Status Page

PROTOCOL 13-482

Permanently
Closed to New Accrual

Closure Effective Date: 01/09/2019
No new subjects may be enrolled in the study as described above. Any questions regarding this closure should be directed to the study's Principal Investigator

Version Date: 3/28/2019

A study to evaluate vascular normalization in patients with recurrent glioblastoma treated with bevacizumab and lomustine using FMISO PET and vascular MRI

Principal Investigator: Elizabeth Gerstner, M.D.
Pappas Center for Neuro-Oncology
55 Fruit Street, Yawkey 9E
Boston, MA 02114
Phone: 617-724-2887
Fax: 617-643-2591
egerstner@mgh.harvard.edu

Co-Investigators:

Umar Mahmood, M.D., Ph.D.
Martinos Center for Biomedical Imaging
Charlestown, MA 02129
Phone : 617-726-6477
umahmood@mgh.harvard.edu

Patrick Wen, M.D.
Center for Adult Neuro-Oncology
Dana-Farber Cancer Institute
44 Binney Street
Boston, MA
pwen@partners.org

Agent(s): FMISO

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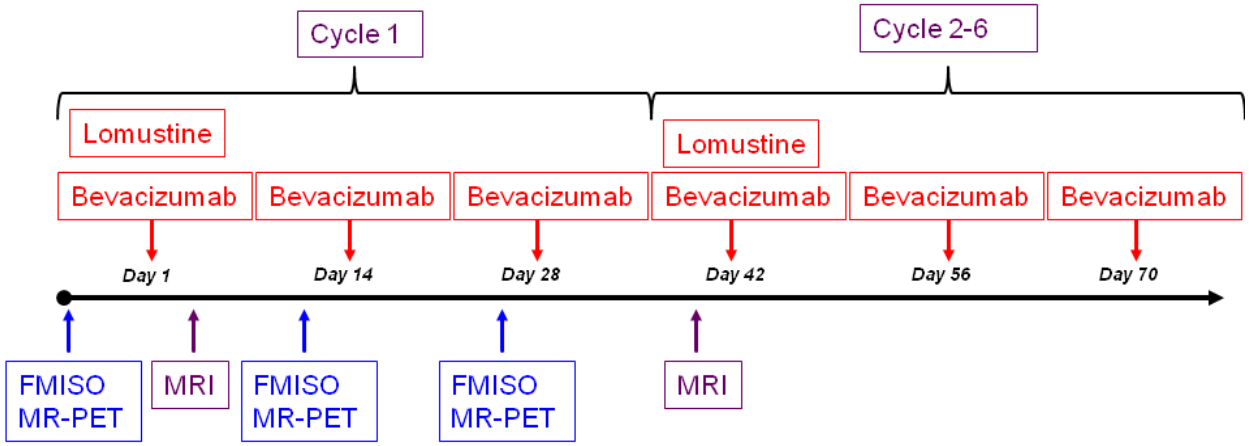
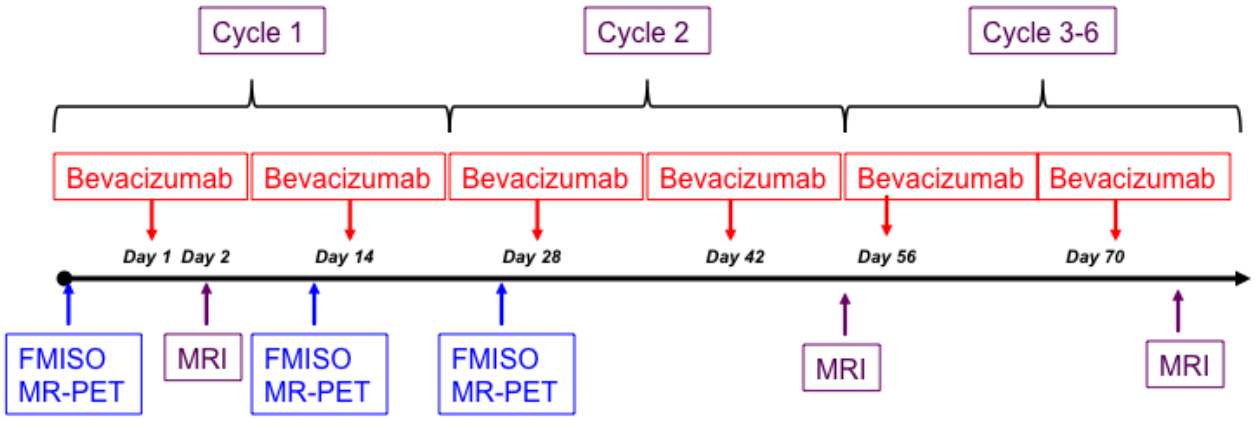
SCHEMA



Population: **Recurrent glioblastoma patients receiving bevacizumab and lomustine.**



Population: **Recurrent glioblastoma patients receiving bevacizumab monotherapy.**

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1. OBJECTIVES

1.1 Study Design

Our goal is to explore changes in tumor vasculature resulting from treatment with bevacizumab and the impact these changes have on tumor oxygenation. Results from this study will shed light on the vascular normalization hypothesis. This study will evaluate patients with recurrent glioblastoma with simultaneous FMISO PET and MRI to assess changes in tumor blood flow, blood volume, vessel caliber, and vascular permeability with advanced MRI while simultaneously measuring tumor oxygenation status with FMISO PET.

We plan to study patients with recurrent glioblastoma whose clinical care plan includes treatment with bevacizumab. We will perform FMISO-PET at 3 time points in all patients: before beginning bevacizumab, prior to the second dose of bevacizumab, and prior to the third dose of bevacizumab. In addition, patients will undergo advanced MRI scans 1 day after start of bevacizumab and monthly for 6 months of treatment.

This study protocol is being amended to account for the publication of recent data which has impacted clinical practice. Based on the published results from the 3-arm BELOB study (Nederlands Trial Register, www.trialregister.nl, number NTR1929) there has been a shift to treating patients with combination bevacizumab and lomustine rather than bevacizumab alone (Taal et al 2014). In this randomized, phase 2, multicenter trial the combination of bevacizumab and lomustine was associated with superior overall survival. Although this study was not formally designed for comparisons across the 3 arms it has influenced the practice of neuro-oncology with some shift away from bevacizumab monotherapy. A randomized, phase 3 trial is underway comparing bevacizumab + lomustine versus lomustine alone (NCT01290939).

For this protocol, there will now be 2 cohorts of patients. Cohort A includes patients with recurrent GBM treated with bevacizumab and lomustine and cohort B includes patients receiving bevacizumab monotherapy. The majority of our patients at MGH are currently treated with combination therapy so will help speed up accrual. However, not every patient can receive combination therapy so we are including the second, exploratory cohort of patients treated with bevacizumab monotherapy.

Lomustine is not expected to have its own anti-angiogenic effects so adding this therapy will not impact the interpretation of the anti-vascular effects of bevacizumab. In general, alkylating agents such as temozolomide or lomustine are not thought to target endothelial cells. In humans treated with alkylating agents, we do not see the remarkable decrease in contrast enhancement seen with anti-VEGF therapy. For example, there was very little change in tumor volume in patients treated with lomustine alone in both the REGAL study and BELOB study) (Batchelor 2013, Taal 2014). If there was a true effect on tumor vasculature, we would see more change in the post-contrast images similar to the responses seen in the ant-VEGF treated arms.

Furthermore, our own data comparing a cohort of patients with newly diagnosed GBM treated with standard radiation and temozolomide to a cohort of patients treated with standard

radiation, temozolomide, and cediranib (an anti-VEGF agent) did not show the same vascular normalization effects (as measured by changes in contrast enhancement, permeability, perfusion, and tumor oxygenation) in the temozolomide + RT alone group (Batchelor 2013).

The PET studies will be performed using our human simultaneous MR-PET imaging camera. No diagnostic decisions or therapy decisions will be based on any results obtained from these PET scans, and we expect no change in the care of these patients. The success of these studies should enable methods that could be used in larger studies to more completely understand the role of anti-angiogenic agents in the treatment of cancer.

1.2 Primary Objectives

1. To determine if bevacizumab improves tumor blood flow/perfusion.
2. To determine if bevacizumab reduces tumor hypoxia.

1.3 Secondary Objectives

1. To explore the link between flow, permeability, and tumor hypoxia.
    a. Measure tumor blood flow with MRI perfusion and compare with tumor hypoxic volume.
    b. Estimate permeability from K^{trans} MRI measurements and compare with tumor hypoxic volume.
2. To explore the link between tumor hypoxia and blood biomarkers of angiogenesis

2. BACKGROUND

2.1 Study Agent(s)

2.2 FMISO

Since the 1980s, interest in radiolabeled nitroimidazoles, and particularly FMISO, as nuclear imaging agents of hypoxia has been growing. Intracellular nitroreductases metabolize nitroimidazoles in the setting of low oxygen levels, thus trapping them in hypoxic cells. Importantly, the metabolism of nitroimidazoles relies on an active electron transport chain so only live cells can trap FMISO. The binding of FMISO is related to the level of hypoxia, and oxygen levels of 3 to 10 mm Hg lead to FMISO binding (Rasey et al, 1990; Gross et al, 1995). Several studies have demonstrated that the uptake of FMISO correlates well with invasive polarographic oxygen electrodes, the gold standard to determine tissue oxygenation (Koh et al, 1992; Bernsen et al, 2000; Rasey et al 1987; Rasey et al, 1996).

Preliminary studies of FMISO uptake in glioma have demonstrated the feasibility of this technique both in animal models and in humans (Valk et al, 1992). In a glioma rat model, FMISO uptake was significantly greater in tumor tissue than in surrounding normal brain (Tochon-Danguy et al 2002). Cher et al showed that all high-grade gliomas have increased FMISO uptake while low-grade gliomas did not, which is consistent with the underlying biology of gliomas—higher-grade

tumors are more hypoxic than lower-grade tumors (Cher et al, 2006). In 22 participants with newly-diagnosed GBM, the greater the volume and degree of hypoxia based on FMISO imaging, the shorter time-to-tumor progression and survival (Spence et al, 2008). In addition, histological studies of tumors have correlated tissue pimonidazole staining, a marker of tissue hypoxia, and CAIX with FMISO uptake (Troost et al, 2006; Dubois et al 2004). In one of these studies, the glioma model had the strongest correlation between FMISO uptake and pimonidazole staining (Troost et al, 2006). Ex vivo autoradiography has also been used to confirm the correlation of FMISO and tumor hypoxia (Sorensen et al, 2005).

2.3 **Study Disease**

An estimated 51,410 primary brain tumors were diagnosed in 2007, and 19% of these tumors were glioblastomas [1]. GBM is the most common malignant primary brain tumor and is a uniformly fatal disease with 5-year survival rates less than 4% despite aggressive treatment with surgery, radiation and chemotherapy. There is no curative therapy for patients with GBM. Current standard of care for patients with newly diagnosed GBM is concomitant involved field radiation and oral temozolomide chemotherapy followed by monthly temozolomide for 6-12 months (Stupp et al., 2005). If patients relapse, there is no standard of care treatment and some patients will be treated with temozolomide again, albeit on a different dosing schedule. Instead of 5 consecutive days every month, patients may be treated with a lower daily dose of temozolomide but for more days every month (Perry et al., 2010). The goal of this dose dense regimen is to saturate the enzyme, MGMT, which is believed to counteract the DNA-damaging effects of temozolomide. Bevacizumab was recently approved by the FDA for recurrent GBM and is ultimately used in most recurrent GBM patients.

2.4 **Rationale**

The dependence of tumor growth and metastasis on angiogenesis — which has been extensively demonstrated in animal models — has provided a powerful rationale for anti-angiogenic approaches to cancer therapy (van de Beek, 2007; Carmeliet & Jain, 2000; Kleihues, Burger, & Scheithauer, 1993). Targeting blood vessels in brain tumors has been a particularly attractive strategy, given the characteristic high degree of endothelial proliferation, vascular permeability, and pro-angiogenic growth-factor expression (for example, VEGF) (Dvorak, 2002; Sundberg, 2001). The approval of bevacizumab, which neutralizes VEGF, in May 2009 for recurrent glioblastoma (rGBM) represented the first new therapy for this disease in many years. A strong anti-edema effect is clearly seen with anti-VEGF therapy in many patients. Whether this conveys a survival benefit is still not clear; cediranib did not improve survival over lomustine in rGBM in a recent phase III trial, and while definitive phase III studies of bevacizumab are underway, recent data suggests that bevacizumab does not improve overall survival when given to newly diagnosed glioblastoma patients (Lai et al., 2010).

Bevacizumab is not an effective monotherapy outside the brain, but it is effective when combined with other drugs. The mechanism of this clinical efficacy is still incompletely understood. Anti-VEGF therapies have been shown to promote vascular "normalization" (Batchelor et al., 2007; Jain, 2005; Willett et al., 2004) in many patients, which could improve local tumor blood flow and therefore cytotoxic chemotherapy delivery to the tumor. We have shown that the 'normalization window' of the anti-VEGF agent cediranib can be detected using serial, non-invasive MRI techniques in rGBM (Batchelor et al., 2007), and we have seen increases in blood flow (Sorensen et al 2012). The outstanding question is whether or not this increased perfusion also translates into improved tumor oxygenation. Since anti-VEGF therapies were originally designed to block blood vessel formation, in theory these agents could potentially also inhibit drug delivery to the tumor and make therapies such as radiation, which rely on oxygenation less effective (Jain, 2005; Jain, 2001).

Our novel hybrid MR-PET technology should allow us to assess both hypoxia and vascular changes to determine the time course and interplay between vascular physiology and tumor oxygenation.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Participants must have histologically confirmed glioblastoma and evidence of recurrence. Patients with low-grade tumors who have progressed to glioblastoma are eligible.

3.1.2 Participants must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$ 10 mm. See section 10 for the evaluation of measureable disease.

3.1.3 For cohort A, only patients for whom their neuro-oncologist has planned to give bevacizumab with lomustine are eligible for this study.

3.1.4 For cohort B, only patients for whom their neuro-oncologist has planned to give bevacizumab monotherapy are eligible for this study.

3.1.5 Age > 18 years. Because no dosing or adverse event data are currently available on the use of FMISO in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric trials.

3.1.6 Life expectancy of greater than 3 months.

3.1.7 Karnofsky performance status > 60 (see Appendix A).

3.1.8 Participants must have normal organ and marrow function as defined below:

- Leukocytes $\geq$ 3,000/mcL
- Absolute neutrophil count $\geq$ 1,500/mcL
- Platelets $\geq$ 75,000/mcL for bevacizumab monotherapy cohort; >100,000 /mcL for bevacizumab + lomustine cohort
- Total bilirubin within normal institutional limits
- AST (SGOT)/ALT (SGPT) $\leq$ 2.5 X institutional upper limit of normal
- Creatinine within normal institutional limits or creatinine clearance $\geq$ 60 mL/min/1.73 m^2 for subjects with creatinine levels about institutional normal.

3.1.9 Patient must be able to undergo MRI and PET scans.

3.1.10 Patients on cortiosteroids must be maintained on a stable corticosteroid regimen for 5 days prior each MR-PET scan.

3.1.11 The effects of FMISO on the developing human fetus are unknown. For this reason and because radiopharmaceuticals agents are known to be teratogenic, women of child-bearing 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.

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

3.2 **Exclusion Criteria**

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 History of allergic reactions attributed to compounds of similar chemical or biologic composition to temozolomide or FMISO.

3.2.2 Participants who have already received anti-VEGF or experimental anti-angiogenic therapy for glioblastoma.

3.2.3 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.

3.2.4 Pregnant women are excluded from this study because FMISO is a radiopharmaceutical agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with radiopharmaceutical agents, breastfeeding should be discontinued if the mother is treated with radiopharmaceutical agents. These potential risks may also apply to other agents used in this study.

3.2.5 HIV-positive individuals on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with FMISO. In addition, these individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

3.2.6 Patients with intracerebral hemorrhage deemed significant by treating physician are excluded.

3.2.7 Patients who are no suitable to undergo MRI or use gadolinium contrast due to:

- Claustrophobia
- Presence of metallic objects or implanted medical devices in body (i.e. cardiac pacemaker, aneurysm clips, surgical clips, prostheses, artificial hearts, valves with steel parts, metal fragments, shrapnel, tattoos near the eye, or steel implants)
- Sickle cell disease
- Renal failure
- Reduced renal function, as determined by creatinine clearance < 30 mL/min based on a serum creatinine level obtained within 28 days prior to registration

3.2.8 Inclusion of Women, Minorities and Other Underrepresented Populations

Both men and women and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

   Exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

4.3 General Guidelines for Other Participating Institutions

N/A

4.4 Registration Process for Other Participating Institutions

N/A

5. STUDY PLAN

This study does not add any additional treatment to patients with malignant glioma who are already scheduled to receive bevacizumab plus lomustine (cohort A) or bevacizumab monotherapy (cohort B). All treatment decisions will be at the discretion of the treating physician. There will be no change in the diagnosis or management of the patient based on any procedures or tests carried out as a part of this study.

In brief, bevacizumab will be administered at a dose of 10 mg/kg i.v. every 14 days per standard of care and the drug label. Lomustine will be given at 90mg/m^2 every 6 weeks for a maximum of 6 cycles. (Taal 2014) A cycle is defined as 6 weeks (42 days). The study duration is 42 weeks (6 cycles). Patients will be treated after 42 weeks or at the time of progression per discretion of their responsible physician. Standard computerized order entry (COE) orders will be utilized for administering this drug.

There are no expected toxicities and potential risks for FMISO. All study related scans will be performed at the Martinos Center for Biomedical Imaging in Charlestown.

5.1 Pre-MR-PET Scan Criteria

5.1.1 Stable dose of corticosteroids for 5 days prior to MR-PET scan (if on corticosteroids).

5.1.2 Creatinine clearance >30 mL/min based on a serum creatinine level obtained within 30 days.

5.1.3 β-HCG in woman of childbearing age (prior to each MR-PET scans).

Please see
http://healthcare.partners.org/phsirb/Guidance/Pregnancy_Testing_in_Research_Involving_Radiation.1.09.pdf for Pregnancy Testing guidelines)

5.1.4 Adequate laboratory values as outlined in eligibility criteria.

5.2 MR-PET Scan Procedure

MA at the Martinos Center. Two lines will be placed for each scan: one venous line to inject the FMISO and gadolinium for the MRI scan, and a second to draw blood to assay for radioactivity and metabolites. If a patient has a port, the port can replace one of the IVs.

5.2.1 FMISO PET scans

The PET scan will be up to 90 minutes. There will be one injection of FMISO in the PET protocol. The blood will be drawn by a nuclear medicine technologist or a nurse trained in radiation safety.

FMISO will be intravenously injected at a dose of 3.7 MBq/kg (0.1 mCi/kg) (maximum 260 MBq, 7 mCi) in $\leq$ 15 mL. The IV will remain in place for injection of the gadolinium for the MRI scan.

Blood draws will be performed at approximately t=20, 40, 60, 115, 120, 125 minutes after the injection. The whole blood will be counted and then centrifuged to separate plasma from cells. Aliquots of plasma and blood will be separately counted for radioactivity. The plasma will be further analyzed using high performance liquid chromatography for radiolabeled metabolites. Blood will be transported to the well counter and HPLC systems in a properly shielded container.

PET volumes will be reconstructed using the ordinary Poisson ordered subsets expectation maximization (OP-OSEM) 3D algorithm. Corrections will be applied for variable detector dead time and efficiency, random coincidences, photon attenuation and scatter, and decay.

5.2.2 MRI Scan

MR scans will be performed with the same sequences and in the same order during each visit, including T1- and T2-weighted volumetric images, fluid attenuated inversion recovery (FLAIR), contrast agent enhanced T1-weighted permeability, diffusion tensor imaging (DTI), T2/T2*-weighted perfusion scans, and MR Spectroscopy. The "Autoalign" package available from the manufacturer will be used to achieve the same slice prescription in the same patient at each visit. Each MRI will last 60-75 minutes versus 45 minutes for standard brain MRIs.

Gadolinium-diethylenetriaminepentaacetic acid (or gadopentetate dimeglumine, Gd-DTPA) will be used as a contrast agent and injected intravenously twice in each scan session. The maximum contrast dose that could be given is 0.3 mmol/kg per visit, in line with the FDA-approved dosing for this class of contrast agents.

5.3 Definition of Dose-Limiting Toxicity

N/A

5.4 General Concomitant Medication and Supportive Care Guidelines

Corticosteroids: Should be used at the lowest possible dose to control cerebral edema and mass effect and discontinued if possible.

Anti-coagulation: Therapeutic anticoagulation with low molecular weight heparin or warfarin is permitted. Patients in warfarin must have INR <3.

Anti-seizure medications: Should be used as indicated.

5.5 Duration of Therapy

Patients may remain on study unless one of the following criteria applies:

- Intercurrent illness that prevents administration of FMISO or bevacizumab
- Unacceptable adverse event(s),
- Unacceptable adverse reaction
- Participant decides to withdraw from the study,
- General or specific changes in the participant's condition render the participant unacceptable for bevacizumab, MRI or PET in the opinion of the treating investigator.

Importantly, the following do NOT remove the patient from the study if they have already undergone the first MR-PET scan:

- Disease progression

5.6 Duration of Follow Up

Participants will be followed for 3 years after removal from study or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients will be followed for survival, subsequent treatments received, and response to treatment by chart review or phone call if patient does not return to clinic. Follow-up will be assessed every 3 months.

5.7 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.5 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant. In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator at 617-724-8770.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the following recommendations. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the agents administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 Adverse Event List(s) for FMISO

There are no known adverse effects of FMISO.

6.1.2 Adverse Event List for lomustine

Nausea, vomiting, poor appetite, myelosuppression, infection, bleeding, pulmonary toxicity, liver toxicity, mucositis, alopecia, optic nerve atrophy, renal toxicity.

6.1.3 Adverse Event List for bevacizumab

Hypertension: An increased incidence of hypertension has been reported. Grade 4 and 5 hypertension events are rare as are hypertensive crisis, hypertensive encephalopathy, and reversible posterior leucoencephalopathy syndrome (RPLS).

Proteinuria: An increased incidence of proteinuria up to 38% has been observed. Patients with HTN may be at increased risk of developing proteinuria.

Thromboembolic events: Both venous and arterial thromboembolic events (TE) have been reported with a range of 2.8-17.3% in bevacizumab treated patients compared to 3.2%-15.6% in chemotherapy alone treated patients.

The following have been additional reported complications of bevacizumab: gastrointestinal perforation, fistula development, wound healing delay, hemorrhage (Grade 3 and 5 events reported in 4.0% of patients), tumor associated hemorrhage, and congestive heart failure.

6.2 Toxicity Management

Patients will be managed symptomatically as clinically indicated. Hypertension in particular should be treated at the first sign of elevated blood pressure.

6.3 Dose Modifications/Delays

6.3.1 FMISO

No dose reduction of FMISO is permitted.

6.3.2 Lomustine

Doses subsequent to the initial dose should be adjusted according to the hematologic response of the patient to the preceding dose. The following schedule is suggested as a guide to dosage adjustment:

Table: Lomustine Dose Modifications for Hematological Toxicity

Nadir After Prior Dose		Dose Reduction
Leukocytes	Platelets	

>4,000	>100,000	None
3,000-3,999	75,000-99,999	None
2,000-2,9999	25,000-74,999	70mg/m2
<2,000	<25,000	50mg/m2

Complete blood counts should be monitored weekly starting 4 weeks after lomustine dose and repeat courses should not be given before 6 weeks as hematologic toxicity is delayed and cumulative. Growth factor support is permitted. Lomustine may be dose reduced a maximum of 2 times.

If toxicities (as determined CTCAE version 4.0) of grade >2 are observed (except alopecia, nausea and vomiting) and related to lomustine, then the dose of lomustine should be held until recovery to grade ≤ 1.

All grade 3 or 4 nonhematological toxicities related to lomustine (except alopecia, anorexia, nausea and vomiting) should have resolved to grade ≤ 1 and lomustine should be re-initiated at a dose of 70mg/m^2. A second dose reduction of lomustine to 50 mg/m^2 is permitted if there are grade 3 or 4 toxicities at the dose of 70 mg/m^2.

If a patient develops unacceptable toxicity to lomustine, bevacizumab may be continued as monotherapy.

Lomustine may be held for a maximum of 12 weeks from the scheduled date of cycle initiation to allow for resolution of toxicity.

The maximum number of cycles of lomustine is 6.

6.3.3 Bevacizumab

Prior to each bevacizumab infusion, the following parameters should be met: platelet count >75,000/mcL, proteinuria < grade 3, creatinine <1.5xULN, hypertension < grade 4.

If toxicities (as determined CTCAE version 4.0) of grade >2 are observed (except alopecia, nausea and vomiting) and related to bevacizumab, then the dose of bevacizumab should held until recovery to grade ≤ 1. All grade 3 or 4 non-hematological toxicities (except hypertension, alopecia, nausea and vomiting) should have resolved to grade ≤ 1 for bevacizumab to be re-initiated at a dose of 5mg/kg. Hypertension should be proactively managed and if cannot be controlled with anti-hypertensive medications to <grade 3, bevacizumab should be dose reduced to 5mg/kg.

Bevacizumab may be held for up to 28 days to allow for resolution of toxicity.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 FMISO

7.1.1 Description

The chemical name is 1H-1-(3-[^{18}F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole.

7.1.2 Form

FMISO is an intravenous injection. The radiopharmaceutical product, FMISO, is the only active ingredient and it is dissolved in a solution of ≤10 mL of 95% isotonic saline: 5% ethanol (v:v). FMISO is provided as a ready to use isotonic, sterile, pyrogen-free, clear, and colorless solution. FMISO is typically packaged in a glass vial and does not contain any preservatives.

7.1.3 Storage and Stability

The FMISO will be produced the day of the scheduled PET scan by either the radiopharmacy lab at Brigham and Women's Hospital (Nuclear Medicine/ Biomedical Imaging Research Core (BICOR)) or the Martinos Center for Biomedical Imaging. Proper transportation will take place according to Martinos Center policies. Following synthesis and quality control, the radiopharmaceutical will be used within 8-12 hours of the end of synthesis.

7.1.4 Compatibility

N/A

7.1.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.1.6 Availability

FMISO will be produced by either the radiopharmacy lab at Brigham and Women's Hospital or the Martinos Center for Biomedical Imaging. Brigham and Women's will provide FMISO if the Martinos Center is unable to produce the FMISO.

7.1.7 **Preparation**

FMISO will be produced as a fully prepared agent ready for intravenous administration. Radioactivity dose can be adjusted by modifying the injection volume. No other changes in constitution should be made.

7.1.8 **Administration**

FMISO is administered to subjects by intravenous injection by bolus of ≤10 mL. The FMISO dose for this protocol should be 3.7 MBq/kg (0.1 mCi/kg) up to a maximum of 260 MBq (7 mCi).

7.1.9 **Ordering**

FMISO will be prepared on a single injection basis as dictated by MR-PET scheduling of studies at the Martinos Center.

7.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent (investigational or free of charge) using the NCI Drug Accountability Record or another comparable drug accountability form. (See the CTEP website at http://ctep.cancer.gov/protocolDevelopment for the "Policy and Guidelines for Accountability and Storage of Investigational Agents" or to obtain a copy of the drug accountability form.)

7.1.11 **Destruction and Return**

At the end of the study, unused supplies of FMISO should be decayed in storage according to institutional policies. This will be documented in the FMISO individual batch record and scan log.

7.2 **Bevacizumab**

7.2.1 **Description**

Please refer to the FDA-approved package insert for more information.

7.2.2 **Form**

Bevacizumab is an intravenous medication.

7.2.3 **Storage and Stability**

Bevacizumab will be stored per standard clinical guidelines.

7.2.4 **Compatibility**

N/A

7.2.5 **Handling**

Bevacizumab will be handled per standard clinical guidelines

7.2.6 **Availability**

Bevacizumab is commercially available.

7.2.7 **Preparation**

Bevacizumab will be prepared per standard guidelines

7.2.8 **Administration**

Administration will be IV per standard clinical guidelines.

7.2.9 **Ordering**

Bevacizumab will be ordered commercially.

7.2.10 **Accountability**

Per standard clinical guidelines

7.2.11 **Destruction and Return**

Per standard clinical guidelines.

7.3 **Lomustine**

7.3.1 **Description**

Please refer to the FDA-approved package insert for more information.

7.3.2 **Form**

Lomustine is an oral medication.

7.3.3 **Storage and Stability**

Lomustine will be stored per standard clinical guidelines.

7.3.4 **Compatibility**

N/A

7.3.5 **Handling**

Lomustine will be handled per standard clinical guidelines

7.3.6 **Availability**

Lomustine is commercially available.

7.3.7 **Preparation**

Lomustine will be prepared per standard guidelines

7.3.8 **Administration**

Administration will be PO per standard clinical guidelines.

7.3.9 **Ordering**

Lomustine will be ordered commercially.

7.3.10 **Accountability**

Per standard clinical guidelines

7.3.11 **Destruction and Return**

Per standard clinical guidelines.

8. CORRELATIVE/SPECIAL STUDIES

8.1 Pharmacokinetic Studies

During PET data acquisition, blood samples will be drawn for analysis of total radioactivity and to determine the proportion of labeled radioactive metabolites.

8.2 Blood Biomarkers

Blood may be collected at any time of the day on these time points

NOTE: The treatment calendar does not include a day 0.

Blood Biomarker Schedule
Baseline (within -14 days of treatment start date)
Cycle 1, 24-72 hours after start of bevacizumab
Approximately day 14 (same day as FMISO PET)
Pre-Subsequent Cycles (within -5 days of start of cycle)

Plasma will be collected for molecular markers (VEGF, PlGF, bFGF, SDF1alpha, Il-1b, Il-6, Il-8, TNF-alpha, collagen IV, Ang1, Ang2, thrombospondin, sVEGFR-1/2, SNPs) and circulating mononuclear cells (CECs, CEPs, VEGFR2 expressing monocytes).

The same blood samples obtained for molecular biomarkers will be used for cell collection.

Collection and Handling Procedures for Dr. Jain's lab (Steele Laboratory)

- Collect ~9 ml of blood into a large Purple top EDTA tube
- Gently invert five to six times to ensure adequate mixing and prevent coagulation.
- Cool the tube immediately in an ice bath.
- Label tube and cover label with freezer tape. Refer to section 8.1.3.2. for information regarding labels.
- Place tube on wet ice and send to Dr. Jain's lab (Steele Laboratory)

For all Lab Samples, prepared label templates will be supplied by MGH. Complete the labels with patient identifiers printing each label with Study-No., patient ID, initials and day/time of sample collection (24-hour clock format, i.e., 6:30 pm = 18:30). A label example is provided below:

Study-No.:	*Investigator:*
Patient-ID:	*Patient Initials:*
Date of sampling: (mm/dd/yy)	*Time of Sampling: (hh:mm) (24-hr)*
Sample Type: (Serum or Plasma)	

Blood Sample Packaging and Shipment

- All blood samples must be contained for safety reasons.
- Place individual samples in a clip-lock bag labeled with the sample ID and seal.
- Individual samples may be batched; seal within a second clip-lock bag labeled with the study ID and seal. Samples should be stored at 4°C in freezer until they are ready for shipping.
- Place samples within another plastic bag labeled with the study ID and institution (treatment site) ID as well as a biosafety label. (Triple bagging provides for ease in further packaging for shipping and protection from damage.)
- Samples should be transported at 4°C with wet ice.

All specimens from DFCI should be shipped to:

Sylvie Roberge or Christina Koppel
MGH, Cox-734
100 Blossom St.
Boston, MA 02114
Phone: (617) 726-1353
Pager: 14082
Email: sylvie@steele.mgh.harvard.edu or christina@steele.mgh.harvard.edu

8.3 **Pharmacodynamic Studies**

N/A

9. STUDY CALENDAR

Lomustine Arm

	Screening	**Pre-bevacizumab and lomustine (Baseline)**	**1 day after bevacizumab and lomustine (post-dose #1)**	**Day 14 [i] (pre-bevacizumab dose #2)**	**Day 28[i] (pre-bevacizumab dose #3)**	**Day 42 (pre-bevacizumab dose #4)**
FMISO PET + MRI [h]		X[a]		X[b]	X[b]	
Vascular MRI scan		X	X	X	X[c]	X[c]
Informed consent	X					
History	X					
Physical exam (Ht, Wt, VS)	X					
Steroid dose	X		X	X	X	X
KPS/MMSE	X					
CBC with diff	X			X	X	X
CMP[d]	X					X
Urine analysis		X		X	X	X

Blood Biomarkers		X	X	X	X [g]	X
B-HCG[e]	X	X		X	X	
Adverse event evaluation [j]		X		X	X	
Follow-up [f]						

a – within 14 days prior to start of bevacizumab
b – 0-3 days prior to dose of bevacizumab (can be performed same day as dose of bevacizumab as long as it is prior to the infusion)
c – Every 6 weeks for 6 cycles per standard of care guidelines. MRI can be performed within 5 days of starting the next cycle of lomustine. If patient stops lomustine, MRI scans will continue every 6 weeks for the remainder of the 6 cycles time frame. After 6 cycles, MRIs will be performed per standard of care guidelines.
d - Sodium, potassium, chloride, glucose, BUN, creatinine, calcium, total protein, albumin, total bilirubin, SGOT [AST], SGPT [ALT], alkaline phosphatase.
e – Serum pregnancy test (for women of childbearing potential) to be performed at baseline and prior to each FMISO PET. See Section 5.1.3 and http://healthcare.partners.org/phsirb/Guidance/Pregnancy_Testing_in_Research_Involving_Radiation.1.09.pdf
f – Follow-up every 3 months will assess subsequent treatments, tumor response to treatment, and survival after completion of MRI on cycle 7 day 1
g – Every 6 weeks for 6 months (within 5 days of starting subsequent cycle)
h – if FMISO is not available, the patient will have an MRI scan only
i – bevacizumab should be given every 14 days +/-2 days but the MRI-PET scan should be done prior to the corresponding bevacizumab dose
j– for AE's related to PET, to be 24±6 hours post PET scan and can be phone call or clinic visit. Otherwise, for lomustine and bevacizumab, per DFHCC SOPs.

Bevacizumab Monotherapy Arm

	Screening	**Pre-bevacizumab and lomustine (Baseline)**	**1 day after bevacizumab and lomustine (post-dose #1)**	**Day 14[i] (pre-bevacizumab dose #2)**	**Day 28[i] (pre-bevacizumab dose #3)**
FMISO PET + MRI [f]		X[a]		X[b]	X[b]
Vascular MRI scan		X	X	X	X[c]
Informed consent	X				
History	X				

Physical exam (Ht, Wt, VS)	X				
Steroid dose	X		X	X	X
KPS/MMSE	X				
CBC with diff	X			X	X
CMP[d]	X				
Urine analysis		X		X	X
Blood Biomarkers		X	X	X	X[h]
B-HCG[e]	X	X		X	X
Adverse event evaluation [k]		X		X	X
Follow-up[g]					

a – within 14 days prior to start of bevacizumab
b – 0-3 days prior to dose of bevacizumab (can be performed same day as dose of bevacizumab as long as it is prior to the infusion)
c – Every 4 weeks for 6 cycles per standard of care guidelines. MRI can be performed within 5 days of bevazicumab dose.
d - Sodium, potassium, chloride, glucose, BUN, creatinine, calcium, total protein, albumin, total bilirubin, SGOT [AST], SGPT [ALT], alkaline phosphatase.
e – Serum pregnancy test (for women of childbearing potential) to be performed at baseline and prior to each FMISO PET. See Section 5.1.3 and http://healthcare.partners.org/phsirb/Guidance/Pregnancy_Testing_in_Research_Involving_Radiation.1.09.pdf
f - if FMISO is not available, the patient will have an MRI scan only
g – Follow-up every 3 months will assess subsequent treatments, tumor response to treatment, and survival after completion of MRI on cycle 7 day 1
h – Every 4 weeks for 6 months (within 5 days of starting subsequent cycle)
j – bevacizumab should be given every 14 days +/-2 days but the MRI-PET scan should be done prior to the corresponding bevacizumab dose
k – for AE's related to PET, to be 24±6 hours post PET scan and can be phone call or clinic visit. Otherwise, for lomustine and bevacizumab, per DFHCC SOPs.

10. ADVERSE EVENT REPORTING REQUIREMENTS

10.1 **Definitions**

10.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

10.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen

- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

10.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

10.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

10.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- 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.

10.2 **Procedures for AE and SAE Recording and Reporting**

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means,

will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse 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 website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

10.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

Investigators must immediately report directly to the RDRC all adverse effects associated with the use of the radioactive drug [21 CFR 361.1 (d) 8]

10.4 Reporting to the Study Sponsor

Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.

- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.

- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Elizabeth Gerstner, M.D.
Phone: 617-724-8770
Page #: 617-726-2000 #13385
Fax: 617-643-2591
egerstner@partners.org

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

10.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

10.6 Reporting to the Food and Drug Administration (FDA)

N/A

10.7 Reporting to the NIH Office of Biotechnology Activities (OBA)

N/A

10.8 Reporting to the Institutional Biosafety Committee (IBC)

N/A

10.9 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

10.10 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

11. DATA AND SAFETY MONITORING

11.1 Data Reporting

11.1.1 Method

The QACT will collect, manage, and monitor data for this study.

Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is indicated in the table below. In addition, investigators must immediately report directly to the RDRC all adverse effects associated with the use of the radioactive drug.

Form	Submission Timeline

Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

11.2 **Safety Meetings**

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

11.3 **Monitoring**

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol,

institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

12. REGULATORY CONSIDERATIONS

12.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

12.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

12.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

- Title 21 Part 11 – Electronic Records; Electronic Signatures
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html
- Title 21 Part 50 – Protection of Human Subjects
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
- Title 21 Part 54 – Financial Disclosure by Clinical Investigators
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
- Title 21 Part 56 – Institutional Review Boards
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
- Title 21 Part 312 – Investigational New Drug Application
  www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws
- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

12.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

12.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

12.6 Multi-center Guidelines

N/A

13. STATISTICAL CONSIDERATIONS

Study Design/Endpoints

The statistical design of the study pertains to Cohort A where the following hypotheses will be specifically tested. The primary statistical analysis will be the comparison of hypoxic volumes measured by the baseline and day 14 [^{18}F]FMISO PET scans. We expect all patients have some hypoxia at baseline, and will express the change day 14 hypoxic volume relative to baseline. We will use two-sided, one-sample Wilcoxon test on log-transformed relative volumes to test the primary hypothesis. We will specifically test if there is a significant decrease in the percentage of the tumor with a HV (as defined as $T/B_{max} \geq 1.2$) from prior to bevacizumab infusion to day 14); thus, this is a continuous endpoint. The 1.2 cut-off is based on prior data from Spence et al showing that HV as defined in this way was predictive of patient survival (Spence et al, 2008). Additional exploratory analyses will look at the change in HV between baseline and day 28. The ROI delineating the contrast-enhancing and non-enhancing tumor will be co-registered to the FMISO PET scan to determine the area of interest on the PET scan and calculate the number of tumor voxels with a $T/B_{max} \geq 1.2$. This will allow us to generate a percentage of the entire tumor volume that is hypoxic. Based on personal communication (Dr. David Mankoff), we expect the SD of the within-person change between FMISO measurements under the null hypothesis to be approximately 10%. Using this value and N=30, we anticipate 80% power to detect an absolute mean change of approximately 5% or greater, based on a Wilcoxon signed rank test at the 0.05 two-tailed significance level. More generally, for any standard deviation for change from baseline, we will be able to detect an effect size of 0.55 standard deviation units or larger.

We will also specifically test if there is a significant change in median tumor CBF from prior to bevacizumab infusion to each of the time points (day 1, day 14). We will use a repeated measures mixed effects model that will jointly model median tumor CBF at baseline, days 1 and 14, without assuming it to follow a parametric function of time, and accounting for correlation within person through a random effect. Based on the model, we will assess the significance of the differences from baseline. For simplicity, we calculate power based on separate, two-sided, 0.05 level paired t-tests which are the simplest versions of the repeated measures models and which should underestimate the power that we will have from the full model. The models and t-tests are applied to median tumor CBF, a measured that is calculated for each subject. We expect that the collection of medians across subjects to be approximately normally distributed. Based on our preliminary data (N=29), we estimate the standard deviation (SD) of a within-subject CBF change averaged over tumor to be 0.2. With 30 patients, we will have 80% power to detect a difference of 0.11 in CBF.

Vascular MRI Secondary Endpoints will include CBV, vessel diameter, and ktrans: Secondary analyses will consist, for each biomarker, of tests of change from baseline (we will apply log-transformation and test for a relative change). We will use two-sided, paired Wilcoxon tests, adjusting p-values by Holm's method to account for multiple time-points, but not for multiple biomarkers — as each biomarker will be of separate

interest. The power for at least one significant outcome is as for the primary analysis of FMISO PET scans given above.

Similar secondary analyses will be conducted with the blood biomarker data in the same manner as with the imaging parameters.

Cohort B is an exploratory cohort and will be summarized using descriptive statistics. Statistical graphics such as boxplots will be used to present the summary statistics at each time point. The differences in HV, CBF, CBV, vessel diameter, and ktrans (same as for Cohort A) before and during treatment will be tested using paired statistics but HV will be the variable of principle interest. Cox regression model or logistic regression model will be used to explore associations between changes of serum biomarker levels/imaging parameters and overall survival or progression-free survival. All estimated associations of biomarker changes during the disease process and the probability of death or disease progression will be reported along with 95% confidence intervals. Using the same assumptions as for Cohort A, for any standard deviation for change from baseline, we will be able to detect an effect size of 1.07 standard deviation units or larger for HV which will be the primary Aim for this cohort. Vascular MRI Secondary Endpoints will be handled as above for Cohort A.

13.1 **Sample Size/Accrual Rate**

30 patients will be enrolled in Cohort A with an accrual rate of ~1 per month. A maximum of 10 patients will be enrolled in the exploratory cohort B.

Stratification Factors

N/A

13.2 **Reporting and Exclusions**

13.2.1 **Evaluation of toxicity.** All participants will be evaluable for toxicity for 24hours +/- 6hours after each MR-PET scan.

14. PUBLICATION PLAN

The Principal Investigator holds primary responsibility for publication of the study results. The results are to be made public via abstract meeting the requirements of the International Committee of Medical Journal Editors or via publication in a peer-reviewed journal within 24 months of the end of data collection and analysis. A full report of the outcomes will be made public no later than three years after the end of data collection and analysis.

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16. APPENDICES

Appendix A: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-	20	Very sick, hospitalization indicated. Death not imminent.

	care. Totally confined to bed or chair.	10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**13-482**
Protocol Name:	**Study to Evaluate Vascular Normalization in Patients with Recurrent Glioblastoma Treated with Bevacizumab Using FMISO PET and Vascular MRI**
DFCI Site PI:	Patrick Wen, M.D.
DFCI Research Nurse:	Debra LaFrankie, RN; Lisa Doherty, NP; Jennifer Rifenburg, NP; Sandra Ruland, RN

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
******Please Check for an Alert Page******

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Design	This study will explore the changes in tumor vasculature resulting from treatment with Bevacizumab and the impact these changes have on tumor oxygenation. The success of these studies should enable methods that could be used in larger studies to more completely understand the role of Anti-angiogenic agents in the treatment of cancer.
Dose Calc	Bevacizumab dose is calculated in mg/kg per standard of care. (Section 5.0) A cycle = 28 Days.
Study Drug Administration	Agent *Administration* Guidelines are found in Section 5.0 ***Bevacizumab*** *will be administered via I.V. infusion, every 14 days per standard of care. (Section 5.0, 7.2)* **MR-PET** • Scans will be performed in Charlestown, MA at the Martinos Center. • 2 IV lines will be placed for each scan: one venous line to inject the FMISO and Gadolinium for the MRI scan, and a second to draw blood to assay for radioactivity & metabolites. • If the Participant has a port, the port can replace one of the IVs. • FMISO PET scans will be approximately 60-75 minutes and begin approximately 90 minutes following the FMISO injection.(Section 5.2.1) • FMISO administration instructions are found in Section 7.1.8. • Gadolinium-diethylenetriaminepentaacetic acid will be used as a contrast agent and injected intravenously twice in each scan session. (Section 5.2.2)
Dose Mod & Toxicity	*Dose Modifications/Dosing Delay for Toxicity* are outlined in Section 6.3 • NCI CTCAE vs. 4.0 will be used in grading toxicities. (Section 6.3.2) • No dose reduction of FMISO is permitted. (Section 6.3.1) • Bevacizumab: Prior to each Bevacizumab infusion, the following parameters should be met: Platelet count >75,000/mcL, proteinuria < grade 3, creatinine <1.5xULN, hypertension < grade 4. • Dose Modifications for Bevacizumab are found in Section 6.3.2. • Bevacizumab may be held for a maximum of 28 days to allow for resolution of toxicity to ≤ 1. (Section 6.3.2)
Concom Meds	*Concomitant Therapy* Guidelines are in Section 5.4 •
Req Data	*Study Calendar and Assessment Required data* found in Sections 8.0 & 9.0 •
Charting Tips	Please be sure to DOCUMENT study medication **actual** UP/DOWN times in medical record • If there is a discrepancy in the infusion time, delay in administration, or the infusion takes longer than is permitted by the guidelines of the protocol, please document the reason for the discrepancy in the medical record. Please be sure to also DOCUMENT any required observation periods, any additional vital signs, routes of administration, or injection sites