 0.5 ml is injected into the stereotactic needle to fill its dead space and the needle is then capped. This operation will be performed on a sterile table that is separate from the main OR and patient tables. The stereotactic needle and syringe are then reinserted into the stereotactic frame and advanced to the target infusion coordinates which will be selected by the surgeon to avoid injections into adjacent motor or speech cortex, the cerebral ventricle, or spillage into the subarachnoid space. Up to 1 ml of NSC-CRAd-S-pk7 is then infused slowly over a period of approximately three to seven minutes. This will require infusion of a chaser of about 0.5 ml of saline (sterile, non-bacteriostatic) into the needle to push the last 0.5 ml of NSC-CRAd-S-pk7 out of the dead space of the stereotactic needle. The needle is then left in place for an additional three to seven minutes before withdrawal. Standard neurosurgical techniques will then be used to close the surgical wound. Staples will not be used due to interference with simulation for radiation therapy.

Arm B – Resectable or partially resectable

A craniotomy with resection of the tumor will be performed. At the neurosurgeon's discretion, this may involve stereotactic methods and/or intraoperative navigational guidance and/or intraoperative MRI or other radiologic guidance. Tumor resection will be performed per routine. After the tumor resection has been completed, freehand injections of 100 microliters of the NSC-CRAd-S-pk7 virus will be performed by the neurosurgeon into up to ten sites in the wall of the resection cavity. The total dose of the NSC-CRAd-S-pk7 will be determined by the appropriate dose escalation scheme. The sites will be at least 1 cm apart and will be selected by the surgeon to avoid injections into adjacent motor or speech cortex, the cerebral ventricle, or spillage into the subarachnoid space. After the injections are completed, the remainder of the operation will consist of routine wound closure.

4.5 Planned Duration of Therapy

Based on projections from current clinical practice, it is anticipated that the accrual will take 12-18 months to complete.

4.6 Concomitant and Adjuvant Medications/Treatments

4.6.1 Peri-Operative Medications

- Antibiotics: All patients will receive a single intravenous dose of cefazolin, or the antibiotic defined by standard care just prior to the surgical procedure.
- Steroids: If clinically indicated, patients may receive dexamethasone prior to and/or following viral injection.
- Anticonvulsant: Anticonvulsant therapy will be administered according to the usual neurosurgical guidelines.
- Analgesics: Pain medication may include codeine and small doses of other narcotic medications.

4.6.2 Radiation Therapy

Radiation will be determined and administered as per standard of care for the patient. It will start 10-14 days after NSC-CRAd-S-pk7 injection. It will consist of standard external field radiation, limited to the area of tumor and brain adjacent to tumor, fractionated at doses of 200 cGy per day for approximately six weeks to a total of 5500-6000 cGy.

4.6.3 Chemotherapy

Standard of care TMZ will be administered concurrent with radiotherapy (75mg/m^2). Patients will sign a drug calendar daily indicating that they have completed the daily TMZ dose during the XRT/TMZ phase (weeks 2 through 7).

Four weeks later, TMZ will be given (150mg/m2 for the first cycle then escalated in the absence of > grade 1 hematotoxicity to 200mg/m^2 if tolerated) during each cycle for a total of 6 cycles. A cycle will consist of 5 days “on”/23 days “off” per Stupp protocol. Dose delays or modifications will be according to the literature at the discretion of the treating oncologist as this is a standard of care treatment.

4.7 Other Modalities or Procedures

Tumor resection or biopsy surgery is a standard of care procedure that will follow Northwestern Medicine institutional guidelines. The tissue collected during surgery will undergo testing to confirm diagnosis and for research purposes, however any other procedures related to surgery are standard of care.

4.8 Duration of Therapy

Within 28 days of screening, patients will receive a one-time intratumoral injection of NSC-CRAd-S-pk7 during the resection or biopsy procedure, at the dose specified in the dose-escalation protocol. Patients will remain inpatient for standard of care post-operative procedures, including an MRI of the brain. Discharge will take place when clinically appropriate.

4.9 Duration of Follow Up

Patients will have a follow-up visits days 1-3 while hospitalized, days 7, 14, and 28 after NSC-CRAd-S-pk7 administration and then at day 56, and every 8 weeks after until disease progression or patient withdrawal. They will be monitored for toxicities by physical exam and laboratory results through day 56. They will also undergo a brain MRI on day 28 and at day 56 to monitor for toxicity and disease progression, then every 8 weeks to monitor for disease progression. Survival status will be monitored until patient death or for up to 5 years.

4.10 Removal of Subjects from Study Treatment and/or Study as a Whole

Patients can be taken off the study treatment and/or study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation must be clearly documented on the appropriate eCRF and may include:

- Patient voluntarily withdraws from treatment (follow-up data collection permitted)
- Patient withdraws consent (no follow-up data collection permitted)
- Patient is unable to comply with protocol requirements
- Patient demonstrates disease progression
- Patient experiences unacceptable toxicity
- Treating physician determines that continuation on the study would not be in the patient’s best interest
- Patient becomes pregnant
- Patient develops a second malignancy that requires treatment which would interfere with this study
- Inability to participate in the medical decision making process.

- New medical information which may adversely affect the health of the patient in the context of the trial.

Long-term follow-up of general health status and any side effects may continue for life after removal from study treatment. Follow-up will be done by telephone contact or letter with the patient, family member, or their primary care physician. Medical records will also be reviewed to assess for any potential side effects and for general health status.

5.0 STUDY PROCEDURES

All patients will undergo all standard of care and clinically indicated evaluations in addition to any study specific evaluations. **Table 3** presents a summary sample schedule. **Table 4** outlines the treatment evaluation schedule. **Table 5** presents the entire study calendar.

Table 3– Sample Schedule	
Screening	
Day -28 until Day -1	Informed consent, medical history and physical exam. baseline laboratory assessments, MRI
Week 1	
Day 0	Intratumoral injection of NSC-CRAd-S-pk7
Days 6-7 (± 3days)	Simulation for RT
Week 2-7	
Days 1-7 (± 3days)	RT (1.8-2 Gy) (Days 1-5 only)
	TMZ (75 mg/m^2)
Week 12	
5 Days on/23 Days off	TMZ (150-200mg/m^2)

Table 4 – Treatment Evaluation Schedule	
History, Physical Exam	≤ 7 Days from Day 0 Weekly 2-4 weeks after completion of XRT At least every three months for first year Annually for five years
Quality of Life questionnaire (FACT-Br version 4) and mini-mental status exam	≤ 7 Days from Day 0 Once during RT administration (week 2 or 3) 2-4 weeks after completion of RT Every three months for the first year or until disease progression
CBC, platelets, PT, PTT, serum electrolytes, creatinine, liver function tests-AST, ALT and bilirubin	≤ 7 Days from Day 0 Weekly during administration (weeks 2, 4, and 6) Annually for five years (CBC Count)
Blood collection for immune research studies*	≤ 7 Days from Day 0 (may be collected in week 2-3 after the NSC-CRAd injection and 2-4 weeks after completion of RT)
Blood collection for RCR testing*	≤ 7 Days from Day 0, 3 months, 6 month, and one year, then annually
Urine/serum pregnancy test (if applicable)	≤ 7 Days from Day 0
Brain MRI	≤ 21 Days from Day 0 2-4 weeks after completion of RT

	Every two-three months for the first year or until disease progression Annually for five years

Table 5. Activities	Screening	Study Drug Administration Period						Follow-up Period			
	Day -28 to -1	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 28	Day 42	Day 56, every 8 weeks thereafter	Yearly
Clinical Assessments											
Informed Consent	X										
Medical/Cancer History	X										
Physical Exam /targeted neurological exam	X	X	X	X	X	X	X	X		X	X
KPS	X	X					X	X		X	X
Height (only at Screen) and Weight	X					X	X	X			
Vital Signs	X	X	X	X	X	X	X	X			
Adverse Events	X	X	X	X	X	X	X	X		X	
Concomitant Meds	X	X	X	X	X	X	X	X		X	
Quality of Life Measures	X						X			X[3]	
Clinical Laboratory											
Urine or serum Pregnancy test	X	X									
Hematology Panel [4]	X	X		X	X	X	X	X	X	X	
Coagulation Panel[4]	X	X			X	X	X				
Serum Chemistry Panel [4]	X	X		X	X	X	X	X	X	X	
Urinalysis with microscopy	X	X					X				
ECG	X	X			X		X				
Registration	X										
Study Drug Administration											
Surgery		X									
NSC-CRAd-S-pk7		X									
Blood/Tissue Testing and Imaging											
Serum Cytokine profile		X			X	X	X	X			
Immune Function blood sample		X				X	X	X			
RCR testing[5]	X									~~X~~	~~X~~
Viral Shedding	X				X						
MRI Scans	X			X				X		X	X
Tumor Sample		X									
CSF Sample		X									

[1] Hematological and metabolic toxicities assessed at week 2, 4, and 6 [2] If available [3]every 8 weeks up to one year
[4] Hematology panel- complete blood count with differential and platelet count; Coagulation panel- prothrombin time/International normalized ratio, partial thromboplastin time; Chemistry panel- glucose, blood urea nitrogen, creatinine, eGFR, sodium, potassium, calcium, chloride, bicarbonate, total bilirubin, alkaline phosphatase, SGOT, SGPT, total protein, albumin; [5]Baseline, day 90(+/- 14 days), 6 months, 1 year, then annually

6.0 ENDPOINT ASSESSMENT

6.1 Primary Endpoint

The primary endpoint is the determination of the dose limiting toxicity and maximum tolerated dose (recommended Phase II dose) using a 3+ 3 design. It was determined by dose escalation schema.

6.2 Secondary Endpoints

This phase 1 study is not powered to detect statistically significant differences in efficacy. However, data will be collected and described descriptively on the following endpoints:

- objective tumor response: objective response to therapy as determined by iRANO criteria (table 9)
- overall survival : time from study project injection to death
- progression-free survival : time from study project injection to first confirmed disease progression as determined by objective tumor response
- quality of life: summary score on patient self-report measure, Functional Assessment of Cancer Therapy- Brain component (as determined by patient self-report on FACT-Br v4, appendix C)

6.3 Exploratory Endpoints

Correlation of cytokine profile and blood immune response with overall survival. Correlation of RCR testing with overall survival.

7.0 ADVERSE EVENTS

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (please refer to Appendices for additional information).

We will institute a data safety monitoring board (DSMB) consisting of at least three experts in the field of neuro-oncology. The DSMB will review any complications arising from the proposed therapy prior to the enrollment of new patients. The DSMB will evaluate deaths that are considered by the PI to be possibly or probably related to the study agent in addition to the death considered related to the study agent.

Death of any patients within the first 56 days of treatment will be reviewed by DSMB within 7 days of the death. Until the review is completed, no new patients will be enrolled in the study. For a patient death that occurs after 56 days, the sponsor/investigator will determine if the death may be a result of the virotherapy. If the death is considered related to the therapy, enrollment will be placed on hold until the DSMB has discussed the case and the PI has discussed the death with the FDA.

In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations.

7.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (from screening through the Day 56 visit). In addition, certain adverse events must be reported in an expedited manner to allow for optimal monitoring and patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be followed until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

7.2 Definitions & Descriptions

7.2.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Recording of AEs should be done in a concise manner using standard, acceptable medical terms. In general, AEs are not procedures or measurements, but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Preexisting conditions that worsen in severity or frequency during the study should also be recorded (a preexisting condition that does not worsen is not an AE). Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, “hepatitis” and not “elevated liver function tests” should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology (e/g/ thrombocytopenia, peripheral edema, QT prolongation).

7.2.2 Severity of AEs

All non-hematologic adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The CTCAE v4 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

7.2.3 Serious Adverse Events (SAEs)

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 56 days after the last administration of study drug, must be reported upon discovery or occurrence.

An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- **Results in *death*.**

If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

- **Is *life-threatening*.**
  The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- **Requires *in-patient hospitalization* or *prolongation of existing hospitalization* for ≥ 24 hours.**
- Results in ***persistent or significant disability or incapacity*.**
- Is a ***congenital anomaly/birth defect.***
- Is an ***important medical event*** not consistent with the risk information described in the general investigational plan or elsewhere in the current IND, and the event is not anticipated from the subject's disease history or status**.**
- **Any significant new neurological deficit in the post-operative period (within 7 days), will be considered an SAE.** For study patients with a history of seizures prior to entry, a single seizure will be considered an AE, but not an SAE. An increase in the seizure frequency or severity in such patients will be considered an SAE
- Secondary malignancy, or
- Any event in which the severity or specificity is
- Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event.” For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependence.

7.2.4 Unanticipated Problems Involving Risks to Subject or Others

Any incident, experience or outcome that meets **all** of the following criteria: [1]

a. Unexpected (in terms of nature, severity, or frequency) given the following:

   (1) The research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigators Brochure (IB); and

   (2) The characteristics of the subject population being studied; and is

b. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); and

c. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized

7.2.4.1 Unanticipated Problems that ARE Adverse Events

Adverse events are any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject’s participation in the research.

7.2.4.2 Unanticipated Problems that ARE NOT Adverse Events

Unanticipated problems that do not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental description of study records, unaccounted for study drug, or black box warnings issued by the FDA. This also includes

unplanned protocol deviations/violations that have already occurred, that may adversely affect the rights, safety or welfare of the research participants, AND for which you did not seek IRB approval.

7.2.4.3 Unanticipated Problems that ARE Adverse Events

Adverse events are any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research.

7.2.4.4 Unanticipated Problems that ARE NOT Adverse Events

Unanticipated problems that do not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental description of study records, unaccounted for study drug, or black box warnings issued by the FDA. This also includes unplanned protocol deviations/violations that have already occurred, that may adversely affect the rights, safety or welfare of the research participants, AND for which you did not seek IRB approval.

7.3 Adverse Event Reporting

7.3.1 Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF. Routine AEs will be reviewed by the Data Monitoring Committee (DMC) according to the study's phase and risk level, as outlined in the DSMP.

7.3.2 Determining if Expedited Reporting is Required

This includes all events that occur within 56 days of the last dose of protocol treatment. Any event that occurs more than 56 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

1) Identify the type of adverse event using the NCI CTCAE v 4.03.
2) Grade the adverse event using the NCI CTCAE v 4.03.
3) Determine whether the adverse event is related to the protocol therapy.
   Attribution categories are as follows:
   - Definite: AE is clearly related to the study treatment.
   - Probable: AE is likely related to the study treatment.
   - Possible: AE may be related to the study treatment.
   - Unlikely: AE not likely to be related to the study treatment.
   - Unrelated: AE is clearly NOT related to the study treatment.
4) Determine the prior experience of the adverse event.
   Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:
   - the current protocol
   - the drug package insert
     - the current Investigator's Brochure

7.3.3 Expedited Reporting of SAEs/Other Events

7.3.3.1 Reporting to the Northwestern University QAM/DMC

All SAEs must be reported to the assigned QAM within 24 hours of becoming aware of the event. Completion of the NU CRO SAE Form, provided as a separate document, is required.

The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)
- The patient's identification number
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)

All SAEs will be reported to, and reviewed by, the DMC at their next meeting.

7.3.3.2 Reporting to the Northwestern University IRB

- Any death of an NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of an NU subject that is actively on study treatment (regardless of whether or not the event is possibly related to study treatment)
- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification.
- All other deaths of NU subjects not previously reported, other non-NU subject deaths that were unanticipated and unrelated, and any other SAEs that were not previously reported as UPIRSOs will be reported to the NU IRB at the time of annual continuing review.

7.3.3.3 Reporting to the FDA

The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.

The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but *not fatal or life-threatening*. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DMC).

All other SAEs will be reported on an annual basis as part of the annual FDA report.

Table 6 summarizes event reporting.

Table 6 – Adverse Event Reporting

Type of Event	Timing of Report*		
	IRB	**IBC (COMS)**	**FDA and NIH OBA**
Fatal or life threatening	As soon as possible, No later than 7 calendar days	As soon as possible, no later than 7 calendar days	As soon as possible. No later than 7 calendar days
SAE resulting in one of the following: 1. Inpatient hospitalization or prolongation of hospitalization 2. A persistent or significant disability of incapacity 3. A congenital anomaly or birth defect	As soon as possible, no later than 15 calendar days	As soon as possible, no later than 15 calendar days	As soon as possible, no later than 15 calendar days

Any other unexpected adverse experiences associated with the use of the product	According to institutional requirements	According to institutional requirements	In the annual report

7.3.3.4 Quarterly IND Reports

Reports containing the following information will be sent on a quarterly basis to City of Hope, the provider of the NSCs:

- Enrollment Information
- Demographics
- Interim Results on the progress toward identifying the MTD
- AE and SAE Data (unlikely, possibly, probably, definitely related)

8.0 DRUG INFORMATION

8.1 Agent NSC-CRAd-S-pk7

8.1.1 Other names- none

8.1.2 Classification- NA

8.1.3 Mode of action

This cell product is an oncolytic vector, CRAd-Survivin-pk7 (CRAd-S-pk7), for the treatment of malignant gliomas. CRAd-S-pk7 is a chimeric vector containing a pk7 fiber modification and a survivin promoter driving E1A replication. CRAd-S-pk7 vector effectively replicates in many glioma cell lines like U-87MG, U-251MG, A172, Kings-1, No. 10 and U-118MG and leads to tumor oncolysis in these cell lines with minimal viral replication and toxicity in normal human brain.

8.1.4 Storage and stability

The HB1.F3.CD_CRAd-S-pk7 cell product suspended in CryoStor 10 was vialed using a volume of 1.3 ml/vial at a concentration of 2.5 x 10^7 per ml (3.25 x 10^7 cells per vial). CryoStor cryopreservation freeze media products have been designed to mitigate temperature-induced molecular cell stress responses during freezing and thawing. All CryoStor products are pre-formulated with USP grade DMSO, a permeant solute cryoprotective agent which helps mitigate damage from the formation of intracellular ice. CryoStor products meet USP <71> Sterility and USP <85> Endotoxin testing standards, and are manufactured under cGMP. The final product has a pH of 8.0 and is a translucent, colorless liquid with no particulates.

The HB1.F3.CD_CRAd-S-pk7 cell product is stored in vapor phase liquid nitrogen at ≤ -140ºC in CryoStor 10 freezing medium. Previous qualification assays performed on the cell product confirm that cells are viable for up to six hours at room temperature. The stability testing plan for the HB1.F3.CD.CRAd-S-pk7 product is described in **Table 7** below.

Table 7 – Stability Testing Plan

Test	Specification	0 month	4 month	12 months June 2016	24 months June 2017
Flow cytometry nestin	Report Results	86% positive (98.5% prior to infection) UAB QC-778	85% positive UAB QC-868	X	X
Flow cytometry for Ad hexon	Report Results	75% positive UAB QC-778	70% Positive UAB QC-868	X	X
Virus Burst Assay	Report Results	2,864 IU/viable cell plated, UAB QC-779	3010 IU/viable cell plated, UAB QC-869	X	X
Viable cell count	Report Results	2.8×10^7 viable cells/vial, UAB QC-777	2.4×10^7 viable cells/vial, UAB QC-867	X	X
Cell viability	> 70% viable	92% viable UAB QC-777	89% viable UAB QC-867	X	X
Sterility: USP <71>, 21 CFR610.12, EP2.6.1 and JP 4.06	No Growth	PASS / BioReliance, AE31BC.510120G MP.BSV_CA	PASS, UAB QC-871	X	X
Endotoxin/LAL	< 5.0 EU/mL	PASS, <0.5 EU/mL, QC 755	PASS, <0.5 EU/mL, QC-870	X	X

8.1.5 Protocol dose specifics

One ml given as a one-time dose.

8.1.6 Preparation

On the day of injection, the HB1.F3.CD_CRAd-S-pk7 cell product will be prepared as described in a separate **SOP (Appendix B)**. Briefly, HB1.F3.CD_CRAd-S-pk7 cells are removed from the vapor phase of liquid nitrogen storage and thawed in a 37º water bath. Cells are then washed with perfusion fluid central nervous system (PFCNS)+2% human serum albumin (HSA) buffer a total of three times, and resuspended in PFCNS+2% HSA and maintained at room temperature in the biosafety cabinet for up to six hours.

8.1.7 Route of administration for this study

Intratumoral at the time of surgical biopsy or resection of the tumor. Refer to Section 4.4 for product administration.

8.1.8 Incompatibilities

NA

8.1.9 Availability & Supply

Human NSCs (HB1.F3) were provided by Dr. Karen Aboody. The HB1.F3 human NSC line was established by retroviral transduction of v-*myc* into primary human NSCs isolated from fetal telencephalon of 15 weeks gestation and provided by Seung U. Kim, MD, PhD. The HB1.F3 line is well-characterized and multipotent, non-tumorigenic and non-immunogenic. A phase I study was recently completed at COH which involved injection of up to 50 million NSCs which confirmed that the risk to benefit ratio merited continued study.

The cell product was manufactured at the University of Alabama and is stored in the Mathews Center for Cell Therapy at Northwestern University. The clinical grade CRAd-S-pk7 vector has been produced in compliance with current Good Manufacturing Practices for gene therapy vectors. This is a replication competent adenoviral vector based on an adenovirus serotype-5 backbone.

8.1.10 Side effects

Toxicities Associated with Adenoviral Injection

In previous trials of intratumoral adenoviral injection, toxicity was rare, and overall, the approach has been well tolerated. A summary of grade 3 and 4 toxicities seen in the previous trials is provided in **Table 8** below. In the Phase I study in recurrent brain

tumors, CNS toxicity (lethargy, confusion, grade 3 hyponatremia, fever, leukocytosis, intratumoral hemorrhage in 1, hydrocephalus in the other) was observed in 2/2 patients at $2x10^{12}$ vp dose level. In the lower dose levels, no DLTs were observed but transient and less severe events included seizure, small intratumoral hemorrhage, worsening of neurologic symptoms, mild hyponatremia. One patient at the highest dose level in the prostate dose escalation study experienced grade 4 transient thrombocytopenia which was considered a DLT and also transient grade 3 elevation in liver transaminases.

Table 8 – Summary of Grade 3 and 4 Toxicities In Trials With Adenovirus

Patient Characteristics	Treatment Specifics	Toxicity
Recurrent malignant gliomas IND 6371 13 patients Trask el al, 2000	**Dose escalation:** 2 x 10^9 vp, (2 pts) 2 x 10^{10} vp, (2 pts) 2 x 10^{11} vp, (7 pts) 2 x 10^{12} vp, (2 pts) **Delivery:** Stereotactic injection **Prodrug:** GCV **Concurrent tx:** none	CNS toxicity in 2/2 patients at 2 x 10^{12} vp dose level Therapy tolerated by other 11 patients including 7/7 patients treated at 2 x 10^{11} vp
Recurrent ovarian cancer, stage **IIIc** IND 7311 10 patients Hasenburg et al, 2001	**Dose escalation:** 2 x 10^{10} vp, (2 pts) 2 x 10^{11} vp, (2 pts) 2 x 10^{12} vp, (2 pts) 2 x 10^{13} vp, (4 pts) **Delivery:** Intraperitoneal **Prodrug:** ACV or Valacyclovir **Concurrent tx:** Topotecan	No significant toxicity attributable to gene therapy.
Recurrent prostate cancer IND 6636 18 patients Herman et al, 1999	**Dose escalation:** 2 x 10^9 vp, (4 pts) 2 x 10^{10} vp, (5 pts) 2 x 10^{11} vp, (4 pts) 2 x 10^{12} vp, (5 pts) **Delivery**: Intraprostatic **Prodrug:** GCV **Concurrent tx:** none	One patient at 2 x 10^{12} vp dose level had transient grade 4 thrombocytopenia and grade 3 hepatotoxity
Recurrent prostate cancer (radiation failures), multiple injections 27 patients (18 new patients plus 9 additional injections in patients from first study) Shalev et al, 2000; Miles et al, 2001	**Dose:** 2-6 x 10^{11} vp **Delivery** Intraprostatic **Prodrug: GCV** **Concurrent tx:** none	Grade 3 hypertension in one patient requiring anti-hypertensive therapy
Pre-prostatectomy 41 patients in 3 studies (1 in Mexico- 10pts; 1 in Netherlands-8pts; 1 in the US-23 pts)	Rojas-Martinez; 10 pts 1 x 10^{11} vp Van der Linden; 8 pts 2 x 10^{10} vp (4pts) 2 x 10^{11} vp (4pts) Ayala et at, 2006: 23 pts 2-8 x 10^{11} vp	No significant toxicity attributable to study drug.
Prostate cancer, combined with radiation therapy IND 8437 71 patients The et al, 2001 (1st 30 patients)	**Dose:** **5x10^{11} vp per injection** **Delivery:** Intraprostatic **Prodrug:** valacyclovir **Concurrent tx**: radiation +/- hormone	1. Grade 3 ALT elevation in 1 patient, resolving after 1 week, no interruption in therapy 2. Grade 3 genitourinary toxicity in 1 patient attributed to the radiation therapy

Potential Biohazard Exposure to Health-Care Personnel

Personnel Involved in Vector Injection: There is a low potential for biohazard exposure to the personnel involved in vector injection. The viral vector is suspended in a small volume (~1.0 ml) of buffer and is contained in a cryovial. The syringe is loaded from the vial with no vector loss. Intratumoral injection is made and the amount of leakage along

the needle track is likely to be very minute. In the event of accidental spills of the vector from the vial common disinfectants can be used to inactivate the agent. All personnel are instructed on the safe use and potential biological hazards of the vector before the procedures are performed.

Post-Surgical Health Care Workers: There is a potential that the virus may escape the patient via urine, feces, saliva, mucus, tears or other excretions. Studies in non-human primates indicate that the potential is low. In studies with six baboons only one plaque was detected in the serum of one baboon injected two days previously with the vector (see IND application 6371 or RAC Protocol 1294-098). In semipermissive cotton rats, only minimal virus was detected after intracardiac injection (Rojas-Martinez et al, 1998). Exposure to shedded replication-competent virus will pose a very limited hazard, as the titers will be extremely low. There is a theoretical possibility that the vector could recombine with wild-type adenovirus although this has not been detected in previous patients treated with the similar adenoviral vectors. If this did occur, infection would be expected to cause a mild, self-limited infection similar to a common adenovirus infection. Health care workers will be instructed on the safe use and potential biological hazards of the virus vector before they are allowed to work with the patients.

8.1.11 Nursing implications

Nurses will not be involved handling or administration of the cell product. After administration, universal precautions should be used during hospitalization. Nurses through their usual care will participate in physical and neurological assessments that may help identify any adverse events.

8.1.12 Return and Retention of Study Drug

Unused prepared study cell product will be disposed of in a Biohazard Waste container, which will be closed and disposed of according to Biohazard Waste disposal policy. Expired unprepared or unused product in the Mathews Center for Cell Therapy will be disposed of according to BSL-2 practices.

8.2 Agent Temozolomide (started after completion of study cell product administration as a standard of care therapy)

8.1.1 Other names

Temodar

8.1.2 Classification - type of agent

Anti-neoplastic

8.1.3 Mode of action

Alkylating agent.

8.1.4 Storage and stability

Temozolomide will be stored in the patient's home at room temperature. The patient will be instructed to keep the drug out of reach of children. Drug stability is determined by the manufacturer and consistent with the drug expiration date printed on the container supplied to the patient.

8.1.5 Protocol dose specifics

Temozolomide is dosed at 75 mg/m^2 daily for up to 49 days, concomitant with focal radiotherapy. After a break of 4 weeks, temozolomide maintenance therapy is given for up to 6 cycles. Initial dose is 150 mg/m^2 with an increase to 200 mg/m^2 if tolerated, given as a single daily dose for 5 days every 28 days. Timing of daily administration will be per routine use and as directed by the treating

oncologist. Temozolomide dose may be delayed or modified by the treating oncologist based on patient tolerance.

8.1.6 Preparation
Temozolomide is supplied in 5, 20, 100, 140, 180 and 250 mg capsules. Daily dose is determined based on BSA calculations, with the appropriate combination of capsules to reach the closest equivalent mg/m^2 dose.

8.1.7 Route of administration for this study
Oral

8.1.8 Incompatibilities
Valproic acid: decreases clearance of oral temozolomide.

8.1.9 Availability & Supply
Commercially available.

8.1.10 Side effects

- The most common adverse reactions (≥10% incidence) are: alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia.
- The most common Grade 3 to 4 hematologic laboratory abnormalities (≥10% incidence) that have developed during treatment with temozolomide are: lymphopenia, thrombocytopenia, neutropenia, and leukopenia. Allergic reactions have also been reported.
- Less likely but severe adverse reactions include myelodysplastic syndrome and hepatotoxicity

8.1.11 Nursing implications
Antiemetic prophylaxis with a 5-HT3 antagonist is recommended prior to administration of the first few temozolomide doses and should be administered orally 30 to 60 minutes before temozolomide treatment. Prophylaxis is at the discretion of the treating oncologist. Monitor complete blood count and liver function tests to determine tolerance.

8.1.12 Return and Retention of Study Drug
Patients are instructed to return any unused temozolomide to the dispensing pharmacy for appropriate disposal.

9.0 CORRELATIVES/SPECIAL STUDIES

An exploratory objective will be to evaluate serum cytokine profile and blood immune response to NSC-CRAd-S-pk7 and to determine whether overall survival rate correlates with extent of immune response. In addition, replication competent retrovirus (RCR) testing will performed by analyzing patient DNA from whole blood for RCR-specific sequences at baseline, 3 months, 6 months, and 1 year after study treatment; and annually thereafter, and to determine whether overall survival rate correlates with persistent gene expression. Real time PCR with 4070A envelope-specific primers will be used to screen DNA extracted from PBMCs from study patients treated with NSCs.

Correlative study (sample type)	Cytokine profile (serum)	Immune response (whole blood)	RCR testing
Mandatory or Optional	Mandatory	Mandatory	Mandatory
Timing (+/- windows)	Baseline, Days 1, 3, 7, 14 and 28 after	Baseline, Days 1, 7, 14 and 28 after study	Baseline, 3 months, 6 months, and 1 year

	study product injection	product injection	after study product injection
Volume Needed (blood only)	4-5 ml	4-5 ml	4-5 ml
Tube type needed (blood only)	EDTA tube	Non-EDTA tube	Non-EDTA tube
Tissue thickness and/or # slides (tissue only)	NA	NA	NA
Processing center	Lurie Research Center (LRC) 3-250	Lurie Research Center (LRC) 3-250	Lurie Research Center (LRC) 3-250
Sample handling/processing instructions	Blood transported directly to LRC within 30 minutes of draw; centrifuge 15 minutes at 3000 rpm; pipette serum into vial and freeze at -80^{o}C until analysis	Blood transported directly to LRC within 30 minutes of draw; freeze at -80^{o}C until analysis	Blood transported directly to LRC within 30 minutes of draw; freeze at -80^{o}C until analysis
Shipping/delivery info	NA	NA	Samples shipped to City of Hope for analysis
Storage needs	Storage available in LRC	Storage available in LRC	Storage available in LRC
Analysis center	LRC	LRC	LRC
Assay methodology	ELISA	ELISA	Real time PCR with 4070A envelope-specific primers

9.1 Sample Collection Guidelines

Additional blood samples as specified above will be drawn at the time of standard of care blood draws by the phlebotomist and transported directly to the LRC within 30 minutes of draw.

9.2 Sample Processing, Storage, and Shipment

Cytokine profile and immune response samples will be processed and stored in Lurie Research Center Neurosurgery Lab, 3-250, 303 E. Superior, Chicago, IL. Samples will be assayed on site and not transported outside Northwestern University. Whole blood samples for RCR testing will be processed and stored in Lurie Research Center Neurosurgery Lab, 3-250, 303 E. Superior, Chicago, IL. Samples will be batch shipped to City of Hope for analysis.

9.3 Assay Methodology

Cytokine profile and immune response samples will be analyzed using enzyme-linked immunosorbent spot (ELISA). RCR samples will be analyzed using real time PCR with 4070A envelope-specific primers.

9.4 Specimen Banking

Tumor tissue (tissue block obtained at surgery flash frozen) and cerebrospinal fluid (3ml), if obtained at the time of surgery, will be banked in the Nervous System Tumor Bank at Northwestern for future analysis.

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Design/Study Endpoints

Please refer to section 2.0 and 6.0.

10.2 Sample Size and Accrual

This phase 1 study is not powered to detect statistically significant differences in efficacy. The primary endpoint of this Phase I dose-escalation study is the MTD of NSC-CRAd-S-pk7 when administered with standard radiation and chemotherapy in patients with malignant glioma. The 3+3 design has a 71% chance (49%, 17%) of dose escalating when the true toxicity rate for that dose is 20% (30%, 50%).

If all three dose levels are administered, the minimum number of patients enrolled will be 9 per arm and the maximum number of patients will be 18 per arm, for total accrual of 18-36 patients enrolled. Based upon the dose escalation schema described, groups of three to six patients will be entered at a dose level in each arm. Using this algorithm, the probabilities of dose escalation for various true DLT rates are given in Table 10. For example, if the true DLT is 50% at a dose level, there is a 17% chance that the dose would be escalated. The maximum tolerated dose determined in this study will be the recommended dose for a Phase II study. In the event the final pathology reading reveals anything other than a malignant glioma, such a patient will not be counted as one of the minimum number required without DLT for advancement to the next dose level but will be counted if a DLT is experienced.

Table 10– Probabilities of Dose Escalation	
20%	71%
30%	49%
40%	31%
50%	17%
60%	8%

10.3 Data Analyses Plans

A formal statistical and analytical plan (SAP) including additional statistical analysis details will be completed prior to database lock.

10.3.1 Analysis Datasets

All the analysis will be conducted in the safety population, including all the patients who received at least one dose of NSC-CRAd-S-pk7.

10.3.2 Description of Statistical Methods

10.3.2.1 General Approach

All study data will be presented in by-subject data listings. Statistical analysis will be descriptive only, with no hypothesis testing. All the endpoint data will be collected and described in summary tables for both two arms at each dose. In general, descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables. Frequencies and percentages will be calculated for categorical and ordinal variables at each category. Statistical analyses will be performed using SAS9.4 (SAS Institute Inc., Cary, NC)

10.3.2.2 Analysis of the Primary Endpoint(S)

The maximum tolerated dose (MTD) will be determined according to the dose escalation algorithm described in Section 4.2. Dose limiting toxicities are defined in Section 4.2.1. If >2 patients do not complete the initial RT/TMZ phase or rule if > 2 patients experience life threatening or fatal neurologic toxicity, the trial will be stopped to analyze, in conjunction with the DMC, whether this regimen is tolerable.

10.3.2.3 Analysis of the Secondary Endpoint(S)

For objective tumor response, a summary table will be produced to present the number and proportion of responders in each arm, together with each of the response categories, namely CR, PR, MR, SD, P and PP.

The analysis of overall survival and progression-free survival are based on the survival function, estimated by the Kaplan-Meier method. Survival will be calculated from the day of study inclusion until progression and death, respectively, or will be censored at the date of last available follow-up information. The survival function will be summarized for 25th percentile, median, and 75th percentile, and the survival curves of the two arms will be presented.

Descriptive statistics will be used to describe the overall quality-of-life score on physical well-being, social/family well-being, emotional well-being, functional well-being and additional concerns, respectively.

10.3.2.4 Analysis of the Exploratory Endpoint(S)

Cytokine profile will be described using descriptive statistics, and blood immune response to NSC-CRAd-S-pk7 will be presented as number (percentage) for each category. The association of extent of immune response with overall survival will be determined by Cox's proportional hazards model.

In addition, retroviral testing (RCR testing) will be performed by analyzing patient DNA from whole blood for RCR-specific sequences prior to surgery; at 3 months, 6 months, and 1 year after study treatment; and annually thereafter.

10.3.3 Safety Analyses

Safety endpoints include adverse events (including serious adverse events), deaths, laboratory parameters, vital signs, physical examination and ECG.

All Adverse event will be coded by System Organ Class (SOC) and Preferred Term using Medical Dictionary for Regulatory Activities (MedDRA) dictionary, and will be presented in frequency tables (overall and by intensity [CTCAE grading]) by SOC. In tables showing the overall incidence of adverse events, subjects who experienced the same event on more than one occasion are counted only once in the calculation of the event frequency. Listings will be presented by subject for all adverse events as well as for serious adverse events, adverse events associated with death, and adverse events leading to discontinuation from the study, unanticipated problems involving risks to subject or others. The following information will be included: start date, stop date, severity, relationship, outcome, and duration. Deaths will be listed and summarized descriptively.

Laboratory data will be presented as summary statistics by dose group using both shift and frequency tables. For each continuous laboratory parameter, results will be categorized as low, normal or high based on the laboratory normal ranges. Subjects who had a shift to low and those who had a shift to high from baseline to any post-dosing assessment will be summarized. All out-of-range and clinically significant laboratory results will be identified in subject data listings.

Individual patient values of vital sign parameters including systolic and diastolic blood pressure and heart rate will be listed by patient. The absolute and percentage changes from baseline will be computed and potentially serious changes will be flagged. Appropriate summary statistics will be provided for all vital sign parameters.

The number and percentages of subjects in each dose group with normal and abnormal ECG performance status and physical examination results will be presented for evaluations at baseline and final visit. For each body system, changes in subjects' findings from baseline to final visit (no change, normal to abnormal, or abnormal to normal) will be tabulated for each dose group.

10.3.4 Baseline Descriptive Statistics

Demographic (age, sex, height and weight) and baseline disease characteristics (including baseline KPS), will be summarized for both arms.

11.0 STUDY MANAGEMENT

11.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

11.2 Amendments

The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies.

11.3 Registration Procedures

11.3.1 Registering a Patient for the Phase I Portion of the Study

For potential patients for the phase I portion of this study, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive an identification number:

- Patient's signed and dated informed consent form (upload to NOTIS and keep original hard copy in a secure location/study chart)
- Eligibility checklist (signed and dated by the treating physician – upload to NOTIS)
- Eligibility eCRF (complete in NOTIS)
- Copy of the pathology report (upload to NOTIS)

The QAM will review all source documentation required to confirm eligibility that is readily available in the patient's electronic medical record (EMR). Any information that is not available in the EMR must be de-identified and emailed to the QAM. Once the QAM confirms the patient is eligible, he or she will register the patient, assign a subject identification number, provide a cohort assignment, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

11.3.2 Registering a Patient to the Phase II Portion of the Study

11.4 Data Submission

Once a subject is confirmed and registered to the study, eCRFs should be submitted according to the detailed data submission guidelines (provided in a separate document). All data for phase I patients during the time period patients are evaluated for Dose Limiting Toxicities (DLTs) must be submitted on a weekly basis.

11.5 Data Management and Monitoring/Auditing

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern. The assigned QAM, with oversight from the Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level. Please refer to the NOTIS for additional data submission instructions.

11.6 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.6.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

11.6.2 Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs.
- Has no substantive effect on the risks to research participants.
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected.
- Did not result from willful or knowing misconduct on the part of the investigator(s).

A protocol deviation may be considered an instance of Promptly Reportable Non-Compliance (PRNC) if it:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

11.7 Investigator Obligations

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The PI is responsible for personally overseeing the treatment of all study patients. The PI must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected, entered onto the appropriate eCRFs, and submitted within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

11.8 Publication Policy

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the policies and processes set forth in the Lurie Cancer Center DSMP. For trials that require high intensity monitoring, the assigned QAM will prepare a preliminary data summary (to be approved by the DMC) no later than 3 months after the study reaches its primary completion date (the date that the final subject is examined or receives an intervention for the purposes of final data collection for the primary endpoint). If the investigator's wish to obtain DMC-approved data prior to this point (or prior to the point dictated by study design), the PI must send a written request for data to the QAM which includes justification. If the request is approved, data will be provided no later than 4 weeks after this request approval. The data will be presented to the DMC at their next available meeting, and a final, DMC-approved dataset will be released along with any DMC decisions regarding publication. The investigators are expected to use only DMC-approved data in future publications. The investigators should submit a copy of the manuscript to the biostatistician to confirm that the DMC-approved data are used appropriately. Once the biostatistician gives final approval, the manuscript may be submitted to external publishers.

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APPENDICES

Appendix A. iRANO criteria	
Term	**Criteria**
Complete Response (CR)	The enhancing tumor is no longer seen by neuroimaging, with the patient off all steroids, or on adrenal maintenance only.
Partial Response (PR)	Decrease of ≥ 50% in the product of two diameters with the patient on a stable or decreasing dose of steroids, with a stable or improving neurologic examination for at least six weeks.
Minor Response (MR)	Decrease in diameter products of < 50% with the patient on a stable or decreasing dose of steroids, with a stable or improving neurologic examination for at least six weeks.
Stable Disease (SD)	The scan shows no change. Patients should be receiving stable or decreasing doses of steroids.
Progression (P)	Increase of ≥ 25% in tumor area (two diameters) provided that the patient has not had his/her dose of steroids decreased since the last evaluation period. A concomitant decrease in steroid dose will rule out a progression designation during the first two months after completion of radiation
Pseudoprogression (PP)	Radiological changes without concomitant neurological changes. It is not unusual to see enhancement in the context of oncolytic viral therapies. If thought to be the case, serial MRI imaging is warranted.