Modification Request Form

PI Name:	Justin Fraser, MD	**IRB Protocol #:**	16-0253-F6A #002
Title of Study:	Low-dose Intra-arterial Bevacizumab for Edema and Radiation necrosis Therapeutic Intervention (LIBERTI)		

PI Signature: _____ Date: 6/21/2016

1. **Address Change for Protocol Correspondence?** ○ Yes ◉ No

If yes, indicate new address: _____

Title change? ○ Yes ◉ No

If yes, indicate new title: _____

3. **Is this a *one-time* request for a deviation from the currently approved protocol or an exception to the currently approved enrollment criteria?** ○ Yes ◉ No

4. **Consent/Assent Form Change?** ○ Yes ◉ No
If yes, be sure changes are reflected in all your revised consent/assent documents **and any applicable HIPAA documents**.

5. **Select One:**
◉ This modification does not increase risk to study participants.
○ This modification may or will increase risk to study participants.

6. **Is this modification request due to an Unanticipated Problem or Adverse Event?** ○ Yes ◉ No

7. **In your professional opinion, does this modification involve information that might relate to a subject's willingness to continue to take part in the research?** ○ Yes ◉ No
If yes, state how the information will be communicated to subjects (i.e., re-consent, send letter, etc.):

_____

8. **Changes Made To:** (check all that apply and attach appropriate documents)

Form A: General Information Sheet
- ☐ Anticipated Project End Date
- ☐ Estimated # of Subjects
- ☐ Subject Population
- ☐ Vulnerable Subject Population
  - ☐ Impaired Consent Capacity (Form T)
  - ☐ Children age 17 or less (Form W)
  - ☐ Pregnant Women (Form U)
  - ☐ Prisoners (Form V)
  - ☐ Other vulnerable population
- ☐ Funding/Support (*some federal agencies have specific requirements – see guidance*)

- ☐ Study Personnel (SP List Template)
- ☐ Medical Device (Form P)
- ☐ Study Drug (Form O)

Other - Describe:

_____

_____

Form B: Research Description & Appendices
- ☐ Objectives
- ☐ Inclusion/Exclusion Criteria
- ☐ Subject Recruitment
- ☐ Procedures/Materials
- ☐ Research Procedures
- ☐ Grant Application
- ☑ Sponsor Protocol; Investigator Brochure

Forms C-F
- ☐ Consent Form (Form C-Medical; Nonmedical) or combined Informed Consent/HIPAA Form
- ☐ Assent Form (Form D-Medical; Nonmedical)
- ☐ Waiver of Informed Consent (Form E)
- ☐ Waiver of Documentation of Informed Consent (Form F)

Forms I-K
- ☐ HIPAA De-Identification Certification (Form I)
- ☐ HIPAA Waiver of Authorization (Form K)

Modification Request Form

PI Name:	Justin Fraser, MD	IRB Protocol #:	16-0253-F6A #002
Title of Study:	Low-dose Intra-arterial Bevacizumab for Edema and Radiation necrosis Therapeutic Intervention (LIBERTI)		

REQUIRED: For each proposed modification, describe the currently approved procedures, forms, etc. and then summarize the proposed change, addition, etc. Include a justification for the modification request. Add additional sheets if necessary.

Example:
Currently Approved: study staff as listed on attached SP List Template

Proposed Revision: add Jane Doe, MD, as co-investigator, Dr. Doe has completed human subject protections training, Dr. Doe is a new faculty member who will be working with subjects on this protocol and she is authorized to obtain consent.

1. Currently Approved: Protocol Version 2
Protocol Version 3 (see highlightes for changes. Added specific outcome measures.

Proposed Revision:

2. Currently Approved:

Proposed Revision:

3. Currently Approved:

Proposed Revision:

4. Currently Approved:

Proposed Revision:

Norton Healthcare/Dept of Neurosurgery
LIBERTI **Version 3**

STUDY TITLE: Low-dose Intra-arterial Bevacizumab for Edema and Radiation necrosis Therapeutic Intervention (LIBERTI)

Participating Centers:

Norton Healthcare
University of Kentucky Healthcare

Sponsor/Principal Investigator:*Shervin R. Dashti, MD, PhD*
4950 Norton Healthcare Blvd. #205
Louisville, KY 40241
Telephone: 502-394-6390
Fax: 502-394-6388
Shervin.dashti@nortonhealthcare.org

Version 3

Date: 20 June 2016

CONFIDENTIAL STATEMENT

SCHEMA

To assess the overall safety and efficacy of intra-arterial (IA) bevacizumab for the treatment of radiation necrosis. A single 2.5 mg/kg dose of bevacizumab will be given intra-arterially after blood-brain-barrier disruption using 25% Mannitol at 4-12 ml/sec for 30 seconds.

INTRA-ARTERIAL DOSING SCHEMA	
Dose NUMBER	**Agent**
Dose#1: Day 0	2.5 mg/kg X 1 dose

INVESTIGATOR'S STATEMENT AND SIGNATURE

I have read and understand this protocol, attachments, and (name of study drug). I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with ICH GCP guidelines and the ethical principles of the Helsinki Declaration.

Sponsor/Principal Investigator:

Signature: _____ Date: _____

Printed Name: _____

CONTACT DETAILS OF KEY PERSONNEL

Role	Name	Contact Information
Sponsor/Principal Investigator	*Shervin R. Dashti, MD, PhD*	4950 Norton Healthcare Blvd. #205 Louisville, KY 40241 *Telephone: 502-394-6390* *Fax: 502-394-6388* *Shervin.dashti@nortonhealthcare.org*
Principal Investigator	Justin Fraser, MD	UK Medical Center Department of Neurosurgery Medical Science Building Room MS 108A Lexington, KY 40536-0298 *Telephone: (859) 323-0616* *Jfr235@uky.edu*
Sponsor Project Manager	Elodie Elayi	UK Center for Clinical and Translational Science 800 Rose Street, Pavilion H, Room C300 Lexington KY, 40536 *Telephone: (859) 323-7939* *Fax: (859) 257-9560* *Elodie.elayi@uky.edu*
Sponsor Study Monitor	Karen Bowen, RN	Bluegrass Research Consultants, Inc. 8020 Sea Hero Run Versailles, KY 40383 *Phone: (859)396-4655* *FAX: (859)873-9963* *Bowen73@windstream.net*
SAE Reporting	*Shervin R. Dashti, MD, PhD*	4950 Norton Healthcare Blvd. #205 Louisville, KY 40241 *Telephone: 502-394-6390* *Fax: 502-394-6388* *Shervin.dashti@nortonhealthcare.org*

1. BACKGROUND

1.1. Radiation Necrosis: Stereotactic radiosurgery has become integral in treatment of brain tumors and arteriovenous malformations (AVM). In up to 10% of cases, this can lead to radiation necrosis (RN) with significant surrounding vasogenic edema and mass effect. Medical treatment for RN includes steroids, vitamin E, pentoxiphylline, and hyperbaric oxygen. Up to 20% of cases however, are medically refractory and experience progressive neurological decline and disabling headaches.

1.2. Bevacizumab: Bevacizumab (Avastin, Genentech BioOncology, South San Francisco, CA) is a recombinant humanized version of a murine anti-human vascular endothelial growth factor (VEGF) monoclonal antibody. Recently, bevacizumab was shown in a small randomized controlled trial (n=14) to be effective in treatment of refractory radiation necrosis after radiation therapy in brain tumors[1]. Patients received 7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles. All patients receiving Bevacizumab and none of the patients receiving placebo had significant clinical and radiographic improvement.

1.3. Preclinical Data

Role of vascular endothelial growth factor (VEGF) in radiation necrosis
VEGF has been implicated in the pathophysiology of radiation necrosis. Reactive astrocytes immediately surrounding the necrotic core in RN are strongly VEGF-positive by immunohistochemistry[2]. It is postulated that radiation causes microvascular injury leading to hypoxia. Hypoxia-induced VEGF up-regulation then drives an increase in vascular permeability, leading to the extensive vasogenic edema seen in RN. Bevacizumab binds circulating VEGF receptors with high specificity, blocking the down-stream signaling cascade.

1.4. Clinical Data: Bevacizumab was originally developed and tested as an anti-angiogenic treatment for various solid tumors. More recently, IV-Bevacizumab was shown in a small, randomized controlled trial (n=14) to be very effective in treatment of refractory radiation necrosis after radiation therapy in brain tumors[1]. Patients received 7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles. All patients receiving Bevacizumab and none of the patients receiving placebo had significant clinical and radiographic improvement. This improvement was durable at 10 months in 8 of 11 patients (4 patients crossed over from the control group). There was however, a very high rate of adverse events (60%), major adverse events (30%. Major adverse events included venous sinus thrombosis, pulmonary embolus, and aspiration pneumonia.

We recently published a case series of two pediatric patients with highly symptomatic steroid refractory radiation necrosis in the brain after stereotactic radiosurgery for treatment of cerebral arteriovenous malformations[3]. Both patients were refractory to all accepted medical therapies. Both were steroid dependent for a prolonged period and severely cushingoid. Both had suffered a significant decline in quality of life with severe headache and need to withdraw from school. In both instances, the patients made a remarkable progressive clinical and radiographic improvement after receiving a single 2.5 mg/kg dose of intra-arterial bevacizumab, which was durable one-year later. To increase bevacizumab penetration into the brain, we used intra-arterial Mannitol to disrupt the blood-brain barrier immediately prior to targeted intra-arterial drug administration.

Case 1: A 12-year-old right handed female presented with severe headaches. MRI of the brain showed a 3.2 cm left posterior frontal arteriovenous malformation (Spetzler-Martin Grade III) with mild surrounding vasogenic edema. There were no signs of hemorrhage. She was initially treated with a 2-week course of steroids with significant improvement in headaches along with decreased edema on MRI. After considering treatment options including observation, endovascular embolization and/or surgical resection, the patient and her family chose stereotactic radiosurgery. She underwent SRS with a dose of 21Gy prescribed to the AVM with a volume of 9.9 cm^3.

Eight months later, the patient presented with severe headaches and focal seizures affecting the right arm and leg. MRI of the brain at this time demonstrated increasing T2 FLAIR signal as well as new Gadolinium signal on the T1 sequences. She was treated with anti-epileptics for her seizures and oral steroids for presumed radiation necrosis. Her seizure episodes resolved, but her severe headache persisted. She was initially started on 24 mg per day of dexamethasone tapering over 3 weeks. Because of worsening headache and progressively worsening right hemiparesis, repeated attempts to reduce dexamethasone dose below 6 mg daily proved unsuccessful over the ensuing 9 months. Courses of pentoxiphylline, vitamin E, and hyperbaric oxygen were all administered per study protocols without success[4,5]. Despite these interventions, she declined to 4/5 strength in the proximal right upper and lower extremities and 0/5 strength in her right hand and foot. She experienced a 60-pound weight gain with severe cushingoid features. She complained of constant severe (10 on the 10-point scale) headache. She also experienced severe emotional lability, causing her to withdraw from school.

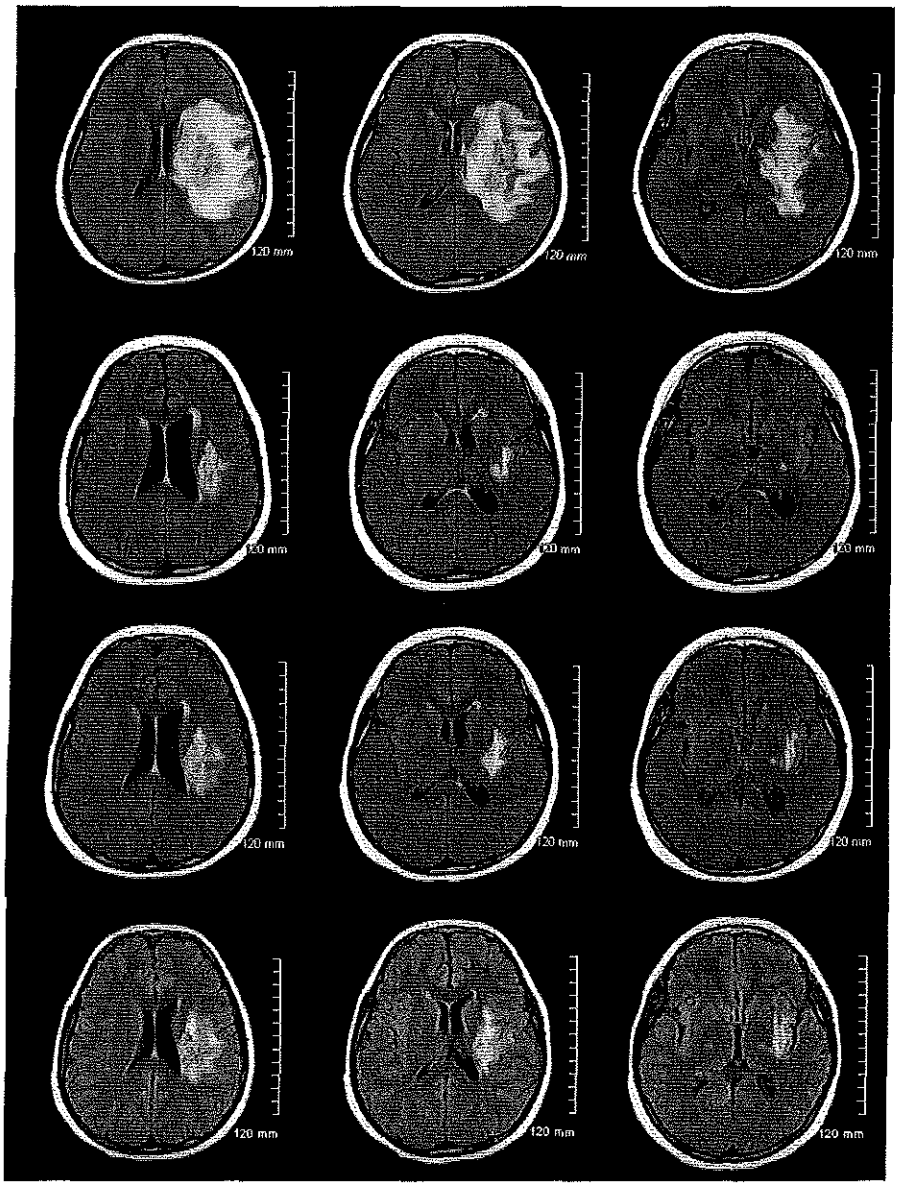
Given the progressive neurological decline and the lack of alternative therapies, the option of IV-bevacizumab treatment was initially explored. However, this proved to be not available for our pediatric patient population. Hence, the option of low dose intra-arterial (IA) bevacizumab treatment was considered. Our rational for this approach was to use a smaller dose of bevacizumab in a more directed fashion (IA-administration after BBB disruption), in order to maintain efficacy while reducing systemic toxicity[6]. The off-label uses of the medication, as well as the risks, including intracranial hemorrhage, were reviewed in detail. An informed written consent was signed by the parents, and assent was provided by the patient. We performed intra-arterial infusion of 2.5 mg/kg bevacizumab after hyperosmotic blood-brain-barrier breakdown. There were no acute complications from the procedure.

Within 12 hours of IA-bevacizumab administration, the patient experienced complete resolution (0 on the 10-point scale) of her previously intractable headaches. She also felt subjectively stronger on her right side. She was started on a long steroid taper under supervision of the Endocrine Service. Two months later, MRI of the brain revealed an 82% decrease in FLAIR signal and a 6% decrease in contrast enhancement (Figures 1A and 1B, middle panel). There was an associated markedly reduced mass effect. MRI of the brain at 5 months revealed 78% decreased FLAIR signal and 22% decreased contrast enhancement **(Figures 1)**. MRI of the brain 5 months and 11 months later showed relative stability of the radiographic improvement, despite active weaning of steroids.

At 18 months clinical follow-up, the patient had made a progressive and prominent improvement in her proximal right arm and leg strength. She was able to walk long distances with the help of an electronic drop-foot unit. She has continued to lose weight (50 pounds so far). She experiences occasional moderate (5-6 on a 10-point scale) headaches that would last several hours, but not the constant very severe headaches she was experiencing before treatment. Her emotional lability resolved and she was able

to go back to school.

Figure 1:



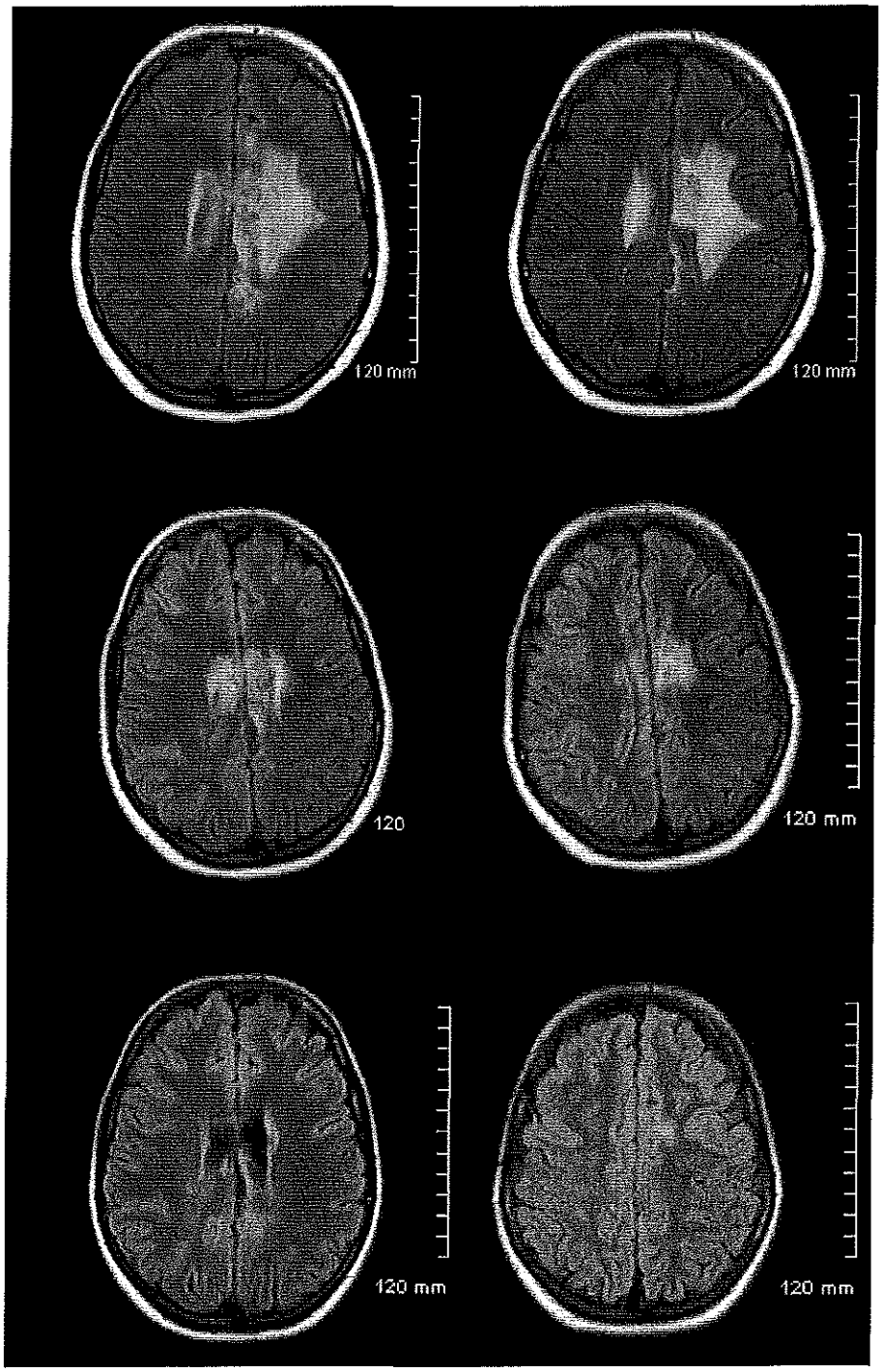
Case 2: An 11-year-old right-handed female presented with right hemiparesis, headache, nausea and vomiting. CT of the head revealed intraparenchymal and intraventricular hemorrhage and hydrocephalus. She required temporary external ventricular drainage. MRI imaging demonstrated a Spetzler-Martin Grade II AVM of the corpus callosum. She underwent embolization with only partial obliteration of the AVM 2 months later. She then underwent stereotactic radiosurgery to the residual AVM nidus with a dose of 18Gy prescribed to the AVM with a volume of 4.3 cm^3. She developed moderate headache within the first month after treatment, which responded to a 2-week course of steroids. Six-months after stereotactic radiosurgery, the patient developed mild to moderate headaches, which initially responded to ibuprofen. MRI showed evidence of mild vasogenic edema surrounding the AVM site. Eight months after radiosurgery, she then developed intractable headaches that were associated with nausea and vomiting. Repeat MRI demonstrated worsening cerebral edema with new contrast enhancement consistent with radiation necrosis in the left frontal lobe. There were no associated motor or sensory symptoms. A 21-day steroid course starting with 24 mg per day of Dexamethasone was initiated. This steroid taper could not however, be weaned below 8 mg of Dexamethasone daily over the ensuing 3 months secondary to recurrent severe headache, nausea and vomiting. Courses of pentoxiphylline and vitamin E were tried without success. Over time, she developed significant steroid related symptoms. The patient also required a hospitalization for fluid overload. In less than 3 months, the patient gained over 30 pounds and had a BMI of 25.5. As a result of her symptoms she too had to withdraw from school.

Based on the initial experience with Case #1, we discussed the option of intra-arterial bevacizumab treatment. The off-label uses of the medication as well as the risks including intracranial hemorrhage were reviewed in detail. An informed written consent was signed by the parents, and the patient provided assent.

The patient underwent intra-arterial infusion of 2.5 mg/kg bevacizumab into the left internal carotid artery after hyperosmotic blood-brain-barrier breakdown. There were no complications.

Immediately after IA-bevacizumab administration, the patient experienced complete resolution of her intractable headache and she was successfully weaned off steroids within 4 weeks. MRI of the brain 3 months later revealed 74% decrease in FLAIR signal volume and a 33.6% decrease in contrast enhancement **(Figure 2)**. MRI of the brain 10 months later showed even further decrease in cerebral edema, which was in fact completely resolved. At 16 months clinical follow-up, the patient was headaches free and was back in school. She lost 30 pounds and was back at her baseline weight.

Figure 2:



1.5. Clinical Pharmacokinetics:

Bevacizumab (Avastin, Genentech BioOncology, South San Francisco, CA) is a recombinant humanized version of a murine anti-human vascular endothelial growth factor (VEGF) monoclonal antibody. VEGF is a tyrosine kinase that plays an important role in angiogenesis and modulation of vascular permeability. VEGF-A binds with high

specificity to VEGF-receptor-1 (VEGFR-1) and VEGF-receptor-2 (VEGFR-2) on vascular endothelial cells. These modulate down-stream signaling pathways affecting various cellular processes. VEGF has recently been implicated in the pathophysiology of radiation necrosis. Reactive astrocytes immediately surrounding the necrotic core in RN are strongly VEGF-positive by immunohistochemistry[2]. It is postulated that radiation causes microvascular injury leading to hypoxia. Hypoxia-induced VEGF up-regulation then drives an increase in vascular permeability, leading to the extensive vasogenic edema seen in RN. Bevacizumab binds circulating VEGF receptors with high specificity, blocking the down-stream signaling cascade. The elimination half-life of bevacizumab is 19 days[7].

1.6. Rationale:

Current IV bevacizumab regimen for RN and its associated morbidity

Current IV-bevacizumab regimens use a dose of 7.5 mg/kg every 3 weeks for 4 cycles. There are significant known side effects of bevacizumab including gastrointestinal perforation, deep venous thrombosis, venous sinus thrombosis, pulmonary embolus, intracranial hemorrhage, wound dehiscence, and severe hypertension [8-14]. These complications are common to the anti-angiogenic class of drugs and reflect systemic exposure to bevacizumab. In our initial clinical experience, we utilized a combination of IA-route of delivery and BBB disruption to reduce bevacizumab dose while maintaining efficacy. This is supported by the durable clinical and radiographic response in our patients after a single 2.5 mg/kg dose of bevacizumab. We believe that this approach will reduce the incidence of serious systemic toxicities compared to the IV-bevacizumab regimens (7.5-15 mg/kg every 2-3 weeks for several weeks to months).

There are multiple recent reports of patients with radiation necrosis who improved with IV-bevacizumab, only to relapse months later. In fact 3/11 patients in the randomized controlled trial discussed above required repeat treatment with IV-bevacizumab because of RN symptom progression[1]. In contrast, the two patients in our series who received IA-bevacizumab continue to show progressive clinical and radiographic improvement more than one year later. We believe that the increased penetration of bevacizumab into the brain because of the intra-arterial administration after blood-brain barrier disruption results in binding of virtually all VEGF molecules. The fact that the results are durable and progressively improving suggests that massive blocking of VEGF activity could have stopped a positive feedback loop of inflammation. Therefore, IA-bevacizumab may result in more effective and durable control of radiation necrosis compared to traditional IV-bevacizumab treatment.

Intra-arterial (IA) route of bevacizumab administration significantly increase drug delivery to the brain

IA-therapy decreases volume dilution of the drug in the circulation and reduces first-pass degradation via proteolytic catabolism[15], resulting in higher drug delivery to target brain tissue. Super-selective IA-injection of 99mTc-HMPAO (Ceretec®) into human cerebral arteries achieves a concentration of radiotracer in brain tissue 50 times higher than with IV injection [16]. In clinical studies of cerebral chemotherapy, the concentration delivered to the tumor by using intra-arterial injection versus intravenous administration of chemotherapeutic agents was five times higher with hydrosoluble drugs and up to 50 times higher with liposoluble drugs. We will infuse bevacizumab in the artery that supplies the territory affected by RN, such as cervical internal carotid artery and/or

cervical vertebral artery.

Blood-brain-barrier breakdown prior to intra-arterial therapy further enhances drug delivery to the brain

The blood-brain-barrier is a selective permeability barrier that block entry of many drugs into the brain. Bevacizumab is a monoclonal antibody with a high molecular weight (149 kDa). There is convincing evidence in the literature that the concentration in the brain of high molecular weight molecules can be significantly increased after osmotic BBB disruption [17,18] [19]. Several tumor clinical trials have shown that localization of monoclonal antibodies to the brain is poor without BBB disruption (0.0006%-0.0043% of the injected dose/g of tumor) [20-23] [24] [21,25]. There is also evidence of a 20-fold increase in permeability to immunoreactive IgM Mab with BBB disruption in rats[24]. We believe that using blood-brain-barrier disruption significantly increases delivery of Bevacizumab to the affected brain. We will use the protocol described by Neuwelt and colleagues, using infusion of 25% Mannitol over 30 seconds[26]. This protocol has been shown to temporarily disrupt the blood brain barrier, peaking at 15 minutes and dissipating in 4 hours. IA-chemotherapy following BBBD has been shown to be feasible and safe across multiple centers with low incidence of complications[27]. The efficacy and safety profile was reproducible across multiple centers. In fact, safety of this protocol has been established in more than 6000 patients treated worldwide with BBBD for intra-arterial chemotherapy infusion[27,28]. The main possible complication is seizure, which occurs in <6% of cases. It is important to note that these seizures generally occurred in patients with widespread malignant pathology such as Glioblastoma and CNS lymphoma who were treated with very toxic chemotherapy agents immediately after BBBD. Recent refinements to the osmotic BBBD protocol have incorporated the use of general anesthesia, as well as prophylaxis with an anti-epileptic agent and Valium to reduce seizure threshold and the chance of seizures.

1.7 Safety of cerebral intra-arterial bevacizumab treatment

Safety of IA-Bevacizumab treatment after hyperosmotic BBBD was recently established in a series of malignant glioma patients[29]. This was done through super-selective injection of intracranial tumor arterial pedicles for purpose of anti-tumor effects. Dose-escalation was performed from 2 mg/kg to 15 mg/kg without reaching maximal tolerated dose. There was a significant decrease in the contrast enhancing and FLAIR signal characteristics of the tumor and surrounding brain at one month after treatment. Overall toxicity for this cohort was comparable to previous reports for IV Bevacizumab therapy. Specifically, hyperosmotic BBB-breakdown followed by IA-Bevacizumab administration did not cause any direct neurotoxicity; there were no cases of intracranial hemorrhage. Multiple other reports of BBBD followed by intra-arterial bevacizumab treatment for other pathologies such as vestibular schwannoma, ependymoma, and malignant brainstem glioma have also demonstrated good safety profile with no obvious neurotoxicity[30-32].

2. OBJECTIVES

2.1. Primary Objective: To assess the efficacy of intra-arterial (IA) bevacizumab for the treatment of radiation necrosis.

2.2. Secondary Objective:

- To assess the degree of clinical and radiographic response to a single treatment
- To assess the durability of response to a single treatment
- To assess time to steroid independence
- To assess neurocognitive improvement

2.3. Tertiary Objective: Cost analysis of a single treatment of low dose intrarterial bevacizumab as compared to conventional intravenous bevaizumab use for radiation necrosis (7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles)

3. STUDY DESIGN: Single Arm Phase II clinical trial

4. PATIENT SELECTION

4.1. Inclusion Criteria

Patients must have radiation necrosis based on radiographic evidence defined as:

- Increased T1 contrast enhancement in the radiated area with central hypointensity
- Increased surrounding vasogenic edema on FLAIR MRI images
- The underlying lesion prompting the radiation can include: Benign lesions such as AVM, Meningioma, schwannoma, trigeminal neuralgia: No biopsy is necessary
- Radiation necrosis must be symptomatic, including severe headache, seizures, and neurological deficits.
- Radiation necrosis must be refractory to steroid treatment; defined as failing a 3-week steroid regiment or not tolerating steroids because of side effects. Beyond 3 weeks, the side effects of steroid therapy worsen rapidly. The patient may receive other therapies such as Vitamine E, Pentoxyfylline, and hyperbaric oxygen during the trial.

Other inclusion criteria include:

- Age $\geq$18 years.
- Ability to understand and the willingness to sign a written informed consent document.
- Both men and women and members of all races and ethnic groups are eligible for this trial.
- Karnofsky Performance Status $\geq$70%.
- Life expectancy of greater *than 3 months.*
- Patients must have normal organ and marrow function as defined below:
  leukocytes $\geq$1,500/mcL
  platelets $\geq$85,000/mcL
  creatinine $\leq$1.8 mg/dl
- Birth Control: The effects of Bevacizumab on the developing human fetus are unknown. For this reason, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Women of childbearing age will have a urine pregnancy test immediately before each IA Bevacizumab treatment.

4.2. Exclusion Criteria

- Patients may not be started on any other investigational agents during the course of this trial. They may however continue previous medical regiments aimed for treatment of radiation necrosis. These include steroids, vitamin E, pentoxiphylline, and hyperbaric oxygen. We feel that these treatments are generally ineffectual and would not confound the results.
- Malignant brain tumor
- Concomitant use of anticoagulation agents including Coumadin, anticoagulation dose Lovenox or Arixtra. Aspirin is acceptable.
- Active bleeding or pathological condition that carries high risk of bleeding.
- Abdominal fistula, abscess, or gastrointestinal tract perforation ≤28 days of study entry.
- Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Any major surgery in the prior 4 weeks. Also any major surgery expected to be performed in the ensuing 4 weeks after treatment.
- Pregnant women are excluded from this study because Bevacizumab is expected to disrupt angiogenesis during pregnancy with the potential for teratogenic or abortive effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Bevacizumab, breastfeeding should be discontinued if the mother is treated with Bevacizumab.
- HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with Bevacizumab.

5. REGISTRATION

5.1. General Guidelines: Eligible patients will be entered into the study at the participating site by the Study Coordinator at that site. Patients must enroll in the study within 4 weeks following registration. Patients should begin protocol treatment within 4 weeks of enrollment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinators should be notified of cancellations as soon as possible.

5.2. Registration Process: To register a patient, the following documents should be completed by the research nurse or data manager:

- Copy of required laboratory tests
- Signed patient consent form
- HIPAA authorization form
- *Other appropriate forms (e.g., Eligibility Screening Worksheet, Registration Form).*
- Assign a patient study number
- Register the patient in the study

6. TREATMENT PLAN

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7.

6.1. Vascular Access, Cerebral Angiogram, and Osmotic Blood-Brain-Barrier Disruption:

We will use the protocol described by Neuwelt and colleagues, using infusion of 25% Mannitol over 30 seconds[26]. The safety of this protocol has been established in more than 6000 patients treated worldwide with BBBD for intra-arterial chemotherapy infusion[27,28].

The patients are to be premedicated with 6 mg Dexamethasone and 1000 mg Keppra. General endotracheal anesthesia will be induced. The femoral artery will be accessed using the Seldinger technique. A 5-French diagnostic catheter will be used to catheterize the cervical internal carotid artery ipsilateral to the area of radiation necrosis. Baseline internal carotid angiogram will be performed.

The anesthesiologist will be instructed to maintain SBP >120 or at pre-operative baseline, whichever value is higher. This is important for efficient bulk flow of drug through the blood brain barrier opening. The catheter is positioned at C1-2 level in the cervical internal carotid artery and C6-7 for a vertebral artery infusion. Optimal rate of Mannitol infusion will be determined by performing injection of contrast at 4 ml/sec for 3 seconds into vessel. If there is no reflux of contrast into the external carotid artery, the injection rate injection will be increased by 2 ml/sec to maximum of 12 ml/sec. The lowest rate at which there is reflux into the external carotid artery will be chosen (the rate to just exceed cerebral blood flow.

Next, 5 mg IV Valium and 0.2 mg IV Atropine are to be administered. Warm (37 degrees C^0) 25% Mannitol is filtered through a 5-micron filter, and then infused into the ipsilateral cervical carotid artery at the rate determined above for a total of 30 seconds.

*It is important to warm the 25% Mannitol for adequate amount of time to prevent crystallization. Filtering with 5-micron filter is important because of the propensity of Mannitol to crystalize at such high concentrations.
**Dexamethasone is given for its anti-inflammatory effect
***Keppra and Valium are given to increase seizure threshold because a small risk of seizure has been observed with osmotic BBBD. This was in the setting of IA-delivery of highly toxic chemotherapy agents in patient with malignant brain tumors such as CNS lymphoma.
**** Atropine is given because of very small potential risk of bradycardia associated with stimulating the carotid bulb during injection of Mannitol.

6.2. Intra-Arterial Bevacizumab Administration

Test injection of contrast will be done in the artery. If there is any evidence of catheter-induced vasospasm, the catheter may be withdrawn more proximally within the artery. Repeat test injection of contrast will be done to document resolution of vasospasm. Within 5 minutes of Mannitol infusion, 2.5 mg/kg bevacizumab in a volume of 100 ml will be administered into the artery over 10 minutes. Repeat angiogram will be performed to document BBBD, as well as to rule out thromboembolic phenomenon.

Angiogram of the femoral artery will then be performed. The femoral sheath will be removed and hemostasis will be achieved using a closure device and/or digital pressure for 20 minutes according to the surgeon's preference.

6.3. Post-Operative Care: The patient is then monitored in the recovery unit with Q15 min neurological exam and continuous heart rate and pulse-oximetric measurement for 2-4 hours. The patient will then be observed overnight in TCU. The patient will be discharged home the following morning if clinically stable.

6.4. General Concomitant Medication and Supportive Care Guidelines: Because there is a potential for interaction of Bevacizumab with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

6.5. Duration of Therapy: One treatment drug dose only

6.6. Duration of Follow-Up: Patients will be followed for 1 year. Patients with adverse events will be followed until resolution or stabilization of the adverse event.

6.7. Definition of Dose-Limiting Toxicity: Development of Grade 3 or 4 major adverse event as described in section 8.2.

*Management and dose modifications associated with the above adverse events are outlined in Section 7.

6.8. Criteria for Removal from the Study: Patients will be removed from study upon request from the patient. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

7. DOSE MODIFICATION: None

8. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

8.1 Adverse Events and Potential Risks List

Category (Body System)	Adverse Events with Possible Relationship to Bevacizumab (CTCAE v3.0 Term)	'Agent Specific Adverse Event List' (ASAEL)
ALLERGY/IMMUNOLOGY		
	Allergic reaction/hypersensitivity (including drug fever)	*Allergic reaction/hypersensitivity (including drug fever)*
	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	*Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)*
BLOOD/BONE MARROW		
	Hemoglobin	*Hemoglobin*
	Leukocytes (total WBC)	*Leukocytes (total WBC)*
	Neutrophils/granulocytes (ANC/AGC)	*Neutrophils/granulocytes (ANC/AGC)*
CARDIAC ARRHYTHMIA		
	Supraventricular arrhythmia NOS	*Supraventricular arrhythmia NOS*
	Ventricular fibrillation	
CARDIAC GENERAL		
	Cardiac ischemia/infarction	*Cardiac ischemia/infarction*
	Cardiac troponin I (cTnI)	
	Hypertension	*Hypertension*
	Hypotension	
	Left ventricular diastolic dysfunction	
	Left ventricular systolic dysfunction	
CONSTITUTIONAL SYMPTOMS		
	Fatigue (asthenia, lethargy, malaise)	*Fatigue (asthenia, lethargy, malaise)*
	Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 x 10^9/L)	*Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 x 10^9/L)*
	Rigors/chills	*Rigors/chills*
	Weight loss	
DERMATOLOGY/SKIN		
	Pruritus/itching	*Pruritus/itching*
	Rash/desquamation	*Rash/desquamation*
	Ulceration	
	Urticaria (hives, welts, wheals)	*Urticaria (hives, welts, wheals)*
	Wound complication, non-infectious	
GASTROINTESTINAL		
	Anorexia	*Anorexia*
	Colitis	
	Constipation	*Constipation*
	Diarrhea	*Diarrhea*
	Fistula, GI - Select	
	Heartburn/dyspepsia	*Heartburn/dyspepsia*
	Ileus (functional obstruction of bowel, i.e., neurogenic constipation)	
	Leak (including anastomotic), GI: large bowel	
	Mucositis/stomatitis (functional/symptomatic) - Select	*Mucositis/stomatitis (functional/symptomatic) - Select*
	Nausea	*Nausea*

Category (Body System)	Adverse Events with Possible Relationship to Bevacizumab (CTCAE v3.0 Term)	'Agent Specific Adverse Event List' (ASAEL)
	Perforation, GI - Select	
	Ulcer, GI - Select	
	Vomiting	*Vomiting*
HEMORRHAGE/BLEEDING		
	Hemorrhage, GI - Select	*Hemorrhage GI - Select*
	Hemorrhage, CNS	*Hemorrhage, CNS*
	Hemorrhage, GU: vagina	*Hemorrhage, GU: vagina*
	Hemorrhage, pulmonary/upper respiratory: lung	*Hemorrhage, pulmonary/upper respiratory: lung*
	Hemorrhage, pulmonary/upper respiratory: nose	*Hemorrhage, pulmonary/upper respiratory: nose*
INFECTION		
	Infection with normal ANC or Grade 1 or 2 neutrophils - Select	
	Infection with normal ANC or Grade 1 or 2 neutrophils - Select (pelvis, peritoneal cavity, rectum, scrotum, skin, wound)	
METABOLIC/LABORATORY		
	Alkaline phosphatase	*Alkaline phosphatase*
	ALT, SGPT (serum glutamic pyruvic transaminase)	*ALT, SGPT (serum glutamic pyruvic transaminase)*
	AST, SGOT (serum glutamic oxaloacetic transaminase)	*AST, SGOT (serum glutamic oxaloacetic transaminase)*
	Bilirubin (hyperbilirubinemia)	*Bilirubin (hyperbilirubinemia)*
	Creatinine	
	Proteinuria	*Proteinuria*
NEUROLOGY		
	CNS cerebrovascular ischemia	*CNS cerebrovascular ischemia*
	Dizziness	*Dizziness*
	Neurology - Other: (Leukoencephalopathy syndrome including reversible posterior leukoencephalopathy syndrome [RPLS])	
PAIN		
	Pain - abdomen NOS	*Pain - abdomen NOS*
	Pain - chest/thorax NOS	*Pain - chest/thorax NOS*
	Pain - head/headache	*Pain - head/headache*
	Pain - joint	*Pain - joint*
	Pain - muscle	
	Pain - NOS	
PULMONARY/UPPER RESPIRATORY		
	Bronchospasm, wheezing	
	Cough	*Cough*
	Dyspnea (shortness of breath)	*Dyspnea (shortness of breath)*
	Fistula, pulmonary/upper respiratory - Select	
	Nasal cavity/paranasal sinus reactions	*Nasal cavity/paranasal sinus reactions*
	Voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis)	*Voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis)*
	Pulmonary/Upper Respiratory - Other (nasal-septal perforation)	
RENAL/GENITOURINARY		
	Fistula, GU - Select	
	Renal failure	
SYNDROMES		
	Cytokine release syndrome/acute infusion reaction	*Cytokine release syndrome/acute infusion reaction*
VASCULAR		
	Thrombosis/thrombus/embolism	*Thrombosis/thrombus/embolism*
	Visceral arterial ischemia (non-myocardial)	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting ADEERSMD@lech-res.com. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

8.2 Adverse Event Definitions:

CTCAE term (AE description) and grade: The descriptions and grading Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Significance of an Adverse Event

Grades 1 are mild adverse events. (e.g., minor event requiring no specific medical intervention; asymptomatic laboratory findings only; marginal clinical relevance)

Grades 2 are moderate adverse events (e.g., minimal intervention; local intervention; non-invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 3 are severe and undesirable adverse events (e.g., significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 4 are life threatening or disabling adverse events (e.g., complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, sepsis; life–threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation).

Grades 5 are fatal adverse event resulting in death.

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

8.2.1 Serious Adverse Events:

- Results in **death.**
- Is a **life-threatening** adverse experience. The term life threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
  - The admission results in a hospital stay of less than 12 hours OR
  - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study) OR
  - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

    However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependant on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
- Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.2.2 Expectedness: Adverse Events can be Expected or Unexpected.

- **An expected adverse event** is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event will be listed in the consent form and research protocol.

- **An unexpected adverse event** is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

8.2.3 Attribution

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

8.3 Reporting Procedures for all Adverse Events

All participating investigators will assess the occurrence of AEs throughout the subject's participation in the study. Subjects will be followed for toxicity for 30 days after treatment has been discontinued or until death, whichever occurs first. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's case report form, subject's medical records, and/or any other institutional requirement. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

The investigator will provide the following for all adverse events:

- Description of the event
- Date of onset and resolution
- Grade of toxicity
- Attribution of relatedness to the investigational agent
- Action taken as a result of the event
- Outcome of event

In this study, descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 available at http://ctep.cancer.gov will be utilized for AE reporting.

Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

8.4 Serious Adverse Event Reporting Procedures

Serious adverse events that occur beginning with the signing of the informed consent form, during treatment, or within 30 days of the last dose of treatment must be reported to the Norton Healthcare Principal Investigator. Investigative sites will report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting serious adverse events.

8.5 Data Safety Toxicity Committee

It is the Norton Healthcare's Principal Investigator's responsibility to ensure that ALL serious adverse events are reported to the Norton Healthcare's Data Safety Toxicity Committee. This submission is simultaneous with submission to the Sponsor or other Regulatory body.

9. PHARMACEEUTICAL INFORMATION

A list of the adverse events and potential risks associated with bevacizumab administered in this study can be found in Section 8.0

Name of Agent

Chemical Name: bevacizumab

Other Names: Avastin

Classification: anti-angiogenic

Molecular Formula: Avastin (bevacizumab) is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF) in *in vitro* and in vivo assay systems. Bevacizumab contains human framework regions and the complementarity-determining regions of a murine antibody that binds to VEGF. Avastin has an approximate molecular weight of 149 kD. Bevacizumab is produced in a mammalian cell (Chinese Hamster Ovary) expression system in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

Mode of Action: Bevacizumab binds VEGF and prevents the interaction of VEGF to its receptors (Flt-1 and KDR) on the surface of endothelial cells. The interaction of VEGF with its receptors leads to endothelial cell proliferation and new blood vessel formation in in vitro models of angiogenesis. Administration of bevacizumab to xenotransplant models of colon cancer in nude (athymic) mice caused reduction of microvascular growth and inhibition of metastatic disease progression.

Metabolism: The pharmacokinetic profile of bevacizumab was assessed using an assay that measures total serum bevacizumab concentrations (i.e., the assay did not distinguish between free bevacizumab and bevacizumab bound to VEGF ligand). Based on a population pharmacokinetic analysis of 491 patients who received 1 to 20 mg/kg of Avastin weekly, every 2 weeks, or every 3 weeks, the estimated half-life of bevacizumab was approximately 20 days (range 11–50 days). The predicted time to reach steady state was 100 days. The accumulation ratio following a dose of 10 mg/kg of bevacizumab every 2 weeks was 2.8.

The clearance of bevacizumab varied by body weight, gender, and tumor burden. After correcting for body weight, males had a higher bevacizumab clearance (0.262 L/day vs. 0.207 L/day) and a larger Vc (3.25 L vs. 2.66 L) than females. Patients with higher tumor burden (at or above median value of tumor surface area) had a higher bevacizumab clearance (0.249 L/day vs. 0.199 L/day) than patients with tumor burdens below the median. In Study 1, there was no evidence of lesser efficacy (hazard ratio for overall survival) in males or patients with higher tumor burden treated with Avastin as compared to females and patients with low tumor burden. The relationship between bevacizumab exposure and clinical outcomes has not been explored.

Product description:
-100 mg per 4 mL single-use vial
-400 mg per 16 mL single-use vial

Solution preparation: Use appropriate aseptic technique. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Withdraw necessary amount of Avastin and dilute in a total volume of 100 mL of 0.9% Sodium Chloride Injection, USP. Discard any unused portion left in a vial, as the product contains no preservatives.

DO NOT ADMINISTER OR MIX WITH DEXTROSE SOLUTION.

Storage requirements: Avastin vials [100 mg (NDC 50242-060-01) and 400 mg (NDC 50242-061-01)] are stable at 2–8°C (36–46°F). Avastin vials should be protected from light. **Do not freeze or shake.**

Stability: Diluted Avastin solutions may be stored at 2–8°C (36–46°F) for up to 8 hours. Store in the original carton until time of use. No incompatibilities between Avastin and polyvinylchloride or polyolefin bags have been observed.

Route of administration:
In this study we will be performing intra-arterial infusion over a 14-minute period.

Drug Procurement: Bevacizumab must be obtained from commercial sources.

Drug Accountability: The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. Under no circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

10. CORRELATIVE / SPECIAL STUDIES

10.1 Imaging correlate

Previous studies have shown that the use of bevacizumab alters the ability of gadolinium to produce enhancement in RN. Bevacizumab also decreases T2 FLAIR signal. In this Pilot Study, precontrast axial and sagittal T1 with the axial with fat-sat, T2, FLAIR, DWI/ADC, susceptibility, post-contrast 3-plane FS T1 images, as well as brain perfusion will be used to evaluate for radiation necrosis and cerebral edema. All scans will be done on a 1.5-Tesla or higher MR. The scans will be performed before the 1st treatment, at 3 months, and 12 months, or as indicated for new clinical symptoms. All MRI's will be sent on CD to Core Lab at Norton Healthcare run by Dr. Kadner (board-certified neuro-radiologist).

If a patient cannot have an MRI for medical reasons, then CT head with and without contrast will be used instead.

10.2 Neuro-cognitive correlate

In order to investigate the immediate and post-operative neurocognitive sequelae of bevacizumab, patients who consent to a formal neuropsychological battery will be tested at baseline (prior to IA bevacizumab administration), 3 and 12 months postoperative follow-up. The test battery was selected by a board certified neuropsychologist in order to thoroughly assess multiple cognitive domains and to be brief enough for patients to complete within one hour at each testing session. The neuropsychologist will conduct appropriate training sessions

with an identified research assistant or nurse at each study site until inter-rater reliability is established (total ICC>.85) to assure both standardized administration and reduction of method error. Subtests from the NAB (Neuropsychological Assessment Battery, Stern & White, PAR Inc.) have been chosen for brevity, sensitivity, and due to the wide range of available normative data (ages 18-97). Subtests of the NAB can be administered alone or as part of the entire battery without loss of appropriate normative data, as each test across forms was administered to the same individuals in the standardization sample. Administration of the entire battery can take up to 4 hours, thus a subset of requisite performance measures was selected for inclusion in this study. In addition, the Wechsler Test of Adult Reading (WTAR, Pearson Inc.) will be administered to provide an estimate of premorbid cognitive functioning. Subtests chosen from the NAB include: Digits Forwards (DF); Digits Backwards (DB); Letter and Numbers (L&N). Together, these will assess attention capacity, working memory, and processing speed. Naming (NAM). This will assess confrontation naming and anomia. Design Construction (DES). This will assess spatial awareness and constructional praxis. List Learning (LL); Story Learning (SL); and Shape Learning (ShL). These will assess auditory and visual episodic learning and memory retrieval and retention skills. Mazes (MZ); Categories (CAT); and Word Generation (WG). These will assess higher order executive functioning with regard to planning, conceptualization, and fluency. We expect the most significant effects to arise from assessment of processing speed, memory, and executive functioning. Each of these subtests will be administered at baseline, time 1, and time 2. The NAB was standardized with 2 alternate, equivalent forms, which will be counterbalanced across administration times while repeating one form per subject.

Given the length of time 2 to time 3, practice effects across the three forms can be minimized. The tests will be administered as follows:

Cognitive Domain	**Time 1 (Baseline)**	**Time 2 week following treatment)**	**Time 3 (3 months following treatment)**
Premorbid Intelligence	WTAR		
Attention / Processing Speed	DF, DB, L&N	DF, DB, L&N	DF, DB, L&N
Language	NAM	NAM	NAM
Visuospatial	DES	DES	DES
Learning and Memory	LL, SL, ShL	LL, SL, ShL	LL, SL, ShL
Executive Functioning	MZ, CAT, WG	MZ, CAT, WG	MZ, CAT, WG

10.3 Headache Correlate

Patients with radiation necrosis often experience severe or intractable headaches. These headaches usually worsen during efforts to wean the patient off corticosteroids, leading to long-term steroid use and all its associated serious morbidities. Our preliminary experience indicates rapid and complete resolution of headaches following IA-bevacizumab treatment. All patients will be tested using a formal headache battery at baseline. The Headache Impact Test (HIT-6) which is a fixed-length 6-item, paper form version of the DYNHA Headache Impact Test will be filled out by the patient. MIDAS 5-item questionnaire will also be completed by the patient before treatment, as well as at 6 weeks, 3 mo, 6 mo, 9 mo, and 12 mo.

11. STUDY PARAMETERS AND CALENDAR

The tests and procedures found in the Study Flow Chart will be performed. Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done 4 weeks prior to the start of therapy.

	Pre-Study	Day 0	Day 1	Week 6	Month 3	Month 6	Month 12
Informed Consent	R						
Demographics	NR						
Medical History	NR	NR	NR		NR		NR
Concurrent Medication	NR	NR	NR		NR		NR
Physical Exam	NR	R	R		R		R
Neurological Exam	NR	R	R		R		R
Carotid Angiogram		R					
Osmotic blood-brain-barrier breakdown		R					
Avastin®		R					
HIT – 6 Survey (may be via phone)	R			R	R	R	R
MIDAS (may be via phone)	R			R	R	R	R
NeuroPsych Testing	R				R		R
KPS Functional Status	R		R		R		R
Screening Lab tests[1]	R						
ECG (if needed)[2]	R						
Adverse Event monitoring		R	R		R		R
Urine Pregnancy Test		R					
Brain MRI w & w/o contrast	NR				NR		NR

R= Research
NR= non-research or standard of care
1 = CBC with differential and platelets count, basic metabolic panel serum chemistry
2= ECG will be done in patients >45 years old or in any patients with history of cardiac problems

Physical Examination: All enrolled participates will have an abbreviated physical examination and vital signs (heart rate, blood pressure and body weight) collected during screening and then again at Day 0, and 1, Month 3 and 12.

Neurological Examination: All enrolled participants will have a detailed neurological examination during screening and then again at Day 0, and 1, Month 3 and 12.

Pregnancy Testing: It is not known whether study drug used in this study may cause side effects to pregnant women, to an unborn child, or to children of nursing women. Because of these unknown risks, all women of childbearing potential will be given a pregnancy test prior to receiving study drug. Testing will be performed either by urine or blood. **Female participants will be informed that they must not become pregnant until 8 weeks following the last dose of study medication**

Karnofsky Performance Status Scale: Will be used to measure the functional status of the participant prior to and following study drug administration

Brain MRI: All subject will undergo a Brain MRI prior to receive study medication (Pre-study) and then again at Months 3, and 12.

CT Scan: Subject who cannot undergo a MRI for medical reasons will have undergo a head CT prior to receiving study medication (Pre-Study) and then again at Months 3 and 12.

12. MEASUREMENT OF EFFECT

12.1 Decrease in Cerebral Edema

We will use pre-contrast axial and sagittal T1 with the axial with fat-sat, T2, FLAIR, DWI/ADC, susceptibility, post-contrast 3-plane FS T1 images, as well as brain perfusion to evaluate for progression. All scans will be done on a 1.5-3.0 Tesla MR. The scans will be performed within 4 weeks before the 1st treatment, at 3 months, and 12 months, or as indicated for new clinical symptoms. A reduction in volume of cerebral edema of > 25% will constitute a positive response.

12.2 Definitions

Please use or modify the following text as appropriate.

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with Bevacizumab.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression treatment will also be considered evaluable.)

12.3 Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Two-stage design, Single-arm Phase II multi-center clinical trial. The participating centers are Norton Healthcare and University of Kentucky Healthcare. This study will determine efficacy of IA-bevacizumab after BBBD treatment for radiation necrosis.

13.2 Sample Size/Accrual Rate

Stage 1: There will be a maximum of 10 patients accrued for stage 1 of this trial. In 2013, there were a total of 5 patients seen and evaluated for medically refractory radiation necrosis at our center. Therefore, the accrual rate would be anticipated as 5 patients per year. The accrual rates are expected to be 3-5 per year in other participating site.

Stage 2: If ≥30% of patients have a positive response, then the number of patients in the trial will escalate to 46 patients. At that point, further participating sites will be considered.

13.3 Stratification Factors: *None.*

13.4 Analysis of Secondary Endpoints: Secondary endpoint findings will be compared to historical controls from published studies of IV-bevacizumab treatment for radiation necrosis.

13.5 Outcome Measures

Primary Outcome Measure: Decrease in radiation necrosis and cerebral edema after a single treatment of low dose intrarterial bevacizumab: Precontrast axial and sagittal T1 with the axial with fat-sat, T2, FLAIR, DWI/ADC, susceptibility, post-contrast 3-plane FS T1 images, as well as brain perfusion will be used to evaluate for radiation necrosis and cerebral edema. All scans will be done on a 1.5-Tesla or higher MR. The scans will be performed before the 1st treatment, at 3 months, and 12 months, or as indicated for new clinical symptoms. All MRI's will be sent on CD to Core Lab at Norton Healthcare run by Dr. Kadner (board-certified neuro-radiologist). If a patient cannot have an MRI for medical reasons, then CT head with and without contrast will be used instead.

Secondary Outcome Measures:

1. Decrease in steroid usage after a single treatment of low dose intrarterial bevacizumab: To assess the utility of intra-arterial bevacizumab treatment in allowing decreased steroid usage, the quantity of steroid use (measured as mg of Decaderon per day) will be noted at 1-day, 3 months, and 12 months.

2. Quantitative improvement in headache after a single treatment of low dose intrarterial bevacizumab: Quantitative improvement in headache will be assessed by performing The Headache Impact Test (HIT-6) which is a fixed-length 6-item, paper

form version of the DYNHA Headache Impact Test will be filled out by the patient. MIDAS 5-item questionnaire will also be completed by the patient before treatment, as well as at 6 weeks, 3 mo, 6 mo, 9 mo, and 12 mo.

3. Neurocognitive improvement after a single treatment of low dose intrarterial bevacizumab: In order to investigate the immediate and post-operative neurocognitive sequelae of intra-arterial bevacizumab, patients who consent to a formal neuropsychological battery will be tested at baseline (prior to IA bevacizumab administration), 3 and 12 months postoperative follow-up. Subtests from the NAB (Neuropsychological Assessment Battery, Stern & White, PAR Inc.) have been chosen for brevity, sensitivity, and due to the wide range of available normative data (ages 18-97). In addition, the Wechsler Test of Adult Reading (WTAR, Pearson Inc.) will be administered to provide an estimate of premorbid cognitive functioning.

4. Safety of a single treatment of low dose intrarterial bevacizumab: Safety of intra-arterial bevacizumab will be assessed by noting the number of participants with treatment-related adverse events as assessed by CTCAE v4.0.

5. Neurological improvement after a single treatment of low dose intrarterial bevacizumab: Objective improvement in neurological exam will be assessed by performing serial neurological exams at baseline, 1 day, 3 months, and 12 months. Also, quantitative improvement in functional status will be assessed by performing Karnofsky Performance Status Scale (KPS) at baseline, 1 day, 3 months, and 12 months.

Other Pre-specified Outcome Measures: Cost analysis of a single treatment of low dose intrarterial bevacizumab compared to conventional IV-bevacizumab regiment: Cost analysis of a single treatment of low dose intrarterial bevacizumab after osmotic blood-brain-barrier disruption as compared to conventional intravenous bevaizumab use for radiation necrosis (7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles).

14. References

1. Levin VA, Bidaut L, Hou P, et al. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. International Journal of radiation Oncology, Biology, Physics 2011;79:1487-95.
2. Nonoguchi N, Miyatake S, Fukumoto M, et al. The distribution of vascular endothelial growth factor-producing cells in clinical radiation necrosis of the brain: pathological consideration of their potential roles. Journal of neuro-oncology 2011;105:423-31.
3. Dashti SR, Spalding A, Kadner RJ, et al. Targeted intraarterial anti-VEGF therapy for medically refractory radiation necrosis in the brain. J Neurosurg Pediatr 2015;15:20-5.
4. Monaco EA, 3rd, Niranjan A, Kano H, Flickinger JC, Kondziolka D, Lunsford LD. Management of adverse radiation effects after radiosurgery for arteriovenous malformations. Progress in neurological surgery 2013;27:107-18.
5. Williamson R, Kondziolka D, Kanaan H, Lunsford LD, Flickinger JC. Adverse radiation effects after radiosurgery may benefit from oral vitamin E and pentoxifylline therapy: a pilot study. Stereotactic and functional neurosurgery 2008;86:359-66.
6. Levin VA, Bidaut L, Hou P, et al. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. Int J Radiat Oncol Biol Phys 2011;79:1487-95.
7. Li J, Gupta M, Jin D, Xin Y, Visich J, Allison DE. Characterization of the long-term pharmacokinetics of bevacizumab following last dose in patients with resected stage II and III carcinoma of the colon. Cancer chemotherapy and pharmacology 2013;71:575-80.
8. Takano M, Kikuchi Y, Kato M, Yoshikawa T, Kita T. Bowel perforation associated with bevacizumab therapy in recurrent ovarian cancers without bowel obstruction or bowel involvement]. Gan To Kagaku Ryoho 2008;35:1981-4.
9. Heinzerling JH, Huerta S. Bowel perforation from bevacizumab for the treatment of metastatic colon cancer: incidence, etiology, and management. Curr Surg 2006;63:334-7.

10. Abbrederis K, Kremer M, Schuhmacher C. [Ischemic anastomotic bowel perforation during treatment with bevacizumab 10 months after surgery]. Chirurg 2008;79:351-5.
11. Suenaga M, Mizunuma N, Kobayashi K, et al. Management of venous thromboembolism in colorectal cancer patients treated with bevacizumab. Med Oncol;27:807-14.
12. Grivas AA, Trafalis DT, Athanassiou AE. Implication of bevacizumab in fatal arterial thromboembolic incidents. J BUON 2009;14:115-7.
13. Vredenburgh JJ, Desjardins A, Kirkpatrick JP, et al. Addition of Bevacizumab to Standard Radiation Therapy and Daily Temozolomide Is Associated with Minimal Toxicity in Newly Diagnosed Glioblastoma Multiforme. Int J Radiat Oncol Biol Phys.
14. Chamberlain MC, Johnston S. Salvage chemotherapy with bevacizumab for recurrent alkylator-refractory anaplastic astrocytoma. J Neurooncol 2009;91:359-67.
15. Gobin YP, Cloughesy TF, Chow KL, et al. Intraarterial chemotherapy for brain tumors by using a spatial dose fractionation algorithm and pulsatile delivery. Radiology 2001;218:724-32.
16. Namba H, Kobayashi S, Iwadate Y, et al. Assessment of the brain areas perfused by superselective intra-arterial chemotherapy using single photon emission computed tomography with technetium-99m-hexamethyl-propyleneamine oxime--technical note. Neurol Med Chir (Tokyo) 1994;34:832-5.
17. Neuwelt EA, Frenkel EP, D'Agostino AN, et al. Growth of human lung tumor in the brain of the nude rat as a model to evaluate antitumor agent delivery across the blood-brain barrier. Cancer Res 1985;45:2827-33.
18. Armstrong BK, Robinson PJ, Rapoport SI. Size-dependent blood-brain barrier opening demonstrated with [14C]sucrose and a 200,000-Da [3H]dextran. Exp Neurol 1987;97:686-96.
19. Ziylan YZ, Robinson PJ, Rapoport SI. Differential blood-brain barrier permeabilities to [14C]sucrose and [3H]inulin after osmotic opening in the rat. Exp Neurol 1983;79:845-57.
20. Bullard DE, Bigner DD. Applications of monoclonal antibodies in the diagnosis and

treatment of primary brain tumors. J Neurosurg 1985;63:2-16.
21. Zalutsky MR, Moseley RP, Benjamin JC, et al. Monoclonal antibody and F(ab')2 fragment delivery to tumor in patients with glioma: comparison of intracarotid and intravenous administration. Cancer Res 1990;50:4105-10.
22. Epenetos AA, Courtenay-Luck N, Pickering D, et al. Antibody guided irradiation of brain glioma by arterial infusion of radioactive monoclonal antibody against epidermal growth factor receptor and blood group A antigen. Br Med J (Clin Res Ed) 1985;290:1463-6.
23. Zalutsky MR, Moseley RP, Coakham HB, Coleman RE, Bigner DD. Pharmacokinetics and tumor localization of 131I-labeled anti-tenascin monoclonal antibody 81C6 in patients with gliomas and other intracranial malignancies. Cancer Res 1989;49:2807-13.
24. Neuwelt EA, Barnett PA, Hellstrom KE, Hellstrom I, McCormick CI, Ramsey FL. Effect of blood-brain barrier disruption on intact and fragmented monoclonal antibody localization in intracerebral lung carcinoma xenografts. J Nucl Med 1994;35:1831-41.
25. Lee YS, Bullard DE, Zalutsky MR, et al. Therapeutic efficacy of antiglioma mesenchymal extracellular matrix 131I-radiolabeled murine monoclonal antibody in a human glioma xenograft model. Cancer Res 1988;48:559-66.
26. Dahlborg SA, Henner WD, Crossen JR, et al. Non-AIDS primary CNS lymphoma: first example of a durable response in a primary brain tumor using enhanced chemotherapy delivery without cognitive loss and without radiotherapy. The cancer journal from Scientific American 1996;2:166-74.
27. Doolittle ND, Miner ME, Hall WA, et al. Safety and efficacy of a multicenter study using intraarterial chemotherapy in conjunction with osmotic opening of the blood-brain barrier for the treatment of patients with malignant brain tumors. Cancer 2000;88:637-47.
28. Neuwelt EA. Mechanisms of disease: the blood-brain barrier. Neurosurgery 2004;54:131-40; discussion 41-2.
29. Boockvar JA, Tsiouris AJ, Hofstetter CP, et al. Safety and maximum tolerated dose of superselective intraarterial cerebral infusion of bevacizumab after osmotic blood-brain barrier disruption for recurrent malignant glioma. J Neurosurg.
30. Rajappa P, Krass J, Riina HA, Boockvar JA, Greenfield JP. Super-selective basilar artery infusion of bevacizumab and cetuximab for multiply recurrent pediatric ependymoma. Interventional neuroradiology : journal of peritherapeutic neuroradiology, surgical procedures and related neurosciences 2011;17:459-65.
31. Riina HA, Burkhardt JK, Santillan A, Bassani L, Patsalides A, Boockvar JA. Short-term clinico-radiographic response to super-selective intra-arterial cerebral infusion of Bevacizumab for the treatment of vestibular schwannomas in Neurofibromatosis type 2. Interventional neuroradiology : journal of peritherapeutic neuroradiology, surgical procedures and related neurosciences 2012;18:127-32.
32. Riina HA, Knopman J, Greenfield JP, et al. Balloon-assisted superselective intra-arterial cerebral infusion of bevacizumab for malignant brainstem glioma. A technical note. Interventional neuroradiology : journal of peritherapeutic neuroradiology, surgical procedures and related neurosciences 2010;16:71-6.

Norton Healthcare/Dept of Neurosurgery
LIBERTI **Version 3**

STUDY TITLE: Low-dose Intra-arterial Bevacizumab for Edema and Radiation necrosis Therapeutic Intervention (LIBERTI)

Participating Centers:

Norton Healthcare
University of Kentucky Healthcare

Sponsor/Principal Investigator: *Shervin R. Dashti, MD, PhD*
4950 Norton Healthcare Blvd. #205
Louisville, KY 40241
Telephone: 502-394-6390
Fax: 502-394-6388
Shervin.dashti@nortonhealthcare.org

Version 3

Date: 20 June 2016

CONFIDENTIAL STATEMENT

SCHEMA

To assess the overall safety and efficacy of intra-arterial (IA) bevacizumab for the treatment of radiation necrosis. A single 2.5 mg/kg dose of bevacizumab will be given intra-arterially after blood-brain-barrier disruption using 25% Mannitol at 4-12 ml/sec for 30 seconds.

INTRA-ARTERIAL DOSING SCHEMA	
Dose NUMBER	**Agent**
Dose#1: Day 0	2.5 mg/kg X 1 dose

INVESTIGATOR'S STATEMENT AND SIGNATURE

I have read and understand this protocol, attachments, and (name of study drug). I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with ICH GCP guidelines and the ethical principles of the Helsinki Declaration.

Sponsor/Principal Investigator:

Signature: _____ Date: _____

Printed Name: _____

CONTACT DETAILS OF KEY PERSONNEL

Role	Name	Contact Information
Sponsor/Principal Investigator	*Shervin R. Dashti, MD, PhD*	4950 Norton Healthcare Blvd. #205 Louisville, KY 40241 *Telephone: 502-394-6390* *Fax: 502-394-6388* *Shervin.dashti@nortonhealthcare.org*
Principal Investigator	Justin Fraser, MD	UK Medical Center Department of Neurosurgery Medical Science Building Room MS 108A Lexington, KY 40536-0298 *Telephone: (859) 323-0616* *Jfr235@uky.edu*
Sponsor Project Manager	Elodie Elayi	UK Center for Clinical and Translational Science 800 Rose Street, Pavilion H, Room C300 Lexington KY, 40536 *Telephone: (859) 323-7939* *Fax: (859) 257-9560* *Elodie.elayi@uky.edu*
Sponsor Study Monitor	Karen Bowen, RN	Bluegrass Research Consultants, Inc. 8020 Sea Hero Run Versailles, KY 40383 *Phone: (859)396-4655* *FAX: (859)873-9963* *Bowen73@windstream.net*
SAE Reporting	*Shervin R. Dashti, MD, PhD*	4950 Norton Healthcare Blvd. #205 Louisville, KY 40241 *Telephone: 502-394-6390* *Fax: 502-394-6388* *Shervin.dashti@nortonhealthcare.org*

1. BACKGROUND

1.1. Radiation Necrosis: Stereotactic radiosurgery has become integral in treatment of brain tumors and arteriovenous malformations (AVM). In up to 10% of cases, this can lead to radiation necrosis (RN) with significant surrounding vasogenic edema and mass effect. Medical treatment for RN includes steroids, vitamin E, pentoxiphylline, and hyperbaric oxygen. Up to 20% of cases however, are medically refractory and experience progressive neurological decline and disabling headaches.

1.2. Bevacizumab: Bevacizumab (Avastin, Genentech BioOncology, South San Francisco, CA) is a recombinant humanized version of a murine anti-human vascular endothelial growth factor (VEGF) monoclonal antibody. Recently, bevacizumab was shown in a small randomized controlled trial (n=14) to be effective in treatment of refractory radiation necrosis after radiation therapy in brain tumors[1]. Patients received 7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles. All patients receiving Bevacizumab and none of the patients receiving placebo had significant clinical and radiographic improvement.

1.3. Preclinical Data

Role of vascular endothelial growth factor (VEGF) in radiation necrosis
VEGF has been implicated in the pathophysiology of radiation necrosis. Reactive astrocytes immediately surrounding the necrotic core in RN are strongly VEGF-positive by immunohistochemistry[2]. It is postulated that radiation causes microvascular injury leading to hypoxia. Hypoxia-induced VEGF up-regulation then drives an increase in vascular permeability, leading to the extensive vasogenic edema seen in RN. Bevacizumab binds circulating VEGF receptors with high specificity, blocking the down-stream signaling cascade.

1.4. Clinical Data: Bevacizumab was originally developed and tested as an anti-angiogenic treatment for various solid tumors. More recently, IV-Bevacizumab was shown in a small, randomized controlled trial (n=14) to be very effective in treatment of refractory radiation necrosis after radiation therapy in brain tumors[1]. Patients received 7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles. All patients receiving Bevacizumab and none of the patients receiving placebo had significant clinical and radiographic improvement. This improvement was durable at 10 months in 8 of 11 patients (4 patients crossed over from the control group). There was however, a very high rate of adverse events (60%), major adverse events (30%. Major adverse events included venous sinus thrombosis, pulmonary embolus, and aspiration pneumonia.

We recently published a case series of two pediatric patients with highly symptomatic steroid refractory radiation necrosis in the brain after stereotactic radiosurgery for treatment of cerebral arteriovenous malformations[3]. Both patients were refractory to all accepted medical therapies. Both were steroid dependent for a prolonged period and severely cushingoid. Both had suffered a significant decline in quality of life with severe headache and need to withdraw from school. In both instances, the patients made a remarkable progressive clinical and radiographic improvement after receiving a single 2.5 mg/kg dose of intra-arterial bevacizumab, which was durable one-year later. To increase bevacizumab penetration into the brain, we used intra-arterial Mannitol to disrupt the blood-brain barrier immediately prior to targeted intra-arterial drug administration.

Case 1: A 12-year-old right handed female presented with severe headaches. MRI of the brain showed a 3.2 cm left posterior frontal arteriovenous malformation (Spetzler-Martin Grade III) with mild surrounding vasogenic edema. There were no signs of hemorrhage. She was initially treated with a 2-week course of steroids with significant improvement in headaches along with decreased edema on MRI. After considering treatment options including observation, endovascular embolization and/or surgical resection, the patient and her family chose stereotactic radiosurgery. She underwent SRS with a dose of 21Gy prescribed to the AVM with a volume of 9.9 cm^3.

Eight months later, the patient presented with severe headaches and focal seizures affecting the right arm and leg. MRI of the brain at this time demonstrated increasing T2 FLAIR signal as well as new Gadolinium signal on the T1 sequences. She was treated with anti-epileptics for her seizures and oral steroids for presumed radiation necrosis. Her seizure episodes resolved, but her severe headache persisted. She was initially started on 24 mg per day of dexamethasone tapering over 3 weeks. Because of worsening headache and progressively worsening right hemiparesis, repeated attempts to reduce dexamethasone dose below 6 mg daily proved unsuccessful over the ensuing 9 months. Courses of pentoxiphylline, vitamin E, and hyperbaric oxygen were all administered per study protocols without success[4,5]. Despite these interventions, she declined to 4/5 strength in the proximal right upper and lower extremities and 0/5 strength in her right hand and foot. She experienced a 60-pound weight gain with severe cushingoid features. She complained of constant severe (10 on the 10-point scale) headache. She also experienced severe emotional lability, causing her to withdraw from school.

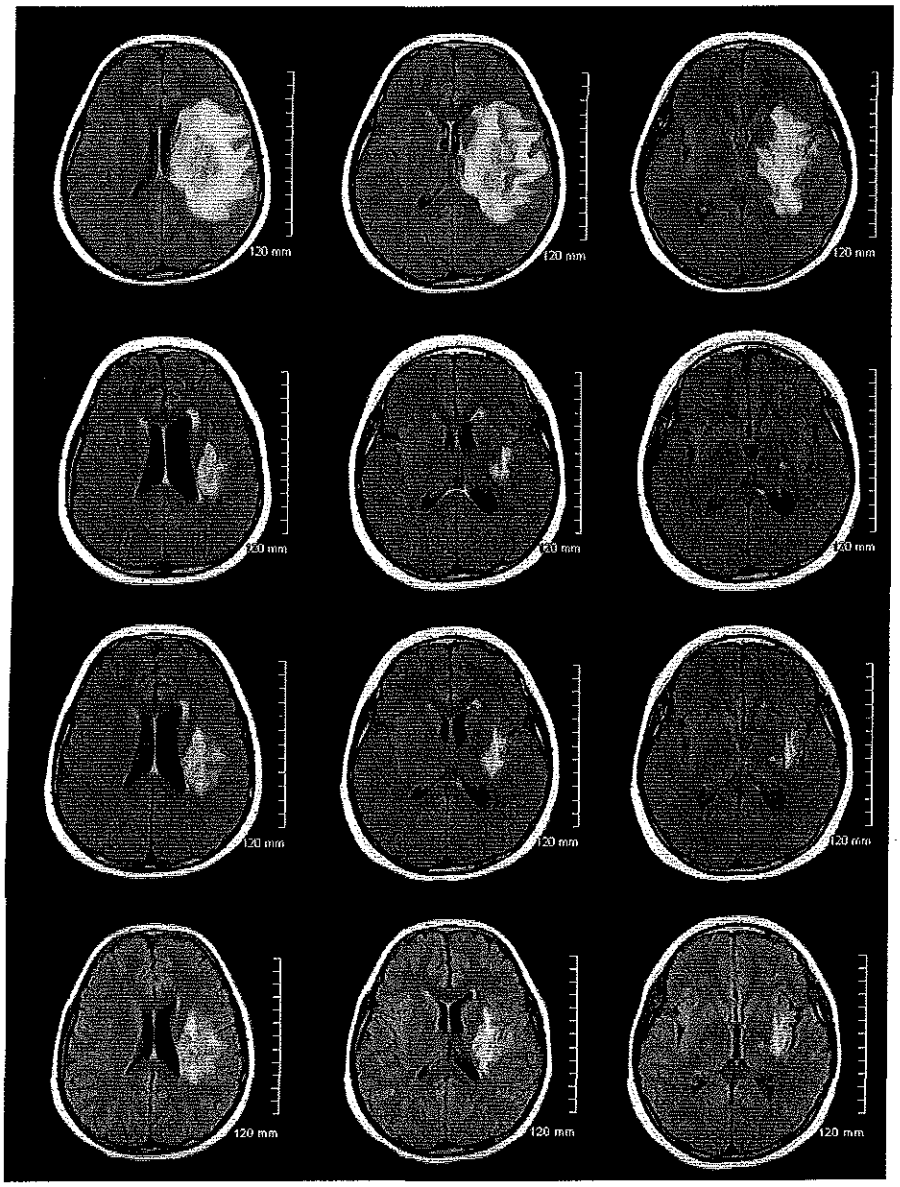
Given the progressive neurological decline and the lack of alternative therapies, the option of IV-bevacizumab treatment was initially explored. However, this proved to be not available for our pediatric patient population. Hence, the option of low dose intra-arterial (IA) bevacizumab treatment was considered. Our rational for this approach was to use a smaller dose of bevacizumab in a more directed fashion (IA-administration after BBB disruption), in order to maintain efficacy while reducing systemic toxicity[6]. The off-label uses of the medication, as well as the risks, including intracranial hemorrhage, were reviewed in detail. An informed written consent was signed by the parents, and assent was provided by the patient. We performed intra-arterial infusion of 2.5 mg/kg bevacizumab after hyperosmotic blood-brain-barrier breakdown. There were no acute complications from the procedure.

Within 12 hours of IA-bevacizumab administration, the patient experienced complete resolution (0 on the 10-point scale) of her previously intractable headaches. She also felt subjectively stronger on her right side. She was started on a long steroid taper under supervision of the Endocrine Service. Two months later, MRI of the brain revealed an 82% decrease in FLAIR signal and a 6% decrease in contrast enhancement (Figures 1A and 1B, middle panel). There was an associated markedly reduced mass effect. MRI of the brain at 5 months revealed 78% decreased FLAIR signal and 22% decreased contrast enhancement **(Figures 1)**. MRI of the brain 5 months and 11 months later showed relative stability of the radiographic improvement, despite active weaning of steroids.

At 18 months clinical follow-up, the patient had made a progressive and prominent improvement in her proximal right arm and leg strength. She was able to walk long distances with the help of an electronic drop-foot unit. She has continued to lose weight (50 pounds so far). She experiences occasional moderate (5-6 on a 10-point scale) headaches that would last several hours, but not the constant very severe headaches she was experiencing before treatment. Her emotional lability resolved and she was able

to go back to school.

Figure 1:



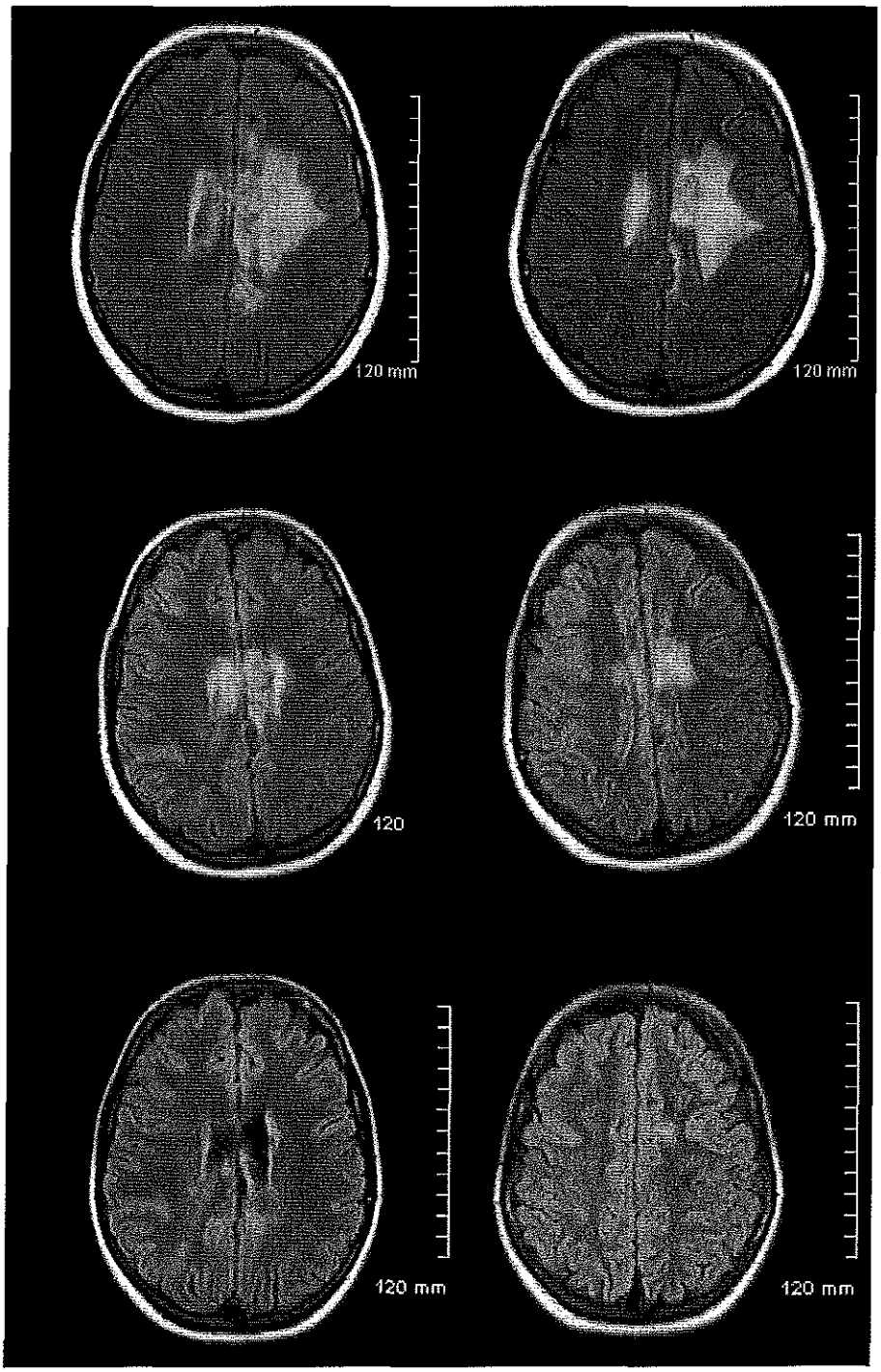
Case 2: An 11-year-old right-handed female presented with right hemiparesis, headache, nausea and vomiting. CT of the head revealed intraparenchymal and intraventricular hemorrhage and hydrocephalus. She required temporary external ventricular drainage. MRI imaging demonstrated a Spetzler-Martin Grade II AVM of the corpus callosum. She underwent embolization with only partial obliteration of the AVM 2 months later. She then underwent stereotactic radiosurgery to the residual AVM nidus with a dose of 18Gy prescribed to the AVM with a volume of 4.3 cm^3. She developed moderate headache within the first month after treatment, which responded to a 2-week course of steroids. Six-months after stereotactic radiosurgery, the patient developed mild to moderate headaches, which initially responded to ibuprofen. MRI showed evidence of mild vasogenic edema surrounding the AVM site. Eight months after radiosurgery, she then developed intractable headaches that were associated with nausea and vomiting. Repeat MRI demonstrated worsening cerebral edema with new contrast enhancement consistent with radiation necrosis in the left frontal lobe. There were no associated motor or sensory symptoms. A 21-day steroid course starting with 24 mg per day of Dexamethasone was initiated. This steroid taper could not however, be weaned below 8 mg of Dexamethasone daily over the ensuing 3 months secondary to recurrent severe headache, nausea and vomiting. Courses of pentoxiphylline and vitamin E were tried without success. Over time, she developed significant steroid related symptoms. The patient also required a hospitalization for fluid overload. In less than 3 months, the patient gained over 30 pounds and had a BMI of 25.5. As a result of her symptoms she too had to withdraw from school.

Based on the initial experience with Case #1, we discussed the option of intra-arterial bevacizumab treatment. The off-label uses of the medication as well as the risks including intracranial hemorrhage were reviewed in detail. An informed written consent was signed by the parents, and the patient provided assent.

The patient underwent intra-arterial infusion of 2.5 mg/kg bevacizumab into the left internal carotid artery after hyperosmotic blood-brain-barrier breakdown. There were no complications.

Immediately after IA-bevacizumab administration, the patient experienced complete resolution of her intractable headache and she was successfully weaned off steroids within 4 weeks. MRI of the brain 3 months later revealed 74% decrease in FLAIR signal volume and a 33.6% decrease in contrast enhancement **(Figure 2)**. MRI of the brain 10 months later showed even further decrease in cerebral edema, which was in fact completely resolved. At 16 months clinical follow-up, the patient was headaches free and was back in school. She lost 30 pounds and was back at her baseline weight.

Figure 2:



1.5. Clinical Pharmacokinetics:

Bevacizumab (Avastin, Genentech BioOncology, South San Francisco, CA) is a recombinant humanized version of a murine anti-human vascular endothelial growth factor (VEGF) monoclonal antibody. VEGF is a tyrosine kinase that plays an important role in angiogenesis and modulation of vascular permeability. VEGF-A binds with high

specificity to VEGF-receptor-1 (VEGFR-1) and VEGF-receptor-2 (VEGFR-2) on vascular endothelial cells. These modulate down-stream signaling pathways affecting various cellular processes. VEGF has recently been implicated in the pathophysiology of radiation necrosis. Reactive astrocytes immediately surrounding the necrotic core in RN are strongly VEGF-positive by immunohistochemistry[2]. It is postulated that radiation causes microvascular injury leading to hypoxia. Hypoxia-induced VEGF up-regulation then drives an increase in vascular permeability, leading to the extensive vasogenic edema seen in RN. Bevacizumab binds circulating VEGF receptors with high specificity, blocking the down-stream signaling cascade. The elimination half-life of bevacizumab is 19 days[7].

1.6. Rationale:

Current IV bevacizumab regimen for RN and its associated morbidity

Current IV-bevacizumab regimens use a dose of 7.5 mg/kg every 3 weeks for 4 cycles. There are significant known side effects of bevacizumab including gastrointestinal perforation, deep venous thrombosis, venous sinus thrombosis, pulmonary embolus, intracranial hemorrhage, wound dehiscence, and severe hypertension [8-14]. These complications are common to the anti-angiogenic class of drugs and reflect systemic exposure to bevacizumab. In our initial clinical experience, we utilized a combination of IA-route of delivery and BBB disruption to reduce bevacizumab dose while maintaining efficacy. This is supported by the durable clinical and radiographic response in our patients after a single 2.5 mg/kg dose of bevacizumab. We believe that this approach will reduce the incidence of serious systemic toxicities compared to the IV-bevacizumab regimens (7.5-15 mg/kg every 2-3 weeks for several weeks to months).

There are multiple recent reports of patients with radiation necrosis who improved with IV-bevacizumab, only to relapse months later. In fact 3/11 patients in the randomized controlled trial discussed above required repeat treatment with IV-bevacizumab because of RN symptom progression[1]. In contrast, the two patients in our series who received IA-bevacizumab continue to show progressive clinical and radiographic improvement more than one year later. We believe that the increased penetration of bevacizumab into the brain because of the intra-arterial administration after blood-brain barrier disruption results in binding of virtually all VEGF molecules. The fact that the results are durable and progressively improving suggests that massive blocking of VEGF activity could have stopped a positive feedback loop of inflammation. Therefore, IA-bevacizumab may result in more effective and durable control of radiation necrosis compared to traditional IV-bevacizumab treatment.

Intra-arterial (IA) route of bevacizumab administration significantly increase drug delivery to the brain

IA-therapy decreases volume dilution of the drug in the circulation and reduces first-pass degradation via proteolytic catabolism[15], resulting in higher drug delivery to target brain tissue. Super-selective IA-injection of 99mTc-HMPAO (Ceretec®) into human cerebral arteries achieves a concentration of radiotracer in brain tissue 50 times higher than with IV injection [16]. In clinical studies of cerebral chemotherapy, the concentration delivered to the tumor by using intra-arterial injection versus intravenous administration of chemotherapeutic agents was five times higher with hydrosoluble drugs and up to 50 times higher with liposoluble drugs. We will infuse bevacizumab in the artery that supplies the territory affected by RN, such as cervical internal carotid artery and/or

cervical vertebral artery.

Blood-brain-barrier breakdown prior to intra-arterial therapy further enhances drug delivery to the brain

The blood-brain-barrier is a selective permeability barrier that block entry of many drugs into the brain. Bevacizumab is a monoclonal antibody with a high molecular weight (149 kDa). There is convincing evidence in the literature that the concentration in the brain of high molecular weight molecules can be significantly increased after osmotic BBB disruption [17,18] [19]. Several tumor clinical trials have shown that localization of monoclonal antibodies to the brain is poor without BBB disruption (0.0006%-0.0043% of the injected dose/g of tumor) [20-23] [24] [21,25]. There is also evidence of a 20-fold increase in permeability to immunoreactive IgM Mab with BBB disruption in rats[24]. We believe that using blood-brain-barrier disruption significantly increases delivery of Bevacizumab to the affected brain. We will use the protocol described by Neuwelt and colleagues, using infusion of 25% Mannitol over 30 seconds[26]. This protocol has been shown to temporarily disrupt the blood brain barrier, peaking at 15 minutes and dissipating in 4 hours. IA-chemotherapy following BBBD has been shown to be feasible and safe across multiple centers with low incidence of complications[27]. The efficacy and safety profile was reproducible across multiple centers. In fact, safety of this protocol has been established in more than 6000 patients treated worldwide with BBBD for intra-arterial chemotherapy infusion[27,28]. The main possible complication is seizure, which occurs in <6% of cases. It is important to note that these seizures generally occurred in patients with widespread malignant pathology such as Glioblastoma and CNS lymphoma who were treated with very toxic chemotherapy agents immediately after BBBD. Recent refinements to the osmotic BBBD protocol have incorporated the use of general anesthesia, as well as prophylaxis with an anti-epileptic agent and Valium to reduce seizure threshold and the chance of seizures.

1.7 Safety of cerebral intra-arterial bevacizumab treatment

Safety of IA-Bevacizumab treatment after hyperosmotic BBBD was recently established in a series of malignant glioma patients[29]. This was done through super-selective injection of intracranial tumor arterial pedicles for purpose of anti-tumor effects. Dose-escalation was performed from 2 mg/kg to 15 mg/kg without reaching maximal tolerated dose. There was a significant decrease in the contrast enhancing and FLAIR signal characteristics of the tumor and surrounding brain at one month after treatment. Overall toxicity for this cohort was comparable to previous reports for IV Bevacizumab therapy. Specifically, hyperosmotic BBB-breakdown followed by IA-Bevacizumab administration did not cause any direct neurotoxicity; there were no cases of intracranial hemorrhage. Multiple other reports of BBBD followed by intra-arterial bevacizumab treatment for other pathologies such as vestibular schwannoma, ependymoma, and malignant brainstem glioma have also demonstrated good safety profile with no obvious neurotoxicity[30-32].

2. OBJECTIVES

2.1. Primary Objective: To assess the efficacy of intra-arterial (IA) bevacizumab for the treatment of radiation necrosis.

2.2. Secondary Objective:

- To assess the degree of clinical and radiographic response to a single treatment
- To assess the durability of response to a single treatment
- To assess time to steroid independence
- To assess neurocognitive improvement

2.3. Tertiary Objective: Cost analysis of a single treatment of low dose intrarterial bevacizumab as compared to conventional intravenous bevaizumab use for radiation necrosis (7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles)

3. STUDY DESIGN: Single Arm Phase II clinical trial

4. PATIENT SELECTION

4.1. Inclusion Criteria

Patients must have radiation necrosis based on radiographic evidence defined as:

- Increased T1 contrast enhancement in the radiated area with central hypointensity
- Increased surrounding vasogenic edema on FLAIR MRI images
- The underlying lesion prompting the radiation can include: Benign lesions such as AVM, Meningioma, schwannoma, trigeminal neuralgia: No biopsy is necessary
- Radiation necrosis must be symptomatic, including severe headache, seizures, and neurological deficits.
- Radiation necrosis must be refractory to steroid treatment; defined as failing a 3-week steroid regiment or not tolerating steroids because of side effects. Beyond 3 weeks, the side effects of steroid therapy worsen rapidly. The patient may receive other therapies such as Vitamine E, Pentoxyfylline, and hyperbaric oxygen during the trial.

Other inclusion criteria include:

- Age $\geq$18 years.
- Ability to understand and the willingness to sign a written informed consent document.
- Both men and women and members of all races and ethnic groups are eligible for this trial.
- Karnofsky Performance Status $\geq$70%.
- Life expectancy of greater *than 3 months.*
- Patients must have normal organ and marrow function as defined below:
  leukocytes $\geq$1,500/mcL
  platelets $\geq$85,000/mcL
  creatinine $\leq$1.8 mg/dl
- Birth Control: The effects of Bevacizumab on the developing human fetus are unknown. For this reason, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. Women of childbearing age will have a urine pregnancy test immediately before each IA Bevacizumab treatment.

4.2. Exclusion Criteria

- Patients may not be started on any other investigational agents during the course of this trial. They may however continue previous medical regiments aimed for treatment of radiation necrosis. These include steroids, vitamin E, pentoxiphylline, and hyperbaric oxygen. We feel that these treatments are generally ineffectual and would not confound the results.
- Malignant brain tumor
- Concomitant use of anticoagulation agents including Coumadin, anticoagulation dose Lovenox or Arixtra. Aspirin is acceptable.
- Active bleeding or pathological condition that carries high risk of bleeding.
- Abdominal fistula, abscess, or gastrointestinal tract perforation ≤28 days of study entry.
- Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
- Any major surgery in the prior 4 weeks. Also any major surgery expected to be performed in the ensuing 4 weeks after treatment.
- Pregnant women are excluded from this study because Bevacizumab is expected to disrupt angiogenesis during pregnancy with the potential for teratogenic or abortive effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Bevacizumab, breastfeeding should be discontinued if the mother is treated with Bevacizumab.
- HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with Bevacizumab.

5. REGISTRATION

5.1. General Guidelines: Eligible patients will be entered into the study at the participating site by the Study Coordinator at that site. Patients must enroll in the study within 4 weeks following registration. Patients should begin protocol treatment within 4 weeks of enrollment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinators should be notified of cancellations as soon as possible.

5.2. Registration Process: To register a patient, the following documents should be completed by the research nurse or data manager:

- Copy of required laboratory tests
- Signed patient consent form
- HIPAA authorization form
- *Other appropriate forms (e.g., Eligibility Screening Worksheet, Registration Form).*
- Assign a patient study number
- Register the patient in the study

6. TREATMENT PLAN

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7.

6.1. Vascular Access, Cerebral Angiogram, and Osmotic Blood-Brain-Barrier Disruption:

We will use the protocol described by Neuwelt and colleagues, using infusion of 25% Mannitol over 30 seconds[26]. The safety of this protocol has been established in more than 6000 patients treated worldwide with BBBD for intra-arterial chemotherapy infusion[27,28].

The patients are to be premedicated with 6 mg Dexamethasone and 1000 mg Keppra. General endotracheal anesthesia will be induced. The femoral artery will be accessed using the Seldinger technique. A 5-French diagnostic catheter will be used to catheterize the cervical internal carotid artery ipsilateral to the area of radiation necrosis. Baseline internal carotid angiogram will be performed.

The anesthesiologist will be instructed to maintain SBP >120 or at pre-operative baseline, whichever value is higher. This is important for efficient bulk flow of drug through the blood brain barrier opening. The catheter is positioned at C1-2 level in the cervical internal carotid artery and C6-7 for a vertebral artery infusion. Optimal rate of Mannitol infusion will be determined by performing injection of contrast at 4 ml/sec for 3 seconds into vessel. If there is no reflux of contrast into the external carotid artery, the injection rate injection will be increased by 2 ml/sec to maximum of 12 ml/sec. The lowest rate at which there is reflux into the external carotid artery will be chosen (the rate to just exceed cerebral blood flow.

Next, 5 mg IV Valium and 0.2 mg IV Atropine are to be administered. Warm (37 degrees C°) 25% Mannitol is filtered through a 5-micron filter, and then infused into the ipsilateral cervical carotid artery at the rate determined above for a total of 30 seconds.

*It is important to warm the 25% Mannitol for adequate amount of time to prevent crystallization. Filtering with 5-micron filter is important because of the propensity of Mannitol to crystalize at such high concentrations.
**Dexamethasone is given for its anti-inflammatory effect
***Keppra and Valium are given to increase seizure threshold because a small risk of seizure has been observed with osmotic BBBD. This was in the setting of IA-delivery of highly toxic chemotherapy agents in patient with malignant brain tumors such as CNS lymphoma.
**** Atropine is given because of very small potential risk of bradycardia associated with stimulating the carotid bulb during injection of Mannitol.

6.2. Intra-Arterial Bevacizumab Administration

Test injection of contrast will be done in the artery. If there is any evidence of catheter-induced vasospasm, the catheter may be withdrawn more proximally within the artery. Repeat test injection of contrast will be done to document resolution of vasospasm. Within 5 minutes of Mannitol infusion, 2.5 mg/kg bevacizumab in a volume of 100 ml will be administered into the artery over 10 minutes. Repeat angiogram will be performed to document BBBD, as well as to rule out thromboembolic phenomenon.

Angiogram of the femoral artery will then be performed. The femoral sheath will be removed and hemostasis will be achieved using a closure device and/or digital pressure for 20 minutes according to the surgeon's preference.

6.3. Post-Operative Care: The patient is then monitored in the recovery unit with Q15 min neurological exam and continuous heart rate and pulse-oximetric measurement for 2-4 hours. The patient will then be observed overnight in TCU. The patient will be discharged home the following morning if clinically stable.

6.4. General Concomitant Medication and Supportive Care Guidelines: Because there is a potential for interaction of Bevacizumab with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies.

6.5. Duration of Therapy: One treatment drug dose only

6.6. Duration of Follow-Up: Patients will be followed for 1 year. Patients with adverse events will be followed until resolution or stabilization of the adverse event.

6.7. Definition of Dose-Limiting Toxicity: Development of Grade 3 or 4 major adverse event as described in section 8.2.

*Management and dose modifications associated with the above adverse events are outlined in Section 7.

6.8. Criteria for Removal from the Study: Patients will be removed from study upon request from the patient. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

7. DOSE MODIFICATION: None

8. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

8.1 Adverse Events and Potential Risks List

Version 1.2, June 19, 2007[1]

Category (Body System)	Adverse Events with Possible Relationship to Bevacizumab (CTCAE v3.0 Term)	'Agent Specific Adverse Event List' (ASAEL)
ALLERGY/IMMUNOLOGY		
	Allergic reaction/hypersensitivity (including drug fever)	*Allergic reaction/hypersensitivity (including drug fever)*
	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	*Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)*
BLOOD/BONE MARROW		
	Hemoglobin	*Hemoglobin*
	Leukocytes (total WBC)	*Leukocytes (total WBC)*
	Neutrophils/granulocytes (ANC/AGC)	*Neutrophils/granulocytes (ANC/AGC)*
CARDIAC ARRHYTHMIA		
	Supraventricular arrhythmia NOS	*Supraventricular arrhythmia NOS*
	Ventricular fibrillation	
CARDIAC GENERAL		
	Cardiac ischemia/infarction	*Cardiac ischemia/infarction*
	Cardiac troponin I (cTnI)	
	Hypertension	*Hypertension*
	Hypotension	
	Left ventricular diastolic dysfunction	
	Left ventricular systolic dysfunction	
CONSTITUTIONAL SYMPTOMS		
	Fatigue (asthenia, lethargy, malaise)	*Fatigue (asthenia, lethargy, malaise)*
	Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 x 10^9/L)	*Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 x 10^9/L)*
	Rigors/chills	*Rigors/chills*
	Weight loss	
DERMATOLOGY/SKIN		
	Pruritus/itching	*Pruritus/itching*
	Rash/desquamation	*Rash/desquamation*
	Ulceration	
	Urticaria (hives, welts, wheals)	*Urticaria (hives, welts, wheals)*
	Wound complication, non-infectious	
GASTROINTESTINAL		
	Anorexia	*Anorexia*
	Colitis	
	Constipation	*Constipation*
	Diarrhea	*Diarrhea*
	Fistula, GI - Select	
	Heartburn/dyspepsia	*Heartburn/dyspepsia*
	Ileus (functional obstruction of bowel, i.e., neuroconstipation)	
	Leak (including anastomotic), GI: large bowel	
	Mucositis/stomatitis (functional/symptomatic) - Select	*Mucositis/stomatitis (functional/symptomatic) - Select*
	Nausea	*Nausea*

Category (Body System)	Adverse Events with Possible Relationship to Bevacizumab (CTCAE v3.0 Term)	'Agent Specific Adverse Event List' (ASAEL)
	Perforation, GI - Select	
	Ulcer, GI - Select	
	Vomiting	*Vomiting*
HEMORRHAGE/BLEEDING		
	Hemorrhage, GI - Select	*Hemorrhage GI - Select*
	Hemorrhage, CNS	*Hemorrhage, CNS*
	Hemorrhage, GU: vagina	*Hemorrhage, GU: vagina*
	Hemorrhage, pulmonary/upper respiratory: lung	*Hemorrhage, pulmonary/upper respiratory: lung*
	Hemorrhage, pulmonary/upper respiratory: nose	*Hemorrhage, pulmonary/upper respiratory: nose*
INFECTION		
	Infection with normal ANC or Grade 1 or 2 neutrophils - Select	
	Infection with normal ANC or Grade 1 or 2 neutrophils - Select (pelvis, peritoneal cavity, rectum, scrotum, skin, wound)	
METABOLIC/LABORATORY		
	Alkaline phosphatase	*Alkaline phosphatase*
	ALT, SGPT (serum glutamic pyruvic transaminase)	*ALT, SGPT (serum glutamic pyruvic transaminase)*
	AST, SGOT (serum glutamic oxaloacetic transaminase)	*AST, SGOT (serum glutamic oxaloacetic transaminase)*
	Bilirubin (hyperbilirubinemia)	*Bilirubin (hyperbilirubinemia)*
	Creatinine	
	Proteinuria	*Proteinuria*
NEUROLOGY		
	CNS cerebrovascular ischemia	*CNS cerebrovascular ischemia*
	Dizziness	*Dizziness*
	Neurology - Other: (Leukoencephalopathy syndrome including reversible posterior leukoencephalopathy syndrome (RPLS))	
PAIN		
	Pain - abdomen NOS	*Pain - abdomen NOS*
	Pain - chest/thorax NOS	*Pain - chest/thorax NOS*
	Pain - head/headache	*Pain - head/headache*
	Pain - joint	*Pain - joint*
	Pain - muscle	
	Pain - NOS	
PULMONARY/UPPER RESPIRATORY		
	Bronchospasm, wheezing	
	Cough	*Cough*
	Dyspnea (shortness of breath)	*Dyspnea (shortness of breath)*
	Fistula, pulmonary/upper respiratory - Select	
	Nasal cavity/paranasal sinus reactions	*Nasal cavity/paranasal sinus reactions*
	Voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis)	*Voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis)*
	Pulmonary/Upper Respiratory - Other (nasal-septal perforation)	
RENAL/GENITOURINARY		
	Fistula, GU - Select	
	Renal failure	
SYNDROMES		
	Cytokine release syndrome/acute infusion reaction	*Cytokine release syndrome/acute infusion reaction*
VASCULAR		
	Thrombosis/thrombus/embolism	*Thrombosis/thrombus/embolism*
	Visceral arterial ischemia (non-myocardial)	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting ADEERSMD@tech-res.com. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

8.2 Adverse Event Definitions:

CTCAE term (AE description) and grade: The descriptions and grading Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Significance of an Adverse Event

Grades 1 are mild adverse events. (e.g., minor event requiring no specific medical intervention; asymptomatic laboratory findings only; marginal clinical relevance)

Grades 2 are moderate adverse events (e.g., minimal intervention; local intervention; non-invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 3 are severe and undesirable adverse events (e.g., significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 4 are life threatening or disabling adverse events (e.g., complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, sepsis; life–threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation).

Grades 5 are fatal adverse event resulting in death.

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

8.2.1 Serious Adverse Events:

- Results in **death.**
- Is a **life-threatening** adverse experience. The term life threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
  - The admission results in a hospital stay of less than 12 hours OR
  - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study) OR
  - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

    However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependant on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
- Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.2.2 Expectedness: Adverse Events can be Expected or Unexpected.

- **An expected adverse event** is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event will be listed in the consent form and research protocol.

- **An unexpected adverse event** is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

8.2.3 Attribution

Definite – The AE *is clearly related* to the study treatment.
Probable – The AE *is likely related* to the study treatment.
Possible – The AE *may be related* to the study treatment.
Unlikely – The AE *is doubtfully related* to the study treatment.
Unrelated – The AE *is clearly NOT related* to the study treatment.

8.3 Reporting Procedures for all Adverse Events

All participating investigators will assess the occurrence of AEs throughout the subject's participation in the study. Subjects will be followed for toxicity for 30 days after treatment has been discontinued or until death, whichever occurs first. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's case report form, subject's medical records, and/or any other institutional requirement. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

The investigator will provide the following for all adverse events:

- Description of the event
- Date of onset and resolution
- Grade of toxicity
- Attribution of relatedness to the investigational agent
- Action taken as a result of the event
- Outcome of event

In this study, descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 available at http://ctep.cancer.gov will be utilized for AE reporting.

Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

8.4 Serious Adverse Event Reporting Procedures

Serious adverse events that occur beginning with the signing of the informed consent form, during treatment, or within 30 days of the last dose of treatment must be reported to the Norton Healthcare Principal Investigator. Investigative sites will report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting serious adverse events.

8.5 Data Safety Toxicity Committee

It is the Norton Healthcare's Principal Investigator's responsibility to ensure that ALL serious adverse events are reported to the Norton Healthcare's Data Safety Toxicity Committee. This submission is simultaneous with submission to the Sponsor or other Regulatory body.

9. PHARMACEEUTICAL INFORMATION

A list of the adverse events and potential risks associated with bevacizumab administered in this study can be found in Section 8.0

Name of Agent

Chemical Name: bevacizumab

Other Names: Avastin

Classification: anti-angiogenic

Molecular Formula: Avastin (bevacizumab) is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF) in *in vitro* and in vivo assay systems. Bevacizumab contains human framework regions and the complementarity-determining regions of a murine antibody that binds to VEGF. Avastin has an approximate molecular weight of 149 kD. Bevacizumab is produced in a mammalian cell (Chinese Hamster Ovary) expression system in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

Mode of Action: Bevacizumab binds VEGF and prevents the interaction of VEGF to its receptors (Flt-1 and KDR) on the surface of endothelial cells. The interaction of VEGF with its receptors leads to endothelial cell proliferation and new blood vessel formation in in vitro models of angiogenesis. Administration of bevacizumab to xenotransplant models of colon cancer in nude (athymic) mice caused reduction of microvascular growth and inhibition of metastatic disease progression.

Metabolism: The pharmacokinetic profile of bevacizumab was assessed using an assay that measures total serum bevacizumab concentrations (i.e., the assay did not distinguish between free bevacizumab and bevacizumab bound to VEGF ligand). Based on a population pharmacokinetic analysis of 491 patients who received 1 to 20 mg/kg of Avastin weekly, every 2 weeks, or every 3 weeks, the estimated half-life of bevacizumab was approximately 20 days (range 11–50 days). The predicted time to reach steady state was 100 days. The accumulation ratio following a dose of 10 mg/kg of bevacizumab every 2 weeks was 2.8.

The clearance of bevacizumab varied by body weight, gender, and tumor burden. After correcting for body weight, males had a higher bevacizumab clearance (0.262 L/day vs. 0.207 L/day) and a larger Vc (3.25 L vs. 2.66 L) than females. Patients with higher tumor burden (at or above median value of tumor surface area) had a higher bevacizumab clearance (0.249 L/day vs. 0.199 L/day) than patients with tumor burdens below the median. In Study 1, there was no evidence of lesser efficacy (hazard ratio for overall survival) in males or patients with higher tumor burden treated with Avastin as compared to females and patients with low tumor burden. The relationship between bevacizumab exposure and clinical outcomes has not been explored.

Product description:
-100 mg per 4 mL single-use vial
-400 mg per 16 mL single-use vial

Solution preparation: Use appropriate aseptic technique. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Withdraw necessary amount of Avastin and dilute in a total volume of 100 mL of 0.9% Sodium Chloride Injection, USP. Discard any unused portion left in a vial, as the product contains no preservatives.

DO NOT ADMINISTER OR MIX WITH DEXTROSE SOLUTION.

Storage requirements: Avastin vials [100 mg (NDC 50242-060-01) and 400 mg (NDC 50242-061-01)] are stable at 2–8°C (36–46°F). Avastin vials should be protected from light. **Do not freeze or shake.**

Stability: Diluted Avastin solutions may be stored at 2–8°C (36–46°F) for up to 8 hours. Store in the original carton until time of use. No incompatibilities between Avastin and polyvinylchloride or polyolefin bags have been observed.

Route of administration:
In this study we will be performing intra-arterial infusion over a 14-minute period.

Drug Procurement: Bevacizumab must be obtained from commercial sources.

Drug Accountability: The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. Under no circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

10. CORRELATIVE / SPECIAL STUDIES

10.1 Imaging correlate

Previous studies have shown that the use of bevacizumab alters the ability of gadolinium to produce enhancement in RN. Bevacizumab also decreases T2 FLAIR signal. In this Pilot Study, precontrast axial and sagittal T1 with the axial with fat-sat, T2, FLAIR, DWI/ADC, susceptibility, post-contrast 3-plane FS T1 images, as well as brain perfusion will be used to evaluate for radiation necrosis and cerebral edema. All scans will be done on a 1.5-Tesla or higher MR. The scans will be performed before the 1st treatment, at 3 months, and 12 months, or as indicated for new clinical symptoms. All MRI's will be sent on CD to Core Lab at Norton Healthcare run by Dr. Kadner (board-certified neuro-radiologist).

If a patient cannot have an MRI for medical reasons, then CT head with and without contrast will be used instead.

10.2 Neuro-cognitive correlate

In order to investigate the immediate and post-operative neurocognitive sequelae of bevacizumab, patients who consent to a formal neuropsychological battery will be tested at baseline (prior to IA bevacizumab administration), 3 and 12 months postoperative follow-up. The test battery was selected by a board certified neuropsychologist in order to thoroughly assess multiple cognitive domains and to be brief enough for patients to complete within one hour at each testing session. The neuropsychologist will conduct appropriate training sessions

with an identified research assistant or nurse at each study site until inter-rater reliability is established (total ICC>.85) to assure both standardized administration and reduction of method error. Subtests from the NAB (Neuropsychological Assessment Battery, Stern & White, PAR Inc.) have been chosen for brevity, sensitivity, and due to the wide range of available normative data (ages 18-97). Subtests of the NAB can be administered alone or as part of the entire battery without loss of appropriate normative data, as each test across forms was administered to the same individuals in the standardization sample. Administration of the entire battery can take up to 4 hours, thus a subset of requisite performance measures was selected for inclusion in this study. In addition, the Wechsler Test of Adult Reading (WTAR, Pearson Inc.) will be administered to provide an estimate of premorbid cognitive functioning. Subtests chosen from the NAB include: Digits Forwards (DF); Digits Backwards (DB); Letter and Numbers (L&N). Together, these will assess attention capacity, working memory, and processing speed. Naming (NAM). This will assess confrontation naming and anomia. Design Construction (DES). This will assess spatial awareness and constructional praxis. List Learning (LL); Story Learning (SL); and Shape Learning (ShL). These will assess auditory and visual episodic learning and memory retrieval and retention skills. Mazes (MZ); Categories (CAT); and Word Generation (WG). These will assess higher order executive functioning with regard to planning, conceptualization, and fluency. We expect the most significant effects to arise from assessment of processing speed, memory, and executive functioning. Each of these subtests will be administered at baseline, time 1, and time 2. The NAB was standardized with 2 alternate, equivalent forms, which will be counterbalanced across administration times while repeating one form per subject.

Given the length of time 2 to time 3, practice effects across the three forms can be minimized. The tests will be administered as follows:

Cognitive Domain	**Time 1 (Baseline)**	**Time 2 week following treatment)**	**Time 3 (3 months following treatment)**
Premorbid Intelligence	WTAR		
Attention / Processing Speed	DF, DB, L&N	DF, DB, L&N	DF, DB, L&N
Language	NAM	NAM	NAM
Visuospatial	DES	DES	DES
Learning and Memory	LL, SL, ShL	LL, SL, ShL	LL, SL, ShL
Executive Functioning	MZ, CAT, WG	MZ, CAT, WG	MZ, CAT, WG

10.3 Headache Correlate

Patients with radiation necrosis often experience severe or intractable headaches. These headaches usually worsen during efforts to wean the patient off corticosteroids, leading to long-term steroid use and all its associated serious morbidities. Our preliminary experience indicates rapid and complete resolution of headaches following IA-bevacizumab treatment. All patients will be tested using a formal headache battery at baseline. The Headache Impact Test (HIT-6) which is a fixed-length 6-item, paper form version of the DYNHA Headache Impact Test will be filled out by the patient. MIDAS 5-item questionnaire will also be completed by the patient before treatment, as well as at 6 weeks, 3 mo, 6 mo, 9 mo, and 12 mo.

11. STUDY PARAMETERS AND CALENDAR

The tests and procedures found in the Study Flow Chart will be performed. Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done 4 weeks prior to the start of therapy.

	Pre-Study	Day 0	Day 1	Week 6	Month 3	Month 6	Month 12
Informed Consent	R						
Demographics	NR						
Medical History	NR	NR	NR		NR		NR
Concurrent Medication	NR	NR	NR		NR		NR
Physical Exam	NR	R	R		R		R
Neurological Exam	NR	R	R		R		R
Carotid Angiogram		R					
Osmotic blood-brain-barrier breakdown		R					
Avastin®		R					
HIT – 6 Survey (may be via phone)	R			R	R	R	R
MIDAS (may be via phone)	R			R	R	R	R
NeuroPsych Testing	R				R		R
KPS Functional Status	R		R		R		R
Screening Lab tests[1]	R						
ECG (if needed)[2]	R						
Adverse Event monitoring		R	R		R		R
Urine Pregnancy Test		R					
Brain MRI w & w/o contrast	NR				NR		NR

R= Research
NR= non-research or standard of care
1 = CBC with differential and platelets count, basic metabolic panel serum chemistry
2= ECG will be done in patients >45 years old or in any patients with history of cardiac problems

Physical Examination: All enrolled participates will have an abbreviated physical examination and vital signs (heart rate, blood pressure and body weight) collected during screening and then again at Day 0, and 1, Month 3 and 12.

Neurological Examination: All enrolled participants will have a detailed neurological examination during screening and then again at Day 0, and 1, Month 3 and 12.

Pregnancy Testing: It is not known whether study drug used in this study may cause side effects to pregnant women, to an unborn child, or to children of nursing women. Because of these unknown risks, all women of childbearing potential will be given a pregnancy test prior to receiving study drug. Testing will be performed either by urine or blood. **Female participants will be informed that they must not become pregnant until 8 weeks following the last dose of study medication**

Karnofsky Performance Status Scale: Will be used to measure the functional status of the participant prior to and following study drug administration

Brain MRI: All subject will undergo a Brain MRI prior to receive study medication (Pre-study) and then again at Months 3, and 12.

CT Scan: Subject who cannot undergo a MRI for medical reasons will have undergo a head CT prior to receiving study medication (Pre-Study) and then again at Months 3 and 12.

12. MEASUREMENT OF EFFECT

12.1 Decrease in Cerebral Edema

We will use pre-contrast axial and sagittal T1 with the axial with fat-sat, T2, FLAIR, DWI/ADC, susceptibility, post-contrast 3-plane FS T1 images, as well as brain perfusion to evaluate for progression. All scans will be done on a 1.5-3.0 Tesla MR. The scans will be performed within 4 weeks before the 1st treatment, at 3 months, and 12 months, or as indicated for new clinical symptoms. A reduction in volume of cerebral edema of > 25% will constitute a positive response.

12.2 Definitions

Please use or modify the following text as appropriate.

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with Bevacizumab.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression treatment will also be considered evaluable.)

12.3 Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Two-stage design, Single-arm Phase II multi-center clinical trial. The participating centers are Norton Healthcare and University of Kentucky Healthcare. This study will determine efficacy of IA-bevacizumab after BBBD treatment for radiation necrosis.

13.2 Sample Size/Accrual Rate

Stage 1: There will be a maximum of 10 patients accrued for stage 1 of this trial. In 2013, there were a total of 5 patients seen and evaluated for medically refractory radiation necrosis at our center. Therefore, the accrual rate would be anticipated as 5 patients per year. The accrual rates are expected to be 3-5 per year in other participating site.

Stage 2: If $\geq$30% of patients have a positive response, then the number of patients in the trial will escalate to 46 patients. At that point, further participating sites will be considered.

13.3 Stratification Factors: *None.*

13.4 Analysis of Secondary Endpoints: Secondary endpoint findings will be compared to historical controls from published studies of IV-bevacizumab treatment for radiation necrosis.

13.5 Outcome Measures

Primary Outcome Measure: Decrease in radiation necrosis and cerebral edema after a single treatment of low dose intrarterial bevacizumab: Precontrast axial and sagittal T1 with the axial with fat-sat, T2, FLAIR, DWI/ADC, susceptibility, post-contrast 3-plane FS T1 images, as well as brain perfusion will be used to evaluate for radiation necrosis and cerebral edema. All scans will be done on a 1.5-Tesla or higher MR. The scans will be performed before the 1st treatment, at 3 months, and 12 months, or as indicated for new clinical symptoms. All MRI's will be sent on CD to Core Lab at Norton Healthcare run by Dr. Kadner (board-certified neuro-radiologist). If a patient cannot have an MRI for medical reasons, then CT head with and without contrast will be used instead.

Secondary Outcome Measures:

1. Decrease in steroid usage after a single treatment of low dose intrarterial bevacizumab: To assess the utility of intra-arterial bevacizumab treatment in allowing decreased steroid usage, the quantity of steroid use (measured as mg of Decaderon per day) will be noted at 1 day, 3 months, and 12 months.

2. Quantitative improvement in headache after a single treatment of low dose intrarterial bevacizumab: Quantitative improvement in headache will be assessed by performing The Headache Impact Test (HIT-6) which is a fixed-length 6-item, paper

form version of the DYNHA Headache Impact Test will be filled out by the patient. MIDAS 5-item questionnaire will also be completed by the patient before treatment, as well as at 6 weeks, 3 mo, 6 mo, 9 mo, and 12 mo.

3. Neurocognitive improvement after a single treatment of low dose intrarterial bevacizumab: In order to investigate the immediate and post-operative neurocognitive sequelae of intra-arterial bevacizumab, patients who consent to a formal neuropsychological battery will be tested at baseline (prior to IA bevacizumab administration), 3 and 12 months postoperative follow-up. Subtests from the NAB (Neuropsychological Assessment Battery, Stern & White, PAR Inc.) have been chosen for brevity, sensitivity, and due to the wide range of available normative data (ages 18-97). In addition, the Wechsler Test of Adult Reading (WTAR, Pearson Inc.) will be administered to provide an estimate of premorbid cognitive functioning.

4. Safety of a single treatment of low dose intrarterial bevacizumab: Safety of intra-arterial bevacizumab will be assessed by noting the number of participants with treatment-related adverse events as assessed by CTCAE v4.0.

5. Neurological improvement after a single treatment of low dose intrarterial bevacizumab: Objective improvement in neurological exam will be assessed by performing serial neurological exams at baseline, 1 day, 3 months, and 12 months. Also, quantitative improvement in functional status will be assessed by performing Karnofsky Performance Status Scale (KPS) at baseline, 1 day, 3 months, and 12 months.

Other Pre-specified Outcome Measures: Cost analysis of a single treatment of low dose intrarterial bevacizumab compared to conventional IV-bevacizumab regiment: Cost analysis of a single treatment of low dose intrarterial bevacizumab after osmotic blood-brain-barrier disruption as compared to conventional intravenous bevaizumab use for radiation necrosis (7.5 mg/kg IV-Bevacizumab every 3 weeks for 4 cycles).

14. References

1. Levin VA, Bidaut L, Hou P, et al. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. International Journal of radiation Oncology, Biology, Physics 2011;79:1487-95.
2. Nonoguchi N, Miyatake S, Fukumoto M, et al. The distribution of vascular endothelial growth factor-producing cells in clinical radiation necrosis of the brain: pathological consideration of their potential roles. Journal of neuro-oncology 2011;105:423-31.
3. Dashti SR, Spalding A, Kadner RJ, et al. Targeted intraarterial anti-VEGF therapy for medically refractory radiation necrosis in the brain. J Neurosurg Pediatr 2015;15:20-5.
4. Monaco EA, 3rd, Niranjan A, Kano H, Flickinger JC, Kondziolka D, Lunsford LD. Management of adverse radiation effects after radiosurgery for arteriovenous malformations. Progress in neurological surgery 2013;27:107-18.
5. Williamson R, Kondziolka D, Kanaan H, Lunsford LD, Flickinger JC. Adverse radiation effects after radiosurgery may benefit from oral vitamin E and pentoxifylline therapy: a pilot study. Stereotactic and functional neurosurgery 2008;86:359-66.
6. Levin VA, Bidaut L, Hou P, et al. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. Int J Radiat Oncol Biol Phys 2011;79:1487-95.
7. Li J, Gupta M, Jin D, Xin Y, Visich J, Allison DE. Characterization of the long-term pharmacokinetics of bevacizumab following last dose in patients with resected stage II and III carcinoma of the colon. Cancer chemotherapy and pharmacology 2013;71:575-80.
8. Takano M, Kikuchi Y, Kato M, Yoshikawa T, Kita T. Bowel perforation associated with bevacizumab therapy in recurrent ovarian cancers without bowel obstruction or bowel involvement]. Gan To Kagaku Ryoho 2008;35:1981-4.
9. Heinzerling JH, Huerta S. Bowel perforation from bevacizumab for the treatment of metastatic colon cancer: incidence, etiology, and management. Curr Surg 2006;63:334-7.

10. Abbrederis K, Kremer M, Schuhmacher C. [Ischemic anastomotic bowel perforation during treatment with bevacizumab 10 months after surgery]. Chirurg 2008;79:351-5.
11. Suenaga M, Mizunuma N, Kobayashi K, et al. Management of venous thromboembolism in colorectal cancer patients treated with bevacizumab. Med Oncol;27:807-14.
12. Grivas AA, Trafalis DT, Athanassiou AE. Implication of bevacizumab in fatal arterial thromboembolic incidents. J BUON 2009;14:115-7.
13. Vredenburgh JJ, Desjardins A, Kirkpatrick JP, et al. Addition of Bevacizumab to Standard Radiation Therapy and Daily Temozolomide Is Associated with Minimal Toxicity in Newly Diagnosed Glioblastoma Multiforme. Int J Radiat Oncol Biol Phys.
14. Chamberlain MC, Johnston S. Salvage chemotherapy with bevacizumab for recurrent alkylator-refractory anaplastic astrocytoma. J Neurooncol 2009;91:359-67.
15. Gobin YP, Cloughesy TF, Chow KL, et al. Intraarterial chemotherapy for brain tumors by using a spatial dose fractionation algorithm and pulsatile delivery. Radiology 2001;218:724-32.
16. Namba H, Kobayashi S, Iwadate Y, et al. Assessment of the brain areas perfused by superselective intra-arterial chemotherapy using single photon emission computed tomography with technetium-99m-hexamethyl-propyleneamine oxime--technical note. Neurol Med Chir (Tokyo) 1994;34:832-5.
17. Neuwelt EA, Frenkel EP, D'Agostino AN, et al. Growth of human lung tumor in the brain of the nude rat as a model to evaluate antitumor agent delivery across the blood-brain barrier. Cancer Res 1985;45:2827-33.
18. Armstrong BK, Robinson PJ, Rapoport SI. Size-dependent blood-brain barrier opening demonstrated with [14C]sucrose and a 200,000-Da [3H]dextran. Exp Neurol 1987;97:686-96.
19. Ziylan YZ, Robinson PJ, Rapoport SI. Differential blood-brain barrier permeabilities to [14C]sucrose and [3H]inulin after osmotic opening in the rat. Exp Neurol 1983;79:845-57.
20. Bullard DE, Bigner DD. Applications of monoclonal antibodies in the diagnosis and

treatment of primary brain tumors. J Neurosurg 1985;63:2-16.
21. Zalutsky MR, Moseley RP, Benjamin JC, et al. Monoclonal antibody and F(ab')2 fragment delivery to tumor in patients with glioma: comparison of intracarotid and intravenous administration. Cancer Res 1990;50:4105-10.
22. Epenetos AA, Courtenay-Luck N, Pickering D, et al. Antibody guided irradiation of brain glioma by arterial infusion of radioactive monoclonal antibody against epidermal growth factor receptor and blood group A antigen. Br Med J (Clin Res Ed) 1985;290:1463-6.
23. Zalutsky MR, Moseley RP, Coakham HB, Coleman RE, Bigner DD. Pharmacokinetics and tumor localization of 131I-labeled anti-tenascin monoclonal antibody 81C6 in patients with gliomas and other intracranial malignancies. Cancer Res 1989;49:2807-13.
24. Neuwelt EA, Barnett PA, Hellstrom KE, Hellstrom I, McCormick CI, Ramsey FL. Effect of blood-brain barrier disruption on intact and fragmented monoclonal antibody localization in intracerebral lung carcinoma xenografts. J Nucl Med 1994;35:1831-41.
25. Lee YS, Bullard DE, Zalutsky MR, et al. Therapeutic efficacy of antiglioma mesenchymal extracellular matrix 131I-radiolabeled murine monoclonal antibody in a human glioma xenograft model. Cancer Res 1988;48:559-66.
26. Dahlborg SA, Henner WD, Crossen JR, et al. Non-AIDS primary CNS lymphoma: first example of a durable response in a primary brain tumor using enhanced chemotherapy delivery without cognitive loss and without radiotherapy. The cancer journal from Scientific American 1996;2:166-74.
27. Doolittle ND, Miner ME, Hall WA, et al. Safety and efficacy of a multicenter study using intraarterial chemotherapy in conjunction with osmotic opening of the blood-brain barrier for the treatment of patients with malignant brain tumors. Cancer 2000;88:637-47.
28. Neuwelt EA. Mechanisms of disease: the blood-brain barrier. Neurosurgery 2004;54:131-40; discussion 41-2.
29. Boockvar JA, Tsiouris AJ, Hofstetter CP, et al. Safety and maximum tolerated dose of superselective intraarterial cerebral infusion of bevacizumab after osmotic blood-brain barrier disruption for recurrent malignant glioma. J Neurosurg.
30. Rajappa P, Krass J, Riina HA, Boockvar JA, Greenfield JP. Super-selective basilar artery infusion of bevacizumab and cetuximab for multiply recurrent pediatric ependymoma. Interventional neuroradiology : journal of peritherapeutic neuroradiology, surgical procedures and related neurosciences 2011;17:459-65.
31. Riina HA, Burkhardt JK, Santillan A, Bassani L, Patsalides A, Boockvar JA. Short-term clinico-radiographic response to super-selective intra-arterial cerebral infusion of Bevacizumab for the treatment of vestibular schwannomas in Neurofibromatosis type 2. Interventional neuroradiology : journal of peritherapeutic neuroradiology, surgical procedures and related neurosciences 2012;18:127-32.
32. Riina HA, Knopman J, Greenfield JP, et al. Balloon-assisted superselective intra-arterial cerebral infusion of bevacizumab for malignant brainstem glioma. A technical note. Interventional neuroradiology : journal of peritherapeutic neuroradiology, surgical procedures and related neurosciences 2010;16:71-6.