PROTOCOL TITLE: Phase I/II Trial of Repeated Super-Selective Intraarterial Cerebral Infusion of Bevacizumab (Avastin) For Treatment of Newly Diagnosed Glioblastoma Multiforme

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IN THE EVENT OF EMERGENCY

Any adverse event experienced by the patient while receiving or within 30 days of receiving treatment under this research protocol, even though the event may not appear to be drug-related, will be reported according to The Feinstein Institute for Medical Research immediate reporting policy.

PATIENT ENROLLMENT ON STUDY

Investigators must notify the Clinical Research Coordinator in advance to pre-qualify a patient for enrollment. Once a patient is identified as meeting the study entry criteria, the investigator or his/her designee will contact the Principal Investigator or designee (one of the individuals listed above) for final approval for patient enrollment.

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PROTOCOL TITLE: PHASE I/II TRIAL OF REPEATED SUPER-SELECTIVE INTRAARTERIAL CEREBRAL INFUSION OF BEVACIZUMAB FOR TREATMENT OF NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

Test Materials: Mannitol (hexan-1,2,3,4,5,6-hexol ($C_6H_8(OH)_6$)
Bevacizumab (Avastin, Genentech Pharmaceuticals)

Dosage Levels: Mannitol 20% 12.5ml over two minutes for blood brain barrier (BBB) disruption

Bevacizumab 15 mg/kg, after BBB disruption, given 1 mL/s

Route of Administration: Repeated Superselective Intraarterial Cerebral Infusion (SIACI)

3. STUDY SYNOPSIS

The current standard of care for newly diagnosed GBM is for patients to undergo tumor resection, if possible, followed by oral Temozolomide and radiation (chemoradiation) for 6 weeks. This is known as the Stupp protocol. Thereafter they receive maintenance oral Temozolomide 5 days on and 23 days off, until their tumor grows more than 25%. At that point, these patients are deemed treatment failures and are given another treatment. Because of the blood brain barrier (BBB) which prevents drugs from penetrating the blood vessel walls well to get into the brain, no one knows for sure if these drugs actually get into the brain after ingestion or infusion.

We have recently completed a Phase I clinical trial that has shown that Superselective Intraarterial Cerebral Infusion (SIACI) of Bevacizumab is safe up to a dose of 15mg/kg in patients with recurrent malignant glioma. Therefore, this one arm open-label, non-randomized trial is a follow up study to that trial and will ask simple questions: Is it safe to deliver ***repeated*** doses of Bevacizumab intraarterially using these super selective intraarterial delivery techniques? Will this IA Bevacizumab treatment regimen increase progression free survival (PFS) and overall survival (OS)? We expect that this project will provide important information regarding the utility of repeated SIACI Bevacizumab therapy for newly diagnosed malignant glioma, and may alter the way these drugs are delivered to our patients in the near future.

Current Standard of Care:

Day 0	Maximum surgical debulking with functional preservation
Day 30	Start radiation and oral Temozolomide for 6 weeks
Day 72	Rest period for one month
Day 100	Start oral Temozolomide (5 days on, 23 days off) and repeat each cycle

Experimental portion of this proposal: This trial will have one experimental arm that will test adding repeated IA Bevacizumab to the chemoradiation treatment at post-op day 30 and every 3 months thereafter

Day 0	Maximum surgical debulking with functional preservation
Day 30*	**Start single dose of IA Bevacizumab** (15mg/kg); followed by radiation and oral Temozolomide for 6 weeks (*May be done at 2 weeks for biopsy patients with well healed incisions)
Day 72	Rest period for one month
Day 100	Start oral Temozolomide (5 days on, 23 days off) and repeat each cycle
Day 120	**Repeat single dose of IA Bevacizumab** (15 mg/kg)
Day 210	**Repeat single dose of IA Bevacizumab** (15 mg/kg)

The experimental aspects of this treatment plan will include:

1. Subjects will first be treated with Mannitol prior to chemotherapy infusion (Mannitol 20%; delivered IA, 12.5 mL over 2 minutes) in order to disrupt the blood brain barrier. This technique has been used in several thousand patients in previous studies for the IA delivery

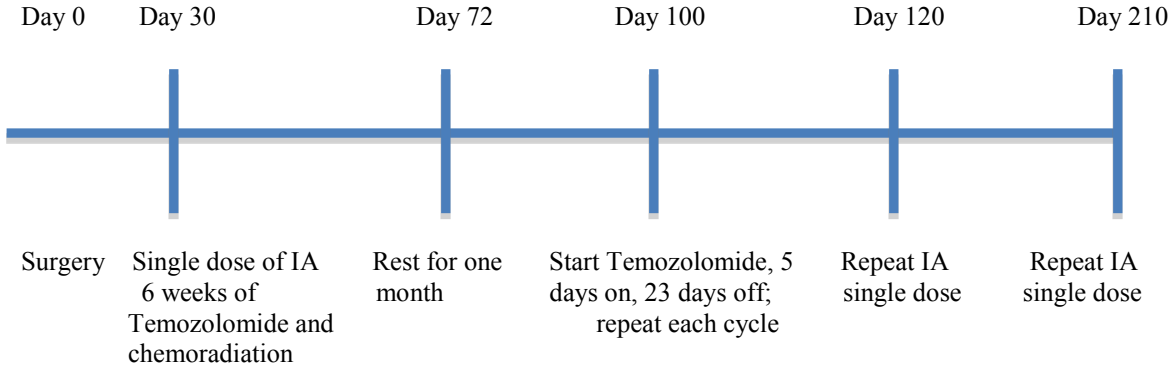
of chemotherapy for malignant glioma. We have used this without complication in the 30 patients from our Phase I protocol as well.

2. Subjects will then be treated with repeated intraarterial delivery (SIACI) of Bevacizumab. Each patient will receive one dose of IA Bevacizumab on day 30, followed by chemoradiation. SIACI of Bevacizumab will be repeated every three months for a total of 3 infusions.

Inclusion criteria: Males or females, ≥18 years of age, with documented histologic diagnosis newly-diagnosed glioblastoma multiforme (GBM). The lesion must be circumscribed to fall within an area targetable by IA therapy. The patient's KPS ≥ 70 and the patient must have a life expectancy greater than 12 weeks.

This treatment may be harmful to a fetus in pregnant subjects. If the subject is a female of childbearing age, she will be asked to practice birth control methods while participating in this research study and for 3 months following her treatment. These methods include oral contraceptives, contraceptive shots, and barrier methods, such as condom use, sponges, and diaphragms. Fertile males are required to use these barrier methods.

The patient may be responsible for any additional costs associated with enrollment in the trial. All costs of the IA delivery and the cost of the drug will be submitted to the patient's insurance provider. The Feinstein Institute for Medical Research will not be named as a sponsor of the study nor will it cover the cost of the experimental procedure.



Toxicity Monitoring/Assessment: Patients will be treated with a previously tested, dose and schedule of Mannitol prior to chemotherapy infusion (Mannitol 20%; 12.5 mL over two minutes) in order to disrupt the BBB. Following BBB disruption, the patient will receive an SIACI with Bevacizumab at a dose of 15mg/kg, given 1mL/minute. Both hematologic and non-hematologic dose limiting toxicity will be determined and scored according to the NCI Common Toxicity Criteria (version 3.0). The dose limiting toxicity will be evaluated for 28 days after the SIACI chemotherapy infusion. DLT will be defined as Grade III or worse non-hematologic and hematologic toxicity or Grade II or worse CNS hemorrhage.

Anti-tumor response: Response will be evaluated after two cycles of chemotherapy (two months), or sooner if deemed necessary by the PI, using an MRI with contrast and the Response Assessment in Neuro-Oncology (RANO) response criteria (Appendix E).

Study Agents: Mannitol and Bevacizumab are registered agents.

Safety/Toxicity Monitoring: Hemorrhagic, Dermatologic, gastrointestinal, respiratory and nervous system disorders are expected to be the primary toxicity in patients receiving this treatment regimen. Patients may at any point during the phase I study receive supportive care, including growth factors, blood cell transfusions, as clinically indicated.

4. INTRODUCTION

In patients with GBM, the expression of VEGF is associated with a poor prognosis. Monoclonal antibodies to VEGF have inhibited the growth of GBM in vitro and in vivo. In fact, intravenous (IV) Bevacizumab, a VEGF inhibitor, has become the standard of care for recurring relapsing GBM.

Bevacizumab is a humanized immunoglobulin (Ig) G1 monoclonal antibody that binds to and inhibits the activity of VEGF. It has been show to work synergistically with chemotherapy in colorectal, lung, brain and breast carcinomas. A preliminary report of the combination of bevacizumab for patients with malignant gliomas demonstrated an encouraging response rate of 43% (Bokstein et al. 2008; Buie and Valgus 2008; Chamberlain 2006; de Groot and Yung 2008; Desjardins et al. 2008; Kang et al. 2008; Narayana et al. 2008; Poulsen et al. 2008; Sathornsumetee and Rich 2007; Vredenburgh et al. 2007; Vredenburgh et al. 2008; Zuniga et al. 2008). In addition, the 6-month overall survival with bevacizumab and irinotecan was improved compared with temozolomide (current first line treatment): 77% versus 60% respectively. Comparison of Bevacizumab and CPT-11 shows a 6-month PFS and median PFS in patients with GBM of 43% and 24 weeks, compared with previous results of 15% and 9 weeks, respectively (Bao et al. 2006; Folkins et al. 2007). The 1-year overall survival with bevacizumab was superior to historical controls: 37% versus 21%, respectively. As a single agent, Bevacizumab achieved a 6-month PFS rate of 42.6% as salvage therapy for recurrent gliomas (Beal et al, 2011). Bevacizumab is also being studied in conjunction with standard chemo-radiation for newly diagnosed tumors (Arko et al, 2010; Leibross et al, 2009). Two recent trials performed in parallel were done to assess the efficacy of IV bevacizumab (Avastin) for newly diagnosed glioblastoma. One by the RTOG (RTOG 0825) in the United States, and one by Roche (AVAGlio), mostly in Europe. Results were presented at the 2013 American Society of Clinical Oncology. The results showed a significantly prolonged progression free survival in both studies and quality of life was preserved in the AVAGlio trial but not the RTOG. Overall survival was not improved, but safety and tolerability were acceptable in both studies. Although the overall survival studies do not support the routine use of IV bevacizumab, our trial examining less frequent intra-arterial dose delivery may improve both PFS and OS by limiting the resistance or escape from VEGF inhibition that appears after prolonged IV bevacizumab chemotherapy.

In conclusion, the use of bevacizumab is strikingly active against recurrent GBM. The standard delivery has been IV, but we have had success in our current studies delivering the drug intraarterially to patients with recurrent disease, in theory giving them treatment directly to the

tumor, with fewer systemic side effects. We hypothesize that in patients with newly diagnosed GBM, superselective intraarterial cerebral infusion of Bevacizumab, up to a dose of 15 mg/kg, will be safe and efficacious and prevent tumor progression in patients.

Clinical Experience with the Individual Chemotherapy Agents

Mannitol: Intraarterial infusion of Mannitol (20%; delivered IA, 12.5 mL over 2 minutes) in order to achieve osmotic disruption of the cerebral circulation has been well described. (Neuwelt et al. 1999) Neuwelt et al. performed 3498 BBB disruption procedures of the internal carotid artery using the same dose in conjunction with alkylating agent chemotherapy infusions in 405 patients. There was no associated risk of intracerebral hemorrhage, seizure or stroke. We have shown in our experience that there was no adverse effect of SIACI of Mannitol.

Bevacizumab: The current standard of care for recurring relapsing GBM is combination or monotherapy IV Bevacizumab chemotherapy. The currently used intravenous dose of Bevacizumab chemotherapy is 10mg/kg IV. In a recent trial of hepatocellular carcinoma, patients with locally advanced disease were treated with transcatheter arterial injection of Bevacizumab at a dose of 5 mg/kg given via superselective catheter. There was no toxicity associated with intraarterial administration of Bevacizumab. In our Phase I clinical trial, we found no adverse effects of IA therapy with Bevacizumab up to 15mg/kg.

Rationale for the dosing schedule: The proposed research trial design utilizes the addition of multiple intracranial superselective intraarterial chemotherapy infusions of Bevacizumab at diagnosis and combining this treatment with the standard chemoradiation.

Choice of Starting the Dose Used in this Study Regimen: The standard IV dose of Bevacizumab for GBM is 10mg/kg. The dose for non-small cell lung cancer is 15mg/kg IV. We have tested the Phase I safety SIACI up to15mg/kg. The dose-limiting toxicity of this dose is grade 2 or worse CNS hemorrhage or Grade 3 or worse non-hematologic toxicity which would be unexpected given the superselective nature of the intraarterial delivery.

Cerebral Angiography

The basic concept of cerebral angiography involves the navigation of a catheter up through the vasculature to the aortic arch. A typical four vessel angiogram studies the major vessels that supply the cerebral circulation either originate from the arch directly or from major tributaries that arise from the arch. These vessels include the Inominate Artery and Subclavian Arteries. Angiography begins with arterial access. By convention this usually involves the puncture and cannulation of the right femoral artery using the Seldinger technique. Local anesthetic is applied to the femoral skin and a single wall puncture needle is used to access the artery. Once the artery is entered then a wire is passed through the puncture needle into the artery and the needle is exchanged for an introducer sheath. The original wire is then removed. The introducer sheath is connected to a continuous heparinized saline flush. This introducer sheath then becomes the portal of entry for the diagnostic catheter. Under direct visualization of fluoroscopy, the diagnostic angiography catheter is then passed through the introducer sheath up the femoral artery, iliac artery and into the descending aorta with the use of a guide wire. This guide wire is later used to access the cerebral

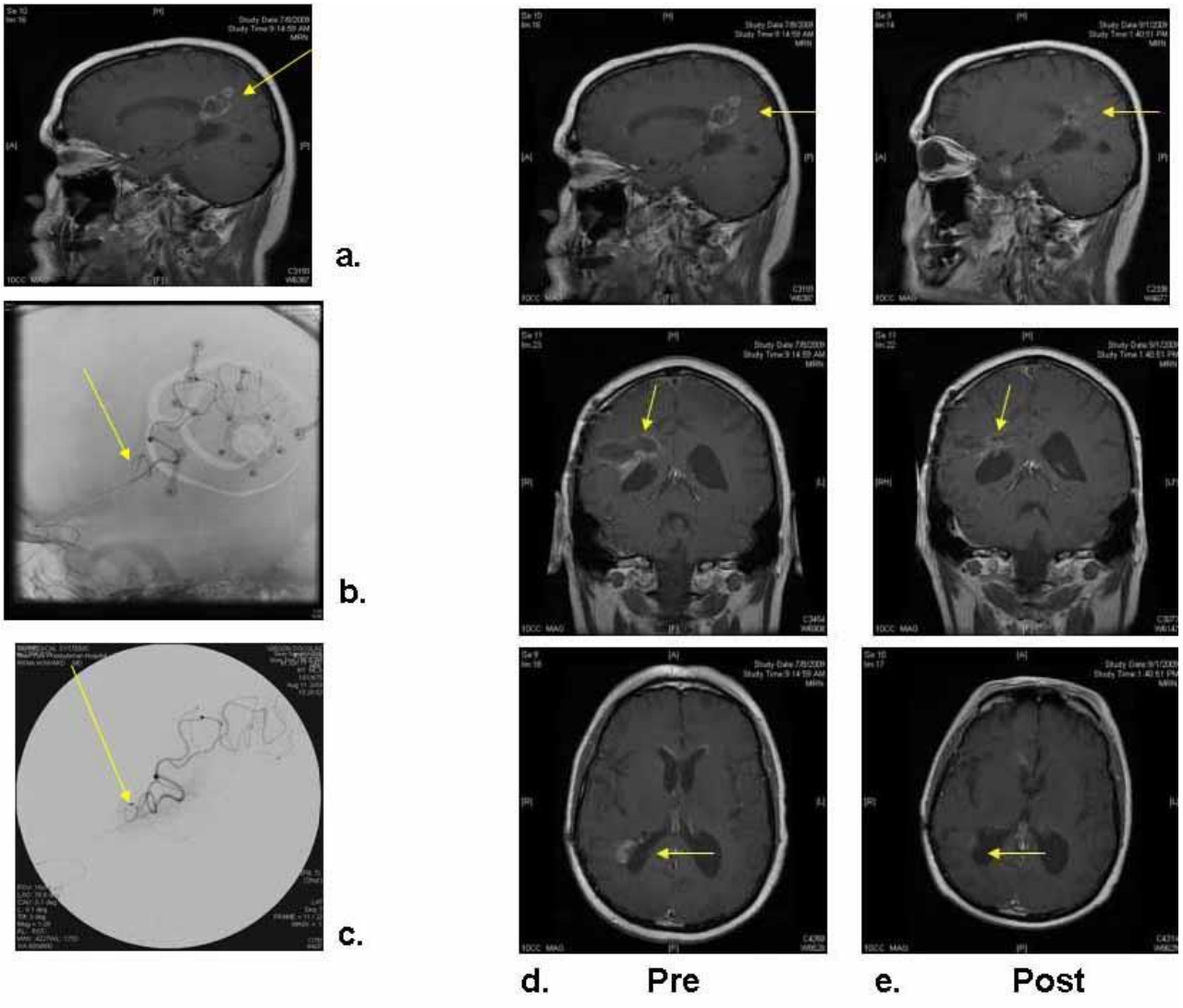
vasculature. The catheter and wire are then advanced together from the descending aorta to the thoracic aorta and into the aortic arch. Once in the arch the catheter wire combination are used to cannulate the Inominate artery. The Inominate artery gives rise to the right Common Carotid artery and the right Subclavian artery. The right vertebral artery usually has its origin from the proximal right Subclavian artery. The guide wire is advanced into the right Common Carotid artery and diagnostic catheter is then advanced over it. The wire is then removed and an angiographic image is obtained of the carotid bifurcation. The wire is then re-introduced and either the external or internal carotid artery is then accessed. For the intracranial circulation, a roadmap, an image generated by injecting contrast and allowing software to subtract out bone, is generated and the guide wire is advanced into the internal carotid artery. With the catheter in the internal carotid artery, a cerebral angiogram is performed over the cranium in AP and Lateral views. The same procedure is repeated on the left side of the cerebral circulation, with the catheter first being placed in the internal carotid artery. The vertebral arteries are also selectively cannulated and images of the posterior cerebral circulation are obtained**.** Decisions regarding which vessels to study will be determined by the angiographers and will be limited to the vessels felt to be supplying the tumor.

Super-selective Angiography

Most endovascular interventions begin with super-selective catheterization of a specific vessel. Prior to this the patient is given a Heparin bolus to obtain an automated coagulation time of approximately three times baseline (Usually 50 units/kg). Next, the diagnostic catheter is exchanged for a guiding catheter. A guiding catheter is stiffer and will provide more support to a micro catheter necessary for super selective catheterization. A new roadmap image is generated and now the micro catheter is introduced with a micro-guide wire. The specific vessel leading to the specific pathology is identified and the micro-guide wire is advanced into this vessel. The micro-catheter is then subsequently advanced over this micro-guide wire and the guide wire is removed. A super-selective angiogram is then performed by injecting contrast through the micro catheter to confirm that it is in the desired vessel, in this case meaning the vessel supplying the territory containing the brain tumor (see Figure 1). Even more super selective catheterization can be performed in order to localize the lesion more specifically. Once the desired catheter position has been achieved, the infusion of Mannitol and Bevacizumab will be performed over the specified time course. On the completion of the infusion, the micro catheter is removed and an angiogram is performed from the guiding catheter to check the cerebral circulation before the catheter and introducer sheath are removed. The guiding catheter is removed and homeostasis is achieved at the femoral artery with either manual compression or the use of an arterial closure device.

Figure 1.

Superselective Intracranial Angiography. **Fig. 1. Patient Response to IA Mannitol and Bevacizumab:** MRI images of patient 1 who underwent SIACI of unlabeled Avastin for recurrent GBM. A. Sagittal post contrast MRI showing tumor recurrence (yellow arrow). B. Superselective Microcatheter delivery (yellow arrow) in relation to the craniotomy site with visible miniplates. C. Contrast injection into the superselective catheter (yellow arrow) supplying the tumor. D. Sagittal, coronal and axial post contrast images prior to a single dose of Intraarterial Avastin (2mg/kg). E. The same images of the tumor 3-weeks after a single superselective intraarterial intracerebral injection (SIACI) showing diminished enhancement of gadolinium in the tumor suggesting tumor response.



5. RISK/BENEFIT OF THE STUDY

Data generated from clinical trials using IV Bevacizumab indicate both safety and efficacy of this drug. Available preclinical and clinical data demonstrate the safety and activity of intraarterial Bevacizumab in other solid cancer models. We will be able to follow prospectively patients who receive IA Bevacizumab and with standard chemoradiation to better understand the safety and ultimate efficacy of this regimen in patients with newly diagnosed glioblastoma.

Intraarterial Bevacizumab use is most likely to result in the following toxicities: CNS hemorrhage (3%) thromboemboloic event (11%), proteinuria (6%), and fatigue (11%). We have not reported any toxicities with superselective intraarterial Avastin infusion.[11]

This treatment would likely be harmful to a gestating embryo or fetus, if pregnancy were to occur. Women of childbearing potential and fertile men will be informed as to the potential risk of

procreation while participating in this research trial and will be advised that they must use effective contraception during the treatment period. A pregnancy test will be performed on each premenopausal female of childbearing potential immediately prior to entry into the research study.

There may be unknown or unanticipated discomforts or risks in addition to those specified above. CNS hemorrhage may lead to death. Because some of these procedures are relatively new and are attempts to advance medical knowledge, however, every precaution will be taken to assure the patients' personal safety and to minimize discomfort. In addition, risks of carotid and cerebral angiography apply to these patients. These include but are not limited to infection, bleeding, stroke, death, vessel injury, groin hematoma and retroperitoneal hematoma. Recent data indicates that the risk of cerebral angiography is less than 1%.

Individual patients may benefit by having a decreased risk of recurrence of brain tumor. They may also live longer. Alternatively, there may be no benefit to the patient's participation in this research study. Society will benefit if an effective first-line therapy is identified for an illness which attacks mainly middle-aged, productive individuals.

6. OBJECTIVES

6.1 Primary study endpoint:

The primary endpoint will be the 6-month composite overall response rate (CORR). We will determine this composite overall response rate (CORR) through the Response Assessment in Neuro-Oncology (RANO) criteria at 6 months (Appendix E), which accounts for treatment response as a composite of MRI changes, clinical response and changes in steroid use. We will define "evaluable" patients as patients who met eligibility requirements, have initiated therapy, and were not removed from the study for non-compliance or patient withdrawal within the first 6 months.

6.2 Primary objective:

In addition to the 6 month CORR, we will assess the 6-month progression-free survival (PFS) and overall survival (OS) by Kaplan-Meier survival analysis, assuming adequate follow-up time. PFS will be measured from the date of the first dose of SIACI Bevacizumab to the date of progression. OS will be measured from the date of the first dose of SIACI Bevacizumab to the date of death. A two-year follow-up time is expected to determine the safety of repeated superselective intracranial intraarterial infusions of Bevacizumab at a dose of 15 mg/kg IA.

6.3 Secondary objectives:

The safety of repeated SIACI of mannitol and Bevacizumab at 15mg/kg, coupled with chemoradiotherapy will be assessed. The descriptive frequency of subjects experiencing toxicities will also be tabulated. Toxicities will be assessed and graded according to the NCI Common Toxicity Criteria, version 3.0.

7. PATIENT SELECTION

7.1 Criteria for Inclusion:

- Male or female patients of ≥18 years of age.
- Patients with documented histologic diagnosis of glioblastoma multiforme (newly diagnosed)
- Patients must have at least one confirmed and evaluable tumor site.*
  *A confirmed tumor site is one which is biopsy-proven. NOTE: Radiographic procedures (*e.g.*, Gd-enhanced MRI or CT scans) documenting existing lesions must have been performed within **three** weeks of treatment on this research study.
- Patients must have a Karnofsky performance status ≥70% (or the equivalent ECOG level of 0-2) (see **Appendix A**; Performance Status Evaluation) and an expected survival of ≥ three months.
- Patients must agree to use a medically effective method of contraception during and for a period of three months after the treatment period. A pregnancy test will be performed on each premenopausal female of childbearing potential immediately prior to entry into the research study.

7.2 Criteria for Exclusion:

- Previous treatment with Bevacizumab.
- Women who are pregnant or lactating.
- Women of childbearing potential and fertile men who decline to use effective contraception during and for a period of **three** months after the treatment period.
- Patients with significant intercurrent medical or psychiatric conditions that would place them at increased risk or affect their ability to receive or comply with treatment or post-treatment clinical monitoring.

8. TEST AGENT PREPARATION AND ADMINISTRATION

8.1 Supplies of research study agent

Bevacizumab (Pharmacy)

8.2 Preparation of the research study agent

1. Bevacizumab

Chemical name: Bevacizumab
Storage: Store vials at 2 degrees to 8 degrees C.
Stability: Protect from light, do not freeze or shake. Do not mix with dextrose-containing solutions. Diluted solutions are stable for up to 8 hours under refrigeration

Half life: 20 days (range: 11-50 days)
Excretion: 2.75-5ml/kg/day

8.3 Doses of Research Study Agent:

Bevacizumab: The dose for IA Bevacizumab will be 15mg/kg

8.4 Route of Administration

- Bevacizumab will be given intracerebrally by superselective intraarterial infusion (SIACI).

8.5 Dosing Procedure

The doses and schedule of treatment for all of the drugs to be used in the research study are given above. This is an outpatient regimen, in which the drug is administered IA. Tumor response will be assessed after every 2 months with new MRI, which is standard of care for patients with GBM, or sooner at the PI's discretion**.**

1. Dose Interruption or Discontinuation:
   If an unacceptable toxicity is experienced during any portion of a patient's infusion, the investigator will follow the procedures outlined (see Section **VII.B**; Management of Toxicity; Dose Interruption or Discontinuation).

9. EXPERIMENTAL PLAN

9.1 Control Methods

There may be multiple research study sites, but no activation of additional sites may occur until there is IRB approval at those sites and either institutional agreements are obtained between The Feinstein Institute for Medical Research and these sites or until site-specific agreements with the drug suppliers are executed.

This is an open-label research trial, non-randomized phase I/II clinical research trial.

9.2 Duration of this Research Trial

To complete the trial approximately 23 patients will be required. The Feinstein Institute for Medical Research will be employed for this research study. Overall, it is anticipated that accrual to complete the study will be 12-18 months.

9.3 Statistical Considerations: Trial Design/Sample Size

For the current standard of care treatment with the Stupp protocol, we estimate that the probability of a Composite Overall Response Rate response (CORR) at 6 months is 60%. Therefore we estimate that a 6-month CORR of at least 85% with intraarterial bevacizumab would be clinically meaningful and worthy of further study.

A Simon two-stage "optimal" design (Simon, 1989) will be used whereby 9 patients would be treated in the first stage. If, amongst these 9 patients, there are 6 or fewer responders, then the study will be terminated. Conversely, if there are 7 or more responders amongst these initial 9 patients, then the study will enroll 14 additional patients for a total of 23 patients. If 18 or more out of the 23 patients respond, then the new treatment will be considered effective and a candidate for further larger clinical trials.

This design corresponds to testing the null hypothesis that the true objective response rate is 60% or less versus the alternative hypothesis that the true response rate is 85% or greater, with alpha = 0.05 and power = 0.80.

9.4 Accrual Rate

The planned sample size is 23 subjects. Assuming that 10% of patients are unevaluable or ineligible, we anticipate that a total of 25 patients will be enrolled in the study. Accrual is expected at a rate of 2-3 subjects per month.

10. PATIENT ENTRY AND TIME ON RESEARCH STUDY

10.1 Enrollment Process

Once a patient is identified as meeting the research study eligibility criteria, the patient will be asked to make an appointment with the PI to discuss treatment options.

The clinical coordinator and PI will maintain a Patient Log in order to ensure compliance with the entry criteria. In order to complete this log and confirm patient eligibility, the following will be documented:

- Patient initials, sex, age, Karnofsky Performance Status.

- Underlying diagnosis.
- Copies of operative and pathology reports.
- Copies of relevant lab reports.
- Copies of imaging reports documenting the location and extent of disease. The Sponsor may request copies of films.
- Confirmation of compliance with all inclusion/exclusion criteria.

10.2 Assignment of Patient Identification

Patients meeting the research study entry criteria will be assigned patient identification code as they enter the research study. Patient identification numbers will be assigned in chronological order.

Information regarding patient identification, as well as the amount of medication given to the subject, will be recorded in a research binder, as well as in the CRFs.

10.3 Inpatient / Outpatient Requirements

Patients will be treated on an outpatient basis.

Patients will be followed routinely by their private physician and oncologist as necessary, but must be evaluated by the investigative site one month after the IA procedure, and as clinically indicated.

10.4 End of Research study

All patients will be monitored for tumor response (by MRI scanning) and toxicity (by physical examination and blood tests) for the duration of bevacizumab therapy.

10.5 Follow-up Monitoring for Response and Safety

Patients in the phase I/II study, will be closely monitored for dermatologic and respiratory toxicity. The primary observation period after IA therapy will be 28 days. After the 28 day period, the patient will continue the oral Temozolomide regimen. Thereafter, toxicities will be monitored by history, physical examination and blood tests, as outlined in Table 1 below. Tumor response assessment by MRI will be performed after every 2 cycles of treatment with Temozolomide using the RANO Response Criteria, sooner if deemed necessary by the PI.

11. TREATMENT PLAN

Superselective intraarterial Dose of Bevacizumab will be given at a dose of 15mg/kg IA after recovery from surgical resection, usually 4 weeks after craniotomy and tumor resection, and 2 weeks after biopsy, then every 90 days until three doses have been given. The patient will start chemoradiation concurrently.

12. SUPPORTIVE PRODUCTS/CONCOMITANT MEDICATIONS

All medications and all concomitant therapy administration during the research study with the reasons for therapy use will be recorded in the data collection form. Surgery will also be recorded. For all subsequent visits, all concomitant therapy, which is continuing or has been **added**, **discontinued** or **had a dosage change** since the previous visit will be recorded. All patients should be maintained on the same medications throughout the research study period, as medically feasible.

1. Antiemetic medications: The specific post-treatment antiemetic therapy will be left to the investigators discretion, but the use of dexamethasone or any other steroids as antiemetics should avoided. Dose and frequency of usage for antiemetic therapy administered to treat patients receiving the research study drug will be recorded in the data collection form. For all subsequent visits, all concomitant therapy, which is continuing or has been added, discontinued or had dosage changed since the previous visit must be recorded.

2. Platelets: Platelet transfusions should be given to all patients if platelets are below 25,000/mm^3, and/or at the investigators discretion if any signs of bleeding occur.

3. Use of growth factors: The use of growth factors is permitted at the clinician's discretion.

4. Corticosteroids: A reasonable dose of corticosteroid (*e.g.*, dexamethasone) will be determined on clinical grounds for each patient if needed to alleviate increased intracranial pressure. An effort will be made to keep the patient on this steroid dose until the next scan is obtained. Changing steroid doses may complicate the interpretation of tumor response. Corticosteroid doses can be tapered as clinically indicated if the patient appears to be responding to therapy, as judged by serial scans, and neurological examination. Patients on steroids will receive prophylaxis for PCP pneumonia with Bactrim, unless they have a history of allergy to sulfa drugs.

13. PARAMETERS TO BE MEASURED

All patients enrolled in this will be evaluated according to the procedures described in Sections A, B and C below. All screening studies are to be performed ≤4 weeks prior to research study entry and, as noted below, some tests will be repeated within two weeks prior to initiation of treatment. Patients will be assessed for the presence of measurable disease and tumor response by MRI of the brain with and without contrast. A tumor response assessment (RANO Response Criteria) will be performed within 2 weeks of completion of every 2^{nd} cycle of treatment of maintenance Temozolomide, sooner if deemed necessary by the PI. Stable disease or better is required for patients to remain on research study.

13.1 Safety Assessments

All evaluations should be performed on or about the indicated research study day.

1. Assessment of Patient Eligibility and Signing of Informed Consent

   · Screening/Baseline: All patients must meet all inclusion criteria and not have any exclusion criteria to be eligible for the research study. All patients must sign an informed consent prior to enrollment, and prior to submitting to any research protocol-related procedure, unless such testing was performed as part of the routine clinical diagnosis or management of the patient.

2. Medical History (complete history at screening, including primary and secondary diagnoses; updates at the indicated times thereafter)

3. Disease Confirmation

   · Screening/Baseline: This needs to include a pathology report as well as reports from imaging studies (particularly MRI scans) to confirm that there is at least one measurable target or index lesion. If there is only one target lesion, it will be required that it show progression or be documented by biopsy. When possible, biopsy tissue will be sent to neuropathology for evaluation of tumor mutations such as VEGF over expression.

4. Performance Status Evaluation, Karnofsky or ECOG (see **Appendix A**)

   · Screening/Baseline
   · Prior to the start of research protocol treatment (within 1-2 weeks)
   · Monthly (*i.e.*, after every cycle) assessments will be made.
   · Every 6 months thereafter for ongoing stable disease or better.
   · For patients who need to go off research study, a final determination will be done.

5. Vital Signs (to include: body temperature, pulse rate, blood pressure, and respiration rate)

   · Screening/Baseline
   · In conjunction with the Physical Examination
   · As clinically indicated

6. Physical Examination (complete at screening, including height and weight; thereafter complete excluding height)

   · Screening/Baseline: This will be performed within 1-2 weeks of initiation of treatment. It will be repeated every cycle and then every 3 months thereafter as specified in Table 1.

7. Hematology (to include CBC with differential and platelet count)

   · Baseline PT and PTT levels as well as complete CBC within 1-2 weeks of initiation of treatment.
   · Thereafter, at the time of the monitoring Physical Examinations.
   · As clinically indicated.
   · Upon early departure from the research study

8. Serum Chemistries (to include: Na, K, Cl, Ca, bicarbonate, BUN, creatinine, glucose, bilirubin, AST, ALT, alkaline phosphatase, LDH, albumin, total protein)

   · Screening/Baseline: this must be within 2 weeks of enrollment on research study.
   · Thereafter, at the time of the monitoring Physical Examinations, if deemed necessary by the PI. (see Table 1).
   · As clinically indicated
   · Upon early departure from the research study

9. Pregnancy Test (urine or serum; in pre-menopausal, non-sterilized females)

   · Screening/Baseline (negative result must be documented on CRF)
   · As clinically indicated

10. Respiratory (Chest x-ray)
    - Screening/Baseline (within 4 weeks prior to procedure)
    - As clinically indicated

13.2 Disease/Tumor Assessment

1. MRI (contrast-enhanced) for Lesion Measurements (*i.e.*, brain) and a response assessment through RANO criteria

   ▪ Screening/Baseline (within 3 weeks prior to research study entry).

   ▪ Response will be assessed after every other cycle of the research protocol or sooner if deemed necessary by the PI, with a contrast-enhanced MRI and response assessment through RANO criteria.

   Contrast-enhanced MRI will be considered the standard method for evaluating tumor involvement. The location and dimensions of "target" (or "index") lesions, as well as other measurable lesions, will be documented on the patient's CRF. Lesion dimensions will also be recorded. MRI imaging must, whenever possible, use the same "cuts" for all follow-up imaging studies to assure accuracy of the data collected. For all objective responses the duration of the response will be determined, from the

day the initial response is observed (using baseline images for comparison) to the time that progression is observed.

14. OVERVIEW OF SCHEDULED PROCEDURES

Table 1:

Protocol Activity	Screening and Enrollment	Prior to IA Procedure	IA Procedure (Day 30[1], 120, and 210 post surgery)	1 Month After Procedure	Every 2 Months
Informed consent	X				
Confirm Inclusion/Exclusion Criteria	X				
Medical history	X				
Physical/Neurological Exam	X			X	
Vital signs	X			X	
KPS	X			X	
Hematology		X[2]		X	
Blood chemistry		X[2]		X	
Pregnancy test		X[2]			
IA Administration of Mannitol and Bevacizumab			X		
ECG		X[3]			
Brain MRI		X[2]			X
Chest X-Ray		X[3]			
CT Scan		X[4]			

Bodily Fluid	Amount	Frequency	Total
Blood	4 tbsp	12	48tbsp
Urine	15-20 mL	Once, then as needed	20 mL or more
Other_____ __			

[1] May be done at 2 weeks for biopsy subjects with well healed incisions

[2] Within 2 weeks of IA procedure

[3] Within 4 weeks of IA procedure

[4] If clinically needed

15. TOXICITY OF INVESTIGATIONAL DRUG

To date, at least 10,000 cancer patients have received bevacizumab at a dose ranging from 3mg/kg up to 20mg/kg IV every two weeks in previous clinical trials. Bevacizumab is used in Colorectal, breast, head and neck, brain, prostate, and renal cancer

Adverse events associated with IV bevacizumab administration reported in two recent clinical trials include hypertension, pain, headache, dizziness, alopecia, nausea/vomiting, stomatitis, fatigue, and thromboembolism. We do not anticipate any problems with intraarterial thrombosis at the catheter insertion site with bevacizumab and have not seen that to date in our patients. We are also aware of the sensitivity to endotoxin when it is delivered intrathecally, particularly at the highest product dose levels. Therefore, we will be alert to any Adverse Events that might be attributable to endotoxin exposure at the highest bevacizumab product dose levels.

16. MANAGEMENT OF TOXICITY

Careful assessment of toxicity experienced by the patient will be carried out throughout the course of this research study. Grades of toxicity (Modified NCI Common Toxicity Criteria 3.0) will be utilized as the criteria to determine appropriate management of the patient with respect to research study status.

All clinical adverse experiences, whether observed by the investigator, or observed or experienced by the patient, will be reported. Any significant change from baseline in a laboratory parameter will be reported to the Sponsor and will be documented. All treatment-emergent clinical and laboratory adverse events must be carefully evaluated for severity (see **Appendix B**; mild, moderate, severe, life-threatening), duration, and relationship to research protocol treatment (see **Appendix C**; unrelated, remote, possible, probable, related). Such information will be documented on the appropriate page of the patient's CRF.

The investigator, co-investigator, or designated health professional must be present at the time of scheduled patient visits for follow-up monitoring and should also to evaluate whether compliance with the research protocol is being maintained. If, at any point during the research study, significant changes occur in either the patient's clinical status or laboratory parameters, such changes will be followed until the parameter either returns to baseline or is adequately explained.

Clinical experience, thus far, indicates that adverse experiences (AEs) related to the use of bevacizumab are mostly cardiovascular (hypertension), and GI (acute nausea and vomiting). Other AEs that may occur but are less common in association with the administration of this agent is: neurotoxicity (including headache), diarrhea, anorexia and fatigue. These AEs are generally mild to moderate, reversible and/or manageable with symptomatic treatment, dose delays or reductions. In our experience with IA Bevacizumab, we have not seen any significant AE's related to the route of administration

16.1 Dose Modification

Toxicity grading is based on NCI-CTCAE, v 3.0.

Table 2

Dosage Modification Criteria and Guidelines for Management of Bevacizumab -Related Toxicities

NCI-CTCAE (v 3.0) Grade	Bevacizumab Dose Modification	Guideline for Management
Diarrhea		
Grade 1	None	Consider Loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until free of diarrhea for 12 hours)
Grade 2	None (Interrupt Bevacizumab if diarrhea persists over 48–72 hours despite optimal medical management)	Loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until diarrhea free for 12 hours)
Grade 3	Interrupt bevacizumab. Bevacizumab should not be started.	Interrupt bevacizumab until resolution to Grade ≤1, and restart at next reduced dose
Grade 4	Discontinue study treatment.	
Pulmonary Events		
All Grades	Temporarily interrupt bevacizumab pending the diagnostic evaluation. If the pulmonary adverse event is assessed as related to Bevacizumab, discontinue the patient from study treatment.	Unexplained dyspnea, either new or progressive, should be aggressively evaluated.
Rash		
Tolerable rash	None	Any of the following: minocycline [a], topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisone (short course) at discretion of investigator
Intolerable rash	Consider interruption reduction if unresponsive to symptomatic management. Re-escalation is allowed.	Manage as described above
Grade 4	Discontinue study treatment.	Manage as described above

[a] Recommended dose: 200 mg po bid (loading dose) followed by 100 mg po bid for 7–10 days.

16.2 Dose Interruption: Any sign of acute allergic reaction to bevacizumab, depending upon the severity, may necessitate temporary interruption or permanent discontinuation of the offending agent.

16.3 Removing Patients From Study

Every effort will be made to keep the patient on research study; however, in the event that a patient is withdrawn from the research study, the investigator should document the reasons for withdrawal as thoroughly as possible. This evaluation should include

final observations, as required by the research protocol at the time of the patient's withdrawal. The reason(s) for early termination must be clearly documented on the appropriate page of the patient's case report form (CRF). A CRF must be completed for any patient who receives ANY amount of treatment on this research study.

1. Criteria for Early Termination of Treatment under this Research Protocol: Patients meeting any of the following criteria will have participation in the research study discontinued:

   a. Significant deviation from the research protocol or eligibility criteria. Such patients will be considered protocol violations and will be removed from research study.

   b. Patients who develop an intercurrent illness, which, in the opinion of the investigator, would prevent their safe completion of treatment or required research study-related evaluations.

   c. Patients who are non-compliant with the research study or follow-up procedures.

   d. Patients who withdraw consent and elect to terminate their participation in the research study.

   e. The occurrence of a grade 3 or greater coagulopathy.

   f. Progression of disease as documented by MRI scan after the 2nd cycle of the treatment regimen of this research protocol.

   g. Termination of the research study by the Sponsor.

   h. Any other reason, which, in the opinion of the investigator, would justify removing the patient from the research study. In such a case, the investigator's reason for a patient's removal must be recorded on the patient's CRF.

2. Replacements: Should a patient withdraw or need to be withdrawn from the research study prior to the second cycle of treatment, a replacement subject will be sought using the stipulated research protocol entry criteria. Patients who have received 2 cycles of treatment and have completed toxicity and tumor response assessments, but have shown disease progression, will be not be eligible to receive additional treatment under this research protocol, but will be considered evaluable and will not be replaced by additional patients.

17. ADVERSE EXPERIENCES

17.1 Definition: An **adverse experience** (AE) is any undesirable, noxious, or pathological change, compared to pre-existing conditions, that occurs to a subject during a clinical research study or the follow-up period, whether or not it is considered to be related to the test drug. Adverse experiences include:

- Suspected adverse drug reactions.

- Reactions from drug overdose, abuse, withdrawal, sensitivity, or toxicity.
- Significant changes or abnormalities, when compared to baseline, in structure (sign), function (symptom), clinical laboratory results, or physiological testing. This includes any worsening of a pre-existing condition temporally associated with the use of research study medication.
- Other medical events, regardless of their relationship to the test drug, such as injury, surgery, accidents, extensions of symptomatology, or apparently unrelated illnesses.

17.2 Evaluation: The investigator will determine the seriousness, intensity, and causality of an adverse experience associated with the use of the test medication (*i.e.*, experiences where there is a reasonable possibility that the experience may have been caused by the test medication) based on the following definitions:

1. Serious Adverse Experiences (notify Sponsor if adverse experience is both **serious and unexpected** within 24 hours [see below]; document on CRF)

   THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE) IS ANY OF THE FOLLOWING:

   - ANY DEATH THAT OCCURS WHILE THE PATIENT IS ENROLLED IN THE STUDY INCLUDING THE FOLLOW-UP PERIOD OR WITHIN 30 DAYS OF COMPLETING THE STUDY.
   - IMMEDIATELY LIFE-THREATENING ADVERSE EVENT.
   - REQUIRES INPATIENT HOSPITALIZATION.
   - PROLONGATION OF AN EXISTING HOSPITALIZATION.
   - CONGENITAL ANOMALY/BIRTH DEFECT
   - MEDICALLY IMPORTANT EVENT*
   - DISABILITY/INCAPACITY (PERSISTENT OR SIGNIFICANT)

   *Medically important events that may not result in death, be live-threatening or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the experience may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition.

2. Unexpected Adverse Experience
   An **unexpected adverse experience** is any adverse drug experience, the specificity or severity of which is not consistent with those noted in the current research protocol and/or Investigator's Brochure. This refers to any adverse drug experience that has not been previously observed, (*e.g.,*not included in the Investigator's Brochure), rather than from the perspective of such an experience not being anticipated from the pharmacologic properties of the product.

3. Non-serious Adverse Experiences

All other adverse experiences, not fulfilling the previous definitions, are classified as non-serious.

17.3 **Documenting Adverse Experiences:** All adverse experiences (including SAEs) are to be accurately recorded on the Adverse Experience page of the patient's CRF. Each experience will be graded on a four-point scale (see **Appendix B**; mild, moderate, severe, life-threatening) as to severity. The date of onset as well as the duration of the experience will be recorded. In addition, the method used to treat the adverse experience and the outcome of the adverse experience will also be noted. The investigator will attempt to assess the relationship of the experience (unrelated, remote, possible, probable, related) to the test drug.

17.4 Reporting SAEs, Unexpected Adverse Experiences, and Patient Deaths

1. All SAE's will be reported according the Northwell Health policy.

2. Information to be Provided by the Investigator

Initial Information: At the time of the initial contact(s), the investigator must transmit information to the Sponsor or designee for completion of a Safety Report. The Sponsor or designee will require that all of the following information about the patient and the adverse experience:

- Patient identification code, sex, age or date of birth
- Height, weight or body surface area (where required for dose calculation)
- Underlying diagnosis and extent of disease
- Dose and frequency of test medications administered
- Dates of test medication administration
- Description of event, including date of onset and duration
- Date of death (if applicable)
- Intervention(s) required
- Concomitant therapy (including regimen(s) and indication)
- Pertinent laboratory data/diagnostic test (including dates)
- Pertinent medical history
- Test medication status (dose interrupted, discontinued)
- Did event abate after interruption of test medication administration (if applicable)?
- Did event recur after test medication was reintroduced (if applicable)?

In addition to the above information, the Sponsor will require the investigator's assessment of the following:

- Severity of the adverse experience
- Relationship of the adverse experience to research study treatment
- Outcome of the adverse experience

E. Follow-up Information on an SAE: Appropriate diagnostic tests should be performed and therapeutic measures, if indicated, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the event has resolved or is otherwise explained. Follow-up data concerning the SAE (*e.g.*, diagnostic test reports, physician's summaries, etc.) must also be submitted to the Sponsor, as they become available, preferably by telefax.

Review of an SAE: The PI will review each serious and unexpected adverse experience report and further evaluate the relationship of this adverse experience to test medications and to the patient's underlying disease. Based on this assessment, a decision will be made concerning the need for further action. The primary consideration governing further action is whether new findings affect the safety of other patients participating in the clinical research study. If the discovery of a new adverse experience related to test medication raises concern over the safety of its continued administration to patients, the PI will take immediate steps to notify the IRB and all investigators participating in clinical studies of the test drugs used in this research protocol

Further action required may include any of the following:

- Modification of the research protocol.
- Discontinuation or suspension of the research study.
- Alteration of the informed consent process by modification of the existing consent form and informing current research study participants of new findings.
- Modification of previously identified expected adverse experiences lists to include adverse experiences newly identified as test medication-related.

18. PRECAUTIONS

18.1 Precautions Regarding Procreation: Women of childbearing potential and fertile men will be informed as to the potential risk of procreation while participating in this research trial and will be advised that they must use effective contraception during the treatment period. A pregnancy test will be performed on each premenopausal, female of childbearing potential immediately prior to entry into the research study. A negative pregnancy test must be documented on the CRF prior to administration of the test material.

18.2. Additional Precautions: Information regarding precautions and adverse experiences associated with this test medication can be found in the Scientific Background section of this document and in the package inserts for the individual research study medications.

19. REGULATORY CONSIDERATIONS

19.1 Conditions for Modifying or Terminating the Research Study

1. Modifications

   In the event that modifications in the experimental design, dosages, parameters, patient selection, etc., are indicated or required, such changes will only be instituted following consultation between the Sponsor and investigator and will be accomplished through formal amendments to this research protocol and approval by the appropriate review committees, except where necessary to eliminate apparent hazards to patients.

   A modification to the research protocol will not be made without the express written approval of the Sponsor. Any amendment prepared by the Sponsor will be implemented according to the Sponsor's standard operation procedures and will be reported to the appropriate IRB(s), and made a formal component of the research protocol document.

2. Termination

   Should the Sponsor and/or the investigator(s) discover conditions, during the course of the research study, that indicate it should be discontinued, an appropriate procedure for termination will be instituted. The principal investigator is responsible for assuring continuing review and approval of the clinical research study. The investigator must also promptly report all changes in the research activity and all unanticipated problems involving risk to the patients or others to his/her IRB. If the research study remains in progress for more than one year, the investigator must obtain annual renewal and re-approval from the IRB. Documentation of renewal must be submitted to the Sponsor.

19.2 Informed Consent

A copy of the IRB-approved consent form document to be utilized during this research study will be maintained by the PI.

Prior to entry into this research study, the purpose and nature of the research study and possible adverse effects must be explained to each patient in the presence of a witness. It is the responsibility of the investigator to obtain written informed consent from each patient. A copy of the signed informed consent document should then be given to the patient. The original executed version must remain in the patient's file and must be available for verification by a representative of the Sponsor.

The study will rely on the Karnofsky Performance Scale and the discussion with the research team to assess the subject's capacity to consent themselves into the study. This discussion will only be performed by clinicians experienced in determining capacity. In addition, the study will utilize a research review questionnaire in the consent form to assist in making this determination. Individuals that are deemed to have the ability to consent themselves will also be required to designate a research proxy to act on their behalf if it is determined that they later lose the capacity to make decisions about participation. In situations that a subject is deemed cognitively impaired, they may be enrolled into the study using a legally authorized representative or research proxy. The subject will then be asked to provide assent, as appropriate.

19.3 Documents to be Submitted Prior to Study Initiation

The following documents must be submitted prior to research study initiation:

- Curriculum vitae of the principal investigator and each sub-investigator. Physician CVs should include medical license numbers.
- Written, signed notification of IRB approval of the research protocol (copy).
- Written, signed notification of the approval of the informed consent document to be utilized during the research study (copy).
- The stamped/signed by IRB informed consent document to be utilized during the research study (copy).
- Signed and dated Investigator Agreement; agreement appears as final page of the research protocol (original).

20. INVESTIGATOR RESPONSIBILITIES

20.1 Medical Supervision: Medical supervision for the conduct of this research protocol is the responsibility of the Principal Investigator. The Principal Investigator may delegate certain day-to-day activities to a sub-investigator, but retains overall responsibility for ensuring that the research study is conducted properly and in accordance with the design and intent herein. The Principal Investigator is responsible for ensuring that drugs and devices are available for treating possible medical emergencies. The Principal Investigator is responsible for ensuring that the research study is conducted according to sound medical practices.

20.2 Confidentiality: The PI affirms that all research study results and information furnished by acquired by the Sponsor during the course of the research study will be maintained in strict confidence. Such information, as well as data generated from this research study, may need to be communicated to the PI's or sub-investigators' review committees, under an appropriate understanding of confidentiality.

20.3 Patient Exclusion Log: A record listing all patients considered for entry into the research

study and subsequently excluded must be maintained by the PI and his department. Patients excluded from the research study will have the reason for exclusion recorded on the Patient Exclusion Log.

20.4 Recording and Processing of Data: Individual CRFs must be completed in black ink. All data must be carefully completed to permit meaningful interpretation. CRFs are designed for computer processing and analysis. Corrections to entered data may be made by drawing a single line through the information to be corrected. All such corrections must be initialed and dated. No correction fluid or obscuring tape may be utilized. A completed CRF is required for every patient who received any amount of test medication. CRFs must be signed by either the PI or his designee.

20.5 Record Retention: Records from the research study must be retained by co-investigators until they can be retrieved by the PI or designee once the data from individual research study patients has been collected and CRFs completed.

20.6 Laboratory Reports: Prior to initiation of this research study, the investigator must supply the PI or designee with the normal laboratory values for the laboratories to be utilized. The corresponding laboratory certification number must also be noted. Laboratory safety evaluations must be performed at the intervals specified. If unexplained laboratory abnormalities occur, corroborative tests will be performed until the laboratory parameter has returned to normal and/or adequate explanation of the abnormality has been provided. Copies of any additional records pertinent to the research study (*e.g.* laboratory data, radiological reports, patient chart summaries, autopsy reports) must be made available to the PI, if requested, with due precaution taken to ensure patient confidentiality.

20.7 Monitoring: This research study will be monitored by representatives of the PI throughout its duration. Monitoring will be in the form of personal visits with the investigator and his/her staff as well as any appropriate communications by telephone, telefax, or mail. Every effort will be made to maintain the anonymity and confidentiality of patients during this clinical research study. In addition to the study's stopping rules and routine medical assessments that are performed at each study visit, the Director of Clinical Research, Cardiovascular Division, Northwell Health, Christina Brennan, MD, will be serving as the study's medical monitor. Dr. Brennan has vast clinical trial experience and extensive endovascular experience. She has the knowledge and expertise required to review the incoming data to determine if aspects of the study need to be changed or stopped. In addition, any unanticipated problems will be reported to the IRB and regulatory agencies as per their specific reporting requirements. Data will be reviewed after each dose limiting toxicity and any unanticipated adverse events. In addition, the IND monitoring will be conducted by Alexis Demopolous, MD and Sherese Fralin, MSN, FNP in adherence to FDA regulations.

21. PROTOCOL DEVIATIONS

Departures from either the research protocol entry criteria or the experimental plan, as outlined herein, must be reported the principal investigator and the Office of the IRB as per their reporting requirements. Anticipated deviations should be submitted to the Principal

Investigator and the IRB prior to its initiation so that a protocol exception may be granted. The investigator must contact the PI or designee to discuss the associated circumstances. The PI will decide as to the subject's continued research protocol eligibility status. All research protocol deviations and the reasons for such deviations must be noted on the appropriate page of the subject's CRF.

22. ANALYSIS OF DATA

Data Review During this Research study: All research study data are to be reviewed at regular meetings of the principal participants to monitor the progress of the project.

23. REFERENCES

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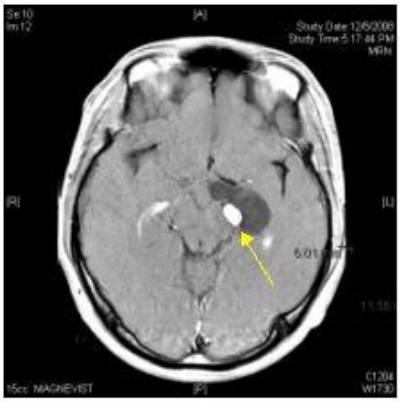
Figure 1.

Superselective Intracranial Angiography. The patient was a 30 year male with seizures. MRI showed a contrast enhancing lesion in the left mesial temporal lobe (Figure 1A and B). Angiography showed a vascular lesion supplied by the anterior choroidal artery (Figure 1C). Superselective catheter angiography (yellow arrows Figure 1D and 1E) allowed selective delivery of contrast only in the distribution of the anterior choroidal artery.



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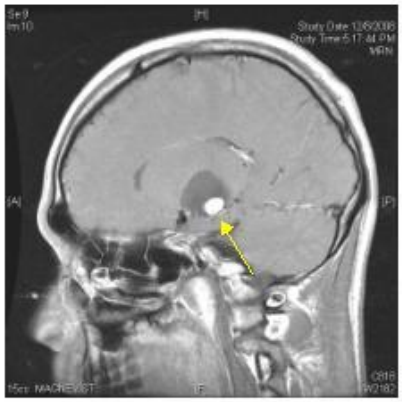

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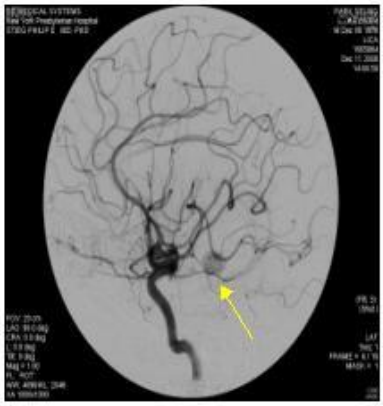

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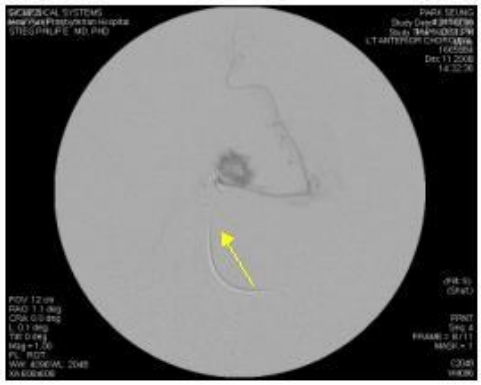

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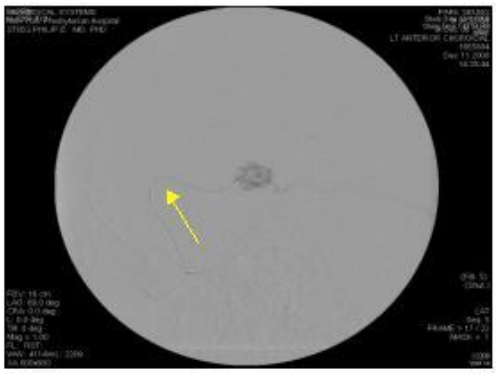

e.

APPENDIX A

Performance Status Evaluation

Percent	Karnofsky Performance Status Description	Level	ECOG*
100	Normal; no complaints, no evidence of disease	0	Normal Activity
90	Able to carry on normal activity; minor signs or symptoms of disease		
80	Normal activity with effort; some signs or symptoms of disease	1	Symptoms but ambulatory
70	Cares for self; unable to carry on normal activity or do active work		
60	Requires occasional assistance but is able to care for most needs	2	In bed < 50% of time
50	Requires considerable assistance and frequent medical care		
40	Disabled; requires special care and assistance	3	In bed $\geq$ 50 % of time
30	Severely disabled; hospitalization is indicated although death is not imminent		
20	Very sick; hospitalization is necessary	4	100% bedridden
10	Moribund; fatal processes progressing rapidly		
Abstracted from: Karnofsky DA, et al., Cancer. 1948; 1:634-656		*ECOG Eastern Cooperative Oncology Group	

APPENDIX B

Clinical Symptomatology and Adverse Experience Grading Scale

- Mild: Awareness of symptom, but, easily tolerated. Usually transient requiring no special treatment; does not interfere with usual status or activities

- Moderate: May be ameliorated by simple therapeutic measures; may interfere with usual activities

- Severe: Incapacitating; unable to perform usual activities

- Life-threatening: Requires immediate intervention; need for emergency treatment

APPENDIX C
Clinical Adverse Experiences: Determining Relationship to Test Drug

Unrelated

This category applies to those adverse experiences which, after careful medical consideration, are clearly felt to be due to extraneous causes (disease, environment, etc.) that are unrelated to the administration of test drug.

Remote (must have first two)

This category applies to those adverse experiences which, after careful medical consideration, are felt unlikely to be related to the administration of the test drug. The relationship of an adverse experience to the test drug can be considered remote if:

- It does not follow a reasonable temporal sequence from administration of the drug.
- It could readily have been a result of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
- It does not follow a known response pattern to the suspected drug.
- It does not reappear or worsen when the drug is re-administered.

Possible (must have first two)

This category applies to those adverse experienceswhich, after careful medical consideration, are felt unlikely to be related to the administration of the test drug, but the possibility cannot be ruled out with certainty. The relationship of an adverse experience to the test drug can be considered possible if:

- It follows a reasonable temporal sequence from administration of the drug.
- It could readily have been a result of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
- It follows a known response pattern to the suspected drug.

Probable (must have first three)

This category applies to those adverse experiences which, after careful medical consideration, are felt with a high degree of certainty to be related to the administration of

the test. The relationship of an adverse experience to the test drug can be considered probable if:

- It follows a reasonable temporal sequence from administration of the drug.
- It could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
- It disappears or decreases upon cessation of drug or reduction in dose*.
- It follows a known response pattern to the suspected drug.

Related (must have first three)

This category applies to those adverse experiences, which, after careful medical consideration, are felt to be related to the administration of the test. The relationship of an adverse experience to the test drug can be considered related if:

- It follows a reasonable temporal sequence from administration of the drug or drug levels have been established in body fluids or tissues.
- It could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
- It disappears or decreases upon cessation of drug or reduction on dose and appears upon re-challenge*.
- It follows a known response pattern to the suspected drug.

*There are exceptions when an adverse experience does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists; *e.g.*, 1) tardive dyskinesia, 2) fixed drug eruptions.

APPENDIX D
Risks

General Anesthesia
Any operation using anesthesia includes a risk of an adverse reaction to anesthetic agents. The risks of anesthesia are the same for any surgical procedure for which anesthesia is used. After receiving a general anesthetic side effects may include the following.

- The most common side effects are nausea and vomiting, from 3 to 5% (1% means one in one hundred).
- Other side effects include dry throat, sore throat, sore jaw, sore muscles and perhaps some short-term memory loss or confusion. The vast majority of patients recover from these side effects in a day or so.
- There is a risk of unexpected and unusual reactions to the anesthesia that can lead to heart problems such as a myocardial infarction (heart attack) or lung problems such as respiratory failure (breathing failure).
- All procedures involving anesthesia carry the risk of death.

Angiography
The super selective delivery of Bevacizumab to the tumor first involves "catheter angiography" via groin. Angiography involves the placement of a catheter in the groin via femoral artery. The catheters are then threaded up to the arteries in the brain. Risks associated with the angiography procedure are very small but include:

- Pain at the groin site, groin hematoma (<1%)
- Stroke in the brain (<0.05%)
- Allergic reaction and damaging of blood vessels that may lead to bleeding

Therapy with Mannitol
Treatment with Mannitol has been used extensively to break down the blood brain barrier or wall between blood vessels and the brain tissue. The risks associated with this therapy include:

- Increased risk of a hemorrhage of blood into either the brain tumor or into adjacent normal brain tissue. This is called a hemorrhagic stroke and has a risk of about 1%.
- The resulting blood clot in the brain is called a "hematoma" and can itself cause weakness on one side of the body, speech problems, memory loss, understanding difficulties. A large hematoma in the brain can also require surgery to remove the hematoma and relieve pressure from the brain (this risk is less than 0.05%).
- If a subject suffers from increased intracerebral pressure, intra-arterial infusion with Mannitol, may increase or decrease that pressure.

Other risks related to Mannitol include:

- Pulmonary congestion, fluid imbalance, acidosis, thirst/dryness of mouth, urinary retention, edema, headache, blurred vision, convulsions, nausea, vomiting, rhinitis, arm pain, skin necrosis, thrombophlebitis, chills, dizziness, urticaria, dehydration, hypotension, tachycardia, fever and angina-like chest pains

Intra-arterial therapy with Bevacizumab

Bevacizumab is a drug that targets the protein on the tumor called VEGF. Intravenous treatment with this drug has been shown to cause some adverse side effects. We hope by delivering this drug "selectively" to the tumor, we may minimize the body's exposure to the drug, however, the safety of this method is unknown and is the purpose of this study.

- The subject's body will be exposed to some of the Bevacizumab as it "re-circulates" in the blood stream. Therefore, although the method of delivery of Bevacizumab is different, subjects may experience some of the systemic risks of Bevacizumab, which are most likely rash and diarrhea. These symptoms can often be managed with medications and rarely cause the need to stop the therapy with Bevacizumab.
- In clinical trials, additional serious side effects in subjects receiving Bevacizumab included gastrointestinal complaints and even perforation of the stomach or intestine.
- Bevacizumab has been shown to slow wound healing in other clinical trials and has been shown to cause bleeding in the lungs. Blood clots in the lungs have also been reported which may cause subjects to have trouble breathing.
- Elevation of blood pressure may occur and congestive heart failure may occur.
- The most common adverse events seen in subjects receiving Bevacizumab across all studies were weakness, pain, abdominal pain, headache, hypertension, diarrhea, nausea, vomiting, loss of appetite, mouth sores, constipation, upper respiratory infection, nosebleeds, difficulty breathing, skin irritation, and proteinuria (a possible sign of kidney malfunction).
- Additional risks associated with bevacizumab include: bleeding of the brain and spinal cord, bleeding of the vagina, rectal bleeding, alteration of taste, dry skin, scaling skin, excessive tearing of the eyes, and back pain.
- Reduction in the dose or interruption of Bevacizumab will be considered if changes in liver function are severe. There may be additional risks as a result of the super selective method of drug delivery; the risks are unknown.

Blood-Drawing

There are no major risks of having blood drawn. It can be uncomfortable and can sometimes cause a bruise. In rare cases, it can cause fainting. Only trained staff will draw blood.

Magnetic Resonance Imaging (MRI) Studies

The magnetic resonance imaging (MRI) machine is a powerful magnet. This magnet may cause any metal in the body to move. Otherwise, there are no known risks of MRI.

- Some people with claustrophobia (fear of closed spaces) may find the MRI scanner too confining. In that case, subjects can ask to be removed from the scanner and this will be done immediately.
- The MRI scanner makes a loud beeping sound. Subjects may be asked to wear protective earplugs during scanning.
- The dye that is injected for the scan is well tolerated.
- Subjects may feel dizzy, queasy or get a headache with it or notice a cold feeling near the injection site.
- There is a rare chance of having an allergic reaction to the dye that very rarely can be serious and life threatening.

Chest x-rays
In this study, subjects will be exposed to radiation during the chest X-ray. While we cannot be sure any dose of radiation is completely safe, the amount subjects will be exposed to in this study is not known to cause health problems.

CT scans
In this study, subjects will be exposed to radiation during the CT scan. Although the amount subjects will be exposed to is higher than that of a usual x-ray, the risk of harmful effects from a single exam is very small.

Collection of Sensitive Information
Some of the questions we will ask subjects are personal. They may feel embarrassed or stressed. Subjects may ask to see the questions before deciding whether or not to take part in this study.

Unknown Side Effects
As with any drug, there might be side effects that are unknown at this time. Subjects will be closely watched for side effects. Subjects should report any unusual events to the study staff.

Risks to Women of Childbearing Potential and Pregnant Women
We do not know the effects of Bevacizumab on fertility or a fetus. For this reason, if subjects believe they are pregnant or have a chance of becoming pregnant, they should not take part in this study. A blood pregnancy test will be performed prior to the start of study procedures. If subjects are pregnant, they will not be allowed to be in the study.

APPENDIX D
Tumor Assessment-RANO Response Criteria

☐Complete Response (CR)	• disappearance of all enhancing measureable and non-measureable disease sustained for at least 4 weeks • no new lesions • stable or improved non-enhancing (T2/FLAIR) lesions • off corticosteroids or on stable physiologic doses only • stable or improved clinically
☐Partial Response (PR)	• ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measureable enhancing lesions sustained for at least 4 weeks • no progression of non-measurable disease • no new lesions • stable or improved non-enhancing (T2/FLAIR) lesions • same or reduced corticosteroid dose compared with baseline • stable or improved clinically
☐Stable Disease (SD)	• does not qualify for complete response, partial response or progression • stable non-enhancing (T2/FLAIR) lesions on same or lower corticosteroid dose compared to baseline scan. • stable clinically
☐ Progressive Disease (PD)	• ≥25% increase of enhancing lesions on stable or increasing doses of corticosteroids • significant increase in T2/FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy not caused by co-morbid events • any new lesion • clear clinical deterioration not attributable to other causes apart from the tumor or changes in corticosteroid dose • clear progression of non-measurable disease

INVESTIGATOR STATEMENT

I have read Research protocol entitled:

PROTOCOL TITLE: PHASE I/II TRIAL OF REPEATED SUPER-SELECTIVE INTRAARTERIAL CEREBRAL INFUSION OF BEVACIZUMAB FOR TREATMENT OF NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

and as the responsible investigator, I agree to conduct this research study as outlined herein.

_____
Principal Investigator (please print)

_____ _____
Principal Investigator (signature) *Date*

_____
Investigator Address

_____
Investigator Telephone Number

Signature on this page assures the Sponsor that, to the best of the investigator's knowledge, the affiliated IRB operates in accordance with the U.S. Code of Federal Regulations, and that the investigator understands, and agrees to abide by, all regulatory obligations while conducting this clinical investigation.

Once signed, the original of this form should be detached from the research protocol and returned to the Sponsor (please retain a copy for your files).