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Title: A study to evaluate vascular normalization in patients with recurrent glioblastoma treated with bevacizumab using [^{11}C]temozolomide PET and Vascular MRI

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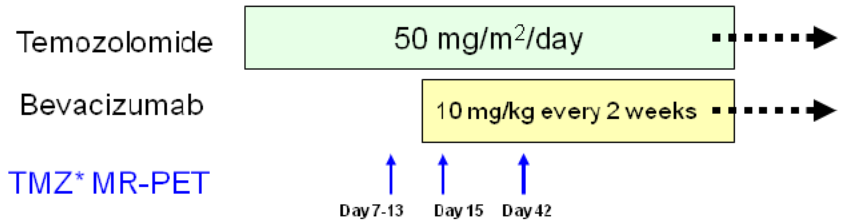
Agent(s): [^{11}C]Temozolomide

SCHEMA



***Population*: Recurrent GBM patients > 2 months from last temozolomide treatment.**

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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 drug delivery. Results from this study will shed light on the vascular normalization hypothesis. Patients with recurrent glioblastoma will undergo simultaneous PET with radiolabeled temozolomide and MRI to assess changes in tumor blood flow, blood volume, vessel caliber, and vascular permeability while simultaneously measuring the pharmacokinetics and brain penetration of temozolomide (TMZ).

We plan to study patients with recurrent glioblastoma whose clinical care plan includes treatment with bevacizumab and temozolomide. Patients taking daily temozolomide 50 mg/m^2/day will undergo a PET scan using radiolabeled temozolomide (TMZ-PET) at 3 time points: 7-13 days after initiation of temozolomide but before beginning bevacizumab ("baseline"- temozolomide steady-state scan), 1 day after initiation of bevacizumab (day 15), and before the third bevacizumab infusion. Arterialized venous blood samples will be collected during the imaging in order to measure radioactivity, blood metabolites, and the relationship between radiotracer uptake and tumor features such as blood-brain barrier (BBB) breakdown and tumor blood flow.

In addition, we will explore the link between flow, permeability, and tumor temozolomide retention. These 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 assess temozolomide delivery before and after bevacizumab in glioblastoma.

1.3 Secondary Objectives

1. To explore the link between flow, permeability, and tumor temozolomide retention.
    a. Measure tumor blood flow with MRI perfusion and compare with temozolomide retention.
    b. Estimate permeability from K^{trans} MRI measurements and compare with temozolomide retention.

2. BACKGROUND

2.1 Study Agent(s)

[^{11}C]Temozolomide

The study agent is a radiopharmaceutical that is chemically identical to the prescription drug temozolomide except that it has a substitution of the positron

emitter ^{11}C for the native ^{12}C at the 3-*N-methyl* position. The purpose of this study is to explore the performance of [^{11}C]temozolomide as a probe of drug delivery and retention in patients with glioblastoma.

Mechanism of Action

Temozolomide was approved in 1999 as a chemotherapeutic agent in cancer. It is thought to be a DNA alkylating agent, thereby inhibiting cell synthesis. Earlier PET studies have identified how temozolomide is converted to the active metabolite, MTIC, and this is illustrated in Figure 1 (from Saleem et al., 2003). Temozolomide undergoes decarboxylation and ring opening in the 3-4 position to produce the highly reactive methyldiazonium ion that alkylates DNA.

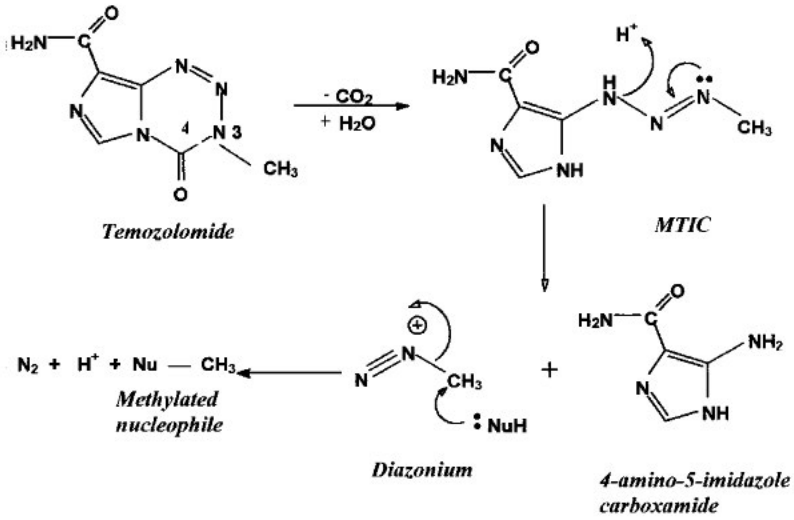

Fig. 1. Mechanism of action of temozolomide. It was postulated that radiolabel in the 3-*N*-methyl position of temozolomide would be retained ultimately by the alkylating species after ring opening of temozolomide. On the other hand the radiolabel in the 4-carbonyl position will get converted to [^{11}C]carbon dioxide, which can be detected in the exhaled air and plasma.

Non-Clinical and Clinical Studies

Positron emission tomography studies have been conducted with tracer doses of temozolomide labeled in the 3-N-methyl positions. A pharmacodynamic relationship has been established between response to temozolomide in recurrent high-grade gliomas and tumor drug concentration in Brock et al. (1998).

Denny et al. (1994) found temozolomide to be robustly stable under acid conditions. However, the rate of degradation rapidly increases on passing through neutral to basic pH. This may provide an important basis of targeted therapy directed toward tumors (i.e. gliomas), which are known to have a higher pH compared to surrounding brain tissue (Vaupel et al., 1989).

Saleem et al. (2003) was the first report of a clinical PET study used to quantify and confirm the in vivo mechanisms of metabolic activation of temozolomide. They

used PET and a dual labeling strategy, where [^{11}C]temozolomide was radiolabeled in two different positions: at the 4-carbonyl and 3-*N-methyl* positions of the molecule. Besides determining the mechanism of action, the study also determined that there was no difference the amount of observed ring opening between tumor and normal tissues ($p>0.05$), suggesting that the ring opening of temozolomide was tissue specific but not tumor specific.

Rosso et al. (2009) used a new PET system analysis method to characterize temozolomide distribution in tissues and predicted normal brain and brain tumor temozolomide concentration profiles for different temozolomide dosing regimens. They concluded higher temozolomide exposures in brain tumor relative to normal brain was attributable to breakdown of the blood brain barrier and possibly to increased intratumoral angiogenesis.

2.2 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 approved by the FDA for recurrent GBM and is ultimately used in most recurrent GBM patients.

2.3 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[2]. However, 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 (Jain, 2005; Jain, 2001). An additional complication in the brain arises due to the presence of efflux pumps (Sharom, 2008). These pumps actively remove chemotherapeutic drugs from the brain tissue and are a key component of the blood-brain barrier. Our data from autopsy specimens obtained after cediranib suggests not only that vascular normalization has occurred but that the efflux protein P-glycoprotein is upregulated (di Tomaso et al, 2011)[3], which suggests that vascular normalization could lead to increased perfusion but decreased retention of temozolomide. If this is the case, drugs or other therapies that are not affected by efflux pumps, such as radiation, might be a better strategy. A tool that could assess the true delivery of the specific therapy under consideration could integrate all these potentially conflicting and/or synergistic effects and provide a summary result.

Beyond the scientific question of the mechanisms of anti-angiogenic therapy, such a tool could potentially have clinical value. Because both bevacizumab and cytotoxic chemotherapy are routinely administered to glioblastoma patients, there could be very high value to tools that could identify whether bevacizumab is changing the delivery of chemotherapy in a given patient. This in turn could provide opportunities to personalize and optimize these specific treatments and dose regimens for each patient.

In previous studies, an increased uptake of [^{11}C]temozolomide in tumor tissue as compared to white and gray matter was observed (Saleem et al, 2003). However, to date it has been not possible to determine if the higher uptake was due to actual higher (specific) retention of temozolomide in tumor tissue, or simply due to a more compromised blood-brain barrier in the tumor location resulting simply in a higher delivery of the drug. Our novel hybrid MR-PET technology should allow us to assess if temozolomide delivery is indeed related to the level of blood-brain barrier disruption, as we will be able to quantify this disruption by the simultaneous acquisition of dynamic contrast enhanced MR data of the tumor area.

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 > 2 months since last cycle of temozolomide or other alkylating agent. Patients with low-grade tumors who have progressed to glioblastoma are eligible.

3.1.2 Patients must have received prior temozolomide or an alkylating agent (ex. CCNU/BCNU).

3.1.3 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.

3.1.4 Only patients for whom their neuro-oncologist has planned to give bevacizumab and temozolomide 50mg/m^2/day as part of their treatment 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 radiolabeled temozolomide 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 $\geq$ 1,000/mcL
- Platelets $\geq$ 100,000/mcL
- 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 must be maintained on a stable corticosteroid regimen for 5 days prior each MR-PET scan.

3.1.11 The effects of radiolabed temozolomide 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.

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 radiolabeled temozolomide 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 radiolabeled temozolomide. 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 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

3.3 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 [redacted] 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 [redacted].

   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 and daily temozolomide as part of their treatment. 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, patients will be administered temozolomide at a dose of 50 mg/m^2 PO on a daily basis starting on Day 1. Bevacizumab will be administered as an infusion at a dose of 10 mg/kg i.v. every two weeks starting no later than Day 14. Standard computerized order entry

(COE) orders will be utilized for administering this drug. A cycle is defined as 28 days. The treatment goal is 12 months of treatment. Treatment beyond 12 months or at the time of disease progression is per discretion of the responsible physician. The administration of both of these FDA-approved drugs will be per standard practice at our hospital.

[^{11}C]temozolomide (and the MR-PET scans) will ONLY be administered (and conducted), respectively, on Cycle 1.

There are no expected toxicities and potential risks for [^{11}C]temozolomide. All scans will be performed at the Martinos Center for Biomedical Imaging in Charlestown on the combined MR-PET scanner.

5.1 Pre-MR-PET Scan Criteria

5.1.1 Stable dose of corticosteroids for 5 days prior to MR-PET scan.

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

Patients will have their scans performed in Charlestown, MA at the Martinos Center. Two lines will be placed for each scan: one venous line to inject [^{11}C]temozolomide) 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 [^{11}C]temozolomide PET scans

The PET scan will be approximately 90 minutes. There will be one injection of radiolabeled temozolomide in the PET protocol. Following the injection of the radiotracer, serial blood samples will be taken from a second IV. The blood will be drawn by a nuclear medicine technologist or a nurse trained in radiation safety.

[^{11}C]temozolomide will be injected at a dose of 7mCi by an infusion pump or hand over approximately 30 seconds followed by a saline flush. The IV will remain in place for injection of the gadolinium for the MRI scan.

PET data will be acquired in list mode format for an additional 70-90minutes after the [^{11}C]temozolomide injection. Serial blood will be drawn from a venous line not used to inject the [^{11}C]temozolomide at times t = 2.5,5,10,20,40,60,75, and 90 minutes after the [^{11}C]temozolomide injection to allow for kinetic modeling. 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 ^{11}C 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 first bolus will be for the dynamic susceptibility contrast sequence. The second bolus will be injected for the dynamic contrast enhanced sequence. 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 will be treated with temozolomide and bevacizumab for up to 1 year. Treatment beyond 1 year is per discretion of responsible physician.

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

- Intercurrent illness that prevents administration of [^{11}C]temozolomide, temozolomide, 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 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 [redacted].

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 Lists(s) for [^{11}C]temozolomide

There are no known adverse effects of [11C]temozolomide, although there are adverse effects of temozolomide. [11C]TMZ will be administered at >0.1 mCi/nmol. Assuming a maximum injection of 15 mCi at the lowest specific activity, 150 nmols (29 micrograms) will be the maximum amount of the active component [11C]TMZ administered.

6.1.2 Adverse Event Lists(s) for temozolomide

Myelosuppression (including neutropenia, lymphopenia, leukopenia, anemia, an thrombocytopenia), has been reported inpatients receiving temozolomide and is a dose-limiting side effect. Thrombocytopenia and leukopenia reach grade 2 or higher in up to 40% of patients on temozolomide therapy. Leukocyte/platelet nadirs usually occur in 3 weeks (about day 22) with a 5-day schedule, although leukocyte nadirs may be seen slightly later (day 29). Dose-limiting thrombocytopenia has persisted for 7 to 42 days, whereas recovery from leukopenia may be quicker. Bleeding has been infrequent with temozolomide therapy. In one large trial involving adult patients with malignant glioma, the predominant effect was lymphopenia, which reached grade 3 and grade 4 in 41% and 15%, respectively, receiving a 5-dayschedule of temozolomide. Corresponding incidences of neutropenia were 2% and 4%. The incidence of grade 3 or 4 thrombocytopenia was 11%. The incidence of myelosuppression may be higher with the use of a dose intense regimen. Opportunistic infections (e.g., pneumocystis jeroveci pneumonia (PCP)) have been reported with the use of temozolomide. Rarely, aplastic anemia and secondary leukemia have been described with temozolomide.

Hepatic toxicity: Mild transaminase elevations (up to 40% of patients) and hyperbilirubinemia (up to 19%) have been reported; increases in alkaline phosphatase have also occurred in some patients. Grade 4 increases in bilirubin have been seen rarely. There are no reported cases of overt hepatotoxicity.

Fatigue is among the most commonly reported adverse effect with the use of temozolomide in clinical trials, and is clearly drug-related. Fatigue with temozolomide therapy may be moderate to severe. The estimated incidence is 34%.

Nausea and vomiting occur in up to 75% of patients with temozolomide therapy, but is not usually severe (mostly grade 1 or 2).These symptoms have often been limited to day 1 of the first cycle of temozolomide. Standard antiemetics have been effective in most patients. The estimated incidence is 42% to 53%. Bedtime administration of temozolomide may help to minimize nausea and vomiting.

6.1.3 Adverse Event Lists(s) 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 [^{11}C]temozolomide

No dose reduction of [^{11}C]temozolomide is permitted.

6.3.2 Temozolomide

Prior to initiation of each temozolomide cycle, the following hematologic parameters are required: platelet ≥100,000 and ANC ≥ 1500.

In the event of a NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 grade 3 or 4 toxicity the temozolomide will be suspended

until recovery to grade ≤ 1 and re-started at a dose reduction of 37.5 mg/m^2.

Temozolomide may be held for up to 28 days.

6.3.3 Bevacizumab

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

If other toxicities (as determined CTCAE version 4.0) of grade >2 are observed (except alopecia, nausea and vomiting) and related to bevazicumab, then the dose of bevazicumab should held until recovery to grade ≤ 1. All grade 3 or 4 non-hematological toxicities (except alopecia, nausea and vomiting) should have resolved to grade ≤ 1 for bevazicumab to be re-initiated at a dose of 5mg/kg.

Bevacizumab may be held for up to 28 days.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 [^{11}C]Temozolomide

7.1.1 Description

The chemical name is: [3-*N*-^{11}C-*methyl*]temozolomide. This agent is a radiolabeled version of temozolomide that can be used to explore the pharmacokinetics of temozolomide therapy.

7.1.2 Form

[^{11}C]Temozolomide is prepared as an intravenous injection in sodium acetate buffer with 5% ethanol (Brown et al., 2002) at a tracer level mass dose that should exhibit no pharmacological effect.

7.1.3 Storage and Stability

[^{11}C]Temozolomide will be produced onsite on the day of the scheduled PET scan. Proper transportation will take place according to Martinos Center policies. Following synthesis and quality control, the radiopharmaceutical will be used immediately (injection time not to exceed expiration of 1 hr post 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

[^{11}C]Temozolomide is an investigational agent and will be produced onsite.

7.1.7 Preparation

[^{11}C]Temozolomide will be produced onsite 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

[^{11}C]Temozolomide will be injected intravenously as a bolus at a dose of 7 (±1) mCi using an infusion pump or by hand over approximately 30 seconds. The bolus will be followed by a 10 mL saline flush.

7.1.9 Ordering

[^{11}C]Temozolomide will not be ordered. [^{11}C]Temozolomide 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 [^{11}C]temozolomide should be decayed in storage according to institutional policies. This will be documented in the [^{11}C]temozolomide individual batch record and scan log.

7.2 Temozolomide

7.2.1 Description

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

Temozolomide is available in commercially available in 5 mg, 20 mg, 100 mg, 140 mg, 180 mg, and 250 mg capsules.

7.2.3 Storage and Stability

Temozolomide capsules should be stored at controlled room temperature.

7.2.4 Compatibility

N/A

7.2.5 Handling

N/A

7.2.6 Availability

Temozolomide is commercially available in 5 mg, 20 mg, 100 mg, 140 mg, 180 mg, and 250 mg capsules.

7.2.7 Preparation

N/A

7.2.8 Administration

The capsules are administered orally and should not be chewed. They should be swallowed whole with a glass of water. Procedures for handling and disposal of anticancer drugs should be considered.

7.2.9 Ordering

Temozolomide will be ordered commercially.

7.2.10 Accountability

N/A

7.2.11 Destruction and Return

N/A

7.3 Bevacizumab

7.3.1 Description

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

7.3.2 Form

Bevacizumab is an intravenous medication.

7.3.3 Storage and Stability

Bevacizumab will be stored per standard clinical guidelines.

7.3.4 Compatibility

N/A

7.3.5 Handling

Bevacizumab will be handled per standard clinical guidelines

7.3.6 Availability

Bevacizumab is commercially available.

7.3.7 Preparation

Bevacizumab will be prepared per standard guidelines

7.3.8 Administration

Administration will be IV per standard clinical guidelines.

7.3.9 **Ordering**

Bevacizumab 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 **Pharmacodynamic Studies**

N/A

9. STUDY CALENDAR

	Screening	**Prior to bevacizumab**	**1 day after initial bevacizumab dose**	**Prior to third dose of bevacizumab**
[^{11}C]TMZ PET/ MRI scan		X[a]	X	Xf
Informed consent	X			
History	X			
Physical exam (Ht, Wt, VS)	X			
Steroid dose	X		X	X
KPS	X			

CBC with diff	X			
CMP[b]	X			
B-HCG[c]	X	X	X	X
Adverse event evaluation[d]		24±6 hours post PET scan	24±6 hours post PET scan	24±6 hours post PET scan
Follow-up[e]				

a – 0-7 days prior to start of bevacizumab. Patient had to have received at least 7 doses of temozolomide prior to the MR-PET (i.e. steady state of temozolomide).
b – Sodium, potassium, chloride, glucose, BUN, creatinine, calcium, total protein, albumin, total bilirubin, SGOT [AST], SGPT [ALT], alkaline phosphatase.
c – Serum pregnancy test (for women of childbearing potential)
d – Can be phone call or clinic visit
e – Patients will be scanned approximately every 2 months per standard of care guidelines. Follow-up every 3 months will assess subsequent treatments, tumor response to treatment, and survival
f – within 3 days prior to the 3rd bevacizumab dose- i.e. can be same day as bevacizumab but must be prior to the infusion or up to 3 days before the 3rd infusion

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.

10.1.4 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.

10.4 Reporting to the Study Sponsor

10.4.1 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.
[redacted]

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.

10.4.2 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.

Investigators must immediately report directly to the Radioactive Drug Research Committees (RDRC) all adverse effects associated with the use of the radioactive drug.

11. DATA AND SAFETY MONITORING

11.1 **Data Reporting**

11.1.1 Method

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

11.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

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

13.1 Study Design/Endpoints

This is a pilot study. The target population of recurrent GBM patients who are 2 months or more from their last temozolomide treatment is a small group of patients, which will limit the number of patients eligible for accrual.

The primary comparison will be for the change in the within-tumor concentration of radiolabelled temozolomide. The change will be expressed as day 15 concentration relative to baseline measurement. Additional analysis will look at the ~day 42 concentration relative to baseline measurement of radiolabeled temozolomide as it is unclear when changes in drug delivery may occur. We will use an exact two-sided, one-sample Wilcoxon signed rank test on log- relative total within-tumor temozolomide concentration to test the primary hypothesis. Due to pilot nature of this study enrollment will be limited to only n=17 patients. Our goal is for 15 evaluable patients so the maximal enrollment will be 17 to allow for 2 nonevaluable patients (i.e. PET data not evaluable) With 15 patients, we would anticipate having a sufficient sample size to identify the subset of patients who respond to bevacizumab with increased perfusion and thus drug delivery. We will have 80% power to detect a difference of 0.81 standard deviation units between baseline and day 15 using a two-sided, 0.05 level Wilcoxon signed rank test with 15 subjects.

13.2 Sample Size/Accrual Rate

A maximum of 17 patients will be enrolled with an accrual rate of 0-1 per month.

13.3 Stratification Factors

N/A

13.4 Reporting and Exclusions

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

13.4.2 **Evaluation of response.** All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

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-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

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