MSK PROTOCOL COVER SHEET

Enhancing Brain Lesions after Radiation Therapy: A Comparison of MRI Perfusion and FDG PET/CT to Distinguish Between Radiation Injury and Tumor Progression

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Some patients treated with brain radiation therapy for tumors located in the head may develop new or increased enhancing mass lesions. These worsening lesions may represent radiation injury/necrosis or tumor progression. This study will examine the utility of MRI perfusion and FDG PET/CT to determine if the enhancing lesions represent radiation injury or tumor progression. Both MRI perfusion and FDG PET/CT scans are part of the standard of care for patients with indeterminate lesions after treatment. The purpose of this study is to prospectively enroll these patients in order to determine if MRI perfusion or FDG PET/CT performs better in predicting if a worsening enhancing brain lesion represents radiation injury or tumor progression. In addition the entire cohort analyses, subcohort analyses will be performed for patients belong to specific clinical scenarios and compared using an interaction test.

Prior to enrolling in the study, each patient will have a CT or MRI scan that shows an increasing indeterminate mass lesion. If the screening scan is a brain MRI perfusion, it can also function as the protocol intervention.

Screening (within 12 weeks of enrollment): Neurological and/or physical examination

Intervention: Brain MRI perfusion and PET/CT performed within 12 weeks of each other. Either MRI perfusion or PET/CT may be performed first. The first of the two scans should be performed within 12 weeks after the screening scan showing an increasing indeterminate mass lesion. If the screening scan is a brain MRI perfusion, it can also function as the protocol intervention. Imaging based diagnosis of radiation injury vs. worsening tumor will be rendered by a study neuroradiologist and by a nuclear medicine radiologist and entered into a secure database.

*Note that clinical reads of the MRI perfusion and PET/CT will be rendered as per the standard of care, usually within 24-48 hours after completion of the study. These reports get dictated in Powerscribe and then become part of the patient's electronic medical record.

Off study: Patients will remain on study until the completion of either the MRI perfusion or PET/CT that are within 12 weeks of each other. After one of these scans, the patient will have no active interventions and will be off study. Patients will obtain a standard of care brain MRI scan about every 2-3 months. These MRI scans will be used to track disease progression.

Follow up: Neurological/physical examination and brain MRI approximately every 2-3 months until death or 1 year after last MRI if lost to follow up. If clinical and radiological follow up is required, determination of the outcome (tumor progression vs. radiation injury) may require 6 months or more from the time of the intervention MRI perfusion and PET/CT scans.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

The primary goal of the proposed study is to directly compare PET/CT with MRI perfusion in distinguishing between radiation injury and tumor progression. These are the two most commonly performed tests for possible radiation injury cases (MRI spectroscopy, diffusion imaging, and experimental PET/CT tracers trail far behind). There has been very little

comparative effectiveness research to determine an optimal imaging strategy, whether both are complementary and necessary, or whether one outperforms the other. This protocol will help us answer those questions, and may lead to dramatic changes in the way we and other institutions manage these diagnostic dilemmas. After the completion of radiation therapy and a follow-up MRI exam demonstrating a worsening enhancing brain lesion, patients will undergo both MRI perfusion and PET/CT exams. If the follow up MRI is a brain MRI perfusion, it will serve as both the screening scan and protocol intervention. In this case, patients will undergo only a PET/CT exam.The findings from each of these imaging studies will be used to predict whether the lesion represents tumor progression or radiation necrosis based on clinical follow-up and/or pathologic tissue sampling.

Primary Endpoint:

1. To assess the utility of PET/CT and MRI perfusion studies in predicting whether worsening enhancing brain lesions seen after radiation therapy represent radiation injury or tumor progression. This study will examine the role of these two imaging techniques in predicting diagnosis and treatment planning.

Exploratory Endpoint:

1. To assess the utility of PET and MRI perfusion studies in predicting tumor progression in patients with tumors with a history of brain radiation therapy receiving anti-angiogenic therapy (such as bevacizumab). Due to direct drug effects upon the vasculature, these tumors often show little or no enhancement despite clinical worsening and increasing non-enhancing abnormalities. The role of advanced imaging in determining progression in lesions that do not show increased enhancement has not been defined. In an exploratory fashion, we will enroll patients with tumors with a history of brain radiation therapy receiving anti-angiogenic therapy with suspected progression and increased nonenhancing lesions.

2. To assess the utility of MR diffusion imaging in predicting radiation injury or tumor progression. Water diffusion occurs independent of tumor blood flow (MRI perfusion) or glucose uptake (FDG PET/CT), and may also be a useful technique to evaluate worsening enhancing brain lesions.

3.0 BACKGROUND AND RATIONALE

Patients with tumors treated by brain radiation therapy may develop new and/or increased enhancing mass lesions within the spectrum of radiation injury. This frequently poses a diagnostic dilemma, as both radiation injury and worsening tumor may fulfill requirements for worsening disease when applying standard response criteria.[1] These criteria usually rely on changes in size of the enhancing lesion on contrast brain MRI scans, which is the current imaging standard for the evaluation and follow up of patients with a history of brain radiation therapy.

Radiation therapy plays an important role in prolonging the survival of patients with primary (malignant gliomas) and secondary (metastases) malignancies of the head. Treatment protocols include administration of whole and/or partial brain radiation therapy as well as stereotactic radiosurgery. Toxicity of radiation therapy on the brain includes radiation-induced injury of healthy brain occurring months to years after treatment. [1] Brain tolerance is related to the prescribed volume, dose, fraction and interval of radiation. Focal radiation injury may manifest with increased or new enhancing mass lesions that mimic worsening tumor. The term focal radiation injury is used here to include pseudoprogression in gliomas and radiation necrosis in both gliomas and metastases – these are related but distinct enhancing entities along a common continuum.

Pseudoprogression is the transient worsening of enhancing disease that occurs soon after completion of radiation therapy in patients with malignant gliomas. This occurs in approximately 31% of treated patients, and in 58% of patients with methylated O^6-methylguanine-methyltransferase enzyme (MGMT) promoter status.[2-5] The increased and/or new enhancing mass lesions are due to early subacute potentiated radiation-induced tissue injury with inflammatory changes and necrosis. This is most common 1-3 months after radiation therapy, with lesions usually spontaneously stabilizing or improving by 6 months. In contrast to tumor progression, pseudoprogression has been associated with prolonged survival.[6, 7]

In contrast, radiation necrosis is an often irreversible process that develops many months to years after completion of radiation therapy. Patients develop increased or new enhancing mass lesions due to late subacute radiation-induced tissue necrosis, inflammatory changes and blood-brain barrier disruption. The lesions are also characterized by marked adjacent brain edema. Surgical resection is the most efficacious treatment. Patients may become dependent on steroids to control the enhancement and edema.

Enhancement is the combined result of tissue vascularity and accumulation of contrast in the extracellular extravascular space secondary to transendothelial diffusion or permeability. Tumors are characterized by neovascularization and angiogenesis with the formation of many new immature and leaky intratumoral blood vessels. While enhancement is usually interpreted to represent tumor, enhancement may also occur due to radiation injury from blood-brain barrier disruption without neovascularization. Rather than relying on contrast enhancement, we propose using two physiological imaging techniques to investigate the biology of the enhancing lesion(s): MRI perfusion and PET/CT.

No study has directly compared MRI perfusion with FDG PET/CT for the diagnosis of focal radiation injury. Although both are commonly performed, their comparative predictive value, sensitivity, specificity and indications remain uncertain. Many studies were performed using small patient cohorts and retrospective designs. We believe that the large numbers of neuro-oncology patients at MSKCC, and the large numbers of scans we (already) perform, leveraged with the benefits of a prospective protocol, will help overcome many of the limitations in the current literature. We hypothesize that MRI perfusion and PET/CT can prospectively predict radiation necrosis from tumor progression. Our goal is to directly compare the two techniques and determine if any particular clinical scenario (such as hemorrhagic lesions) would favor one over the other. Early diagnosis would help clinical decision-making, as the treatments for these two entities are very different.

The gold standard for the diagnosis of radiation injury is histopathology. This is an invasive technique, however, which may be limited by sampling error or may not be possible for inaccessible lesions in eloquent brain. A safe, noninvasive imaging biomarker to determine radiation injury is therefore desirable.

3.1 MRI

Conventional MRI provides exquisite high resolution images and detailed structural information about the size, location and effects of a tumor on the surrounding brain. When evaluating patients who have undergone radiation therapy, however, lesion size and enhancement characteristics are of relatively limited utility in distinguishing tumor

progression from radiation necrosis[8], both of which can present as enhancing mass lesions on conventional MRI.[9, 10] Enhancement in these cases reflects disruption or absence of the normal blood-brain barrier,[9, 10] and does not necessarily correlate with residual or progressive tumor. Furthermore, contrast enhancement alone will likely become a less reliable marker with the increasing use of anti-angiogenesis agents such as Avastin (bevacizumab) that directly induce rapid and profound decreases in tumor enhancement.

3.1.2 Rational for the use of MRI Perfusion

MRI perfusion provides physiologic information about brain tissue biology, allowing the *in vivo* measurement of increased microvascularity and permeability to suggest tumor.[11, 12] There is growing evidence that MRI perfusion can improve the diagnosis and prognosis of patients with brain tumors over conventional imaging alone.[13-15] MRI perfusion correlates with microvessel density and vascular endothelial growth factor (VEGF) expression at histopathology and catheter based angiography.[16, 17] Two main methods of MR perfusion are currently being utilized to evaluate brain tumors – dynamic susceptibility contrast (DSC) perfusion imaging and dynamic contrast enhanced (DCE) perfusion imaging. DSC MRI perfusion is a T2* technique that relies on signal changes detected during the rapid bolus of contrast through brain tissues. Although small retrospective studies have suggested a role for MRI perfusion,[18-21] no prospective trial has been designed to distinguish between radiation injury and tumor progression.

DCE MRI perfusion is a T1 technique with superior spatial resolution that is beneficial in regions where hemorrhage or bone/air interfaces may otherwise limit DSC imaging. In addition, DCE provides potentially more robust absolute measurements of cerebral perfusion, flow and permeability – as opposed to the only relative measurements obtained by DSC. We propose studying the role of DCE MRI perfusion in distinguishing between focal radiation injury and tumor progression.

3.1.2 Rationale for the use of PET/CT

PET/CT is a commonly used technique in oncologic imaging that relies on combining the functional/physiologic information provided by PET imaging with the anatomic information provided by the concurrently acquired CT scan. As a proxy for cellular glucose uptake, FDG PET/CT can identify hypermetabolic lesions to suggest tumor. After the administration of a radiolabeled glucose analogue (F-18 FDG), relative metabolic activity in the brain can be imaged with tracer uptake directly correlated to the level of cellular glucose metabolism. In the brain, abnormal uptake can be determined by visual inspection and comparison to uptake in normal cortex or via quantification by measurement of standardized uptake values (SUV). No consensus SUV cut-off values for tumor detection have been established in the literature.[22] Quantification of abnormal FDG uptake may also be performed by textural and topographc analysis using feature-based image analysis tools. Although retrospective reports[23-29] suggest that PET imaging may play a role in distinguishing radionecrosis from tumor progression, this role has yet to be well defined in a prospective trial.

3.2 Preliminary Data

MRI perfusion and PET/CT are already routinely performed for patients with tumors with a history of brain radiation at MSKCC. In 2010, we performed 1,474 MRI perfusion scans and 212 PET/CT scans, for determining radiation injury as well as a variety of other reasons that include for staging, preoperative planning, preradiation therapy planning, and treatment follow up. The vast majority of the MRI perfusion scans were performed using

the DSC technique, with very few (<50) performed using the potentially more robust DCE technique.

In a preliminary retrospective analysis, we identified 12 consecutive patients over a one year period with indeterminate enhancing lesions after radiation therapy who underwent DSC MRI perfusion, FDG PET/CT, and subsequent surgical resection with pathologic diagnosis (Hatzoglou V, Ulaner G, Beal K, Zhigang Z, Young RJ; Abstract 9012072, Radiological Society of North America, Chicago, IL, December 2010). Both primary (n=3) and secondary (n=9) brain neoplasms were included. Seven patients received stereotactic radiosurgery (SRS, 18-21 Gy), 2 patients whole brain radiation (35-52.5 Gy) and 3 patients partial brain radiation therapy (59.4-60 Gy). From the PET/CT scans, standardized uptake values (SUV) of the lesion and background were measured by a board-certified nuclear medicine radiologist and ratios (SUV_{ratio}) calculated. From the DSC MRI perfusion images, maximal relative cerebral blood volume ($rCBV_{max}$) values were measured by a board-certified neuroradiologist. P-values were obtained using bootstrap or permutation tests, and receiver operating characteristic (ROC) analyses and areas under curve (AUC) were calculated.

We found that the SUV_{ratio} had the highest predictive value (AUC=0.943) for tumor progression, although this was not statistically better than any perfusion metric (AUC=0.757-0.829). After excluding two patients with hemorrhagic lesions, the AUC of the perfusion metrics improved slightly to 0.917-0.938.

This preliminary study found similar accuracy for PET/CT and MRI perfusion in distinguishing recurrent tumor from treatment necrosis. MRI perfusion performed better when patients with hemorrhagic lesions were excluded. These results have compelled us to develop a prospective trial, which will allow us to determine the optimal roles for PET/CT and MRI perfusion in the evaluation of patients with worsening enhancing lesions after treatment. We plan to perform DCE T1 perfusion instead of DSC T2* perfusion, as the DCE technique provides absolute measures of perfusion and permeability and is not limited by hemorrhage.

A systemic feature- or texture-based approach has been applied to PET scans in gynecological and head and neck cancers. This is similar to increasingly popular histogram analyses for other imaging data,[30] and has shown promise in treatment response analyses using multiparameter predictive models over single metrics such as simple SUV descriptive statistics. In a pilot study, correlation between feature-based predictive models and outcome improved by 14% in gynecological and 12% in head and neck cancers over SUV statistics.[31]

3.3 Exploratory use in patients treated with anti-angiogenic therapy

Bevacizumab is an anti-angiogenic therapy that is commonly used to treat patients with malignant gliomas (usually recurrent) as well as some systemic tumors (e.g., breast). In these cases, the use of contrast enhancement to determine response as performed in daily clinical practice and as defined by many clinical protocols, is less reliable.[32] This is because bevacizumab may induce rapid decreases in contrast enhancement at imaging not accompanied by actual reductions in tumor. An alternative imaging marker to determine tumor progression in these patients is necessary.

Several studies have described decreased perfusion and decreased enhancement after anti-angiogenic therapy.[33-35] This has been postulated to be due to normalization of the blood-brain barrier rather than decreased tumor burden. The exploratory endpoint concerns the growing use of anti-angiogenesis therapy (such as bevacizumab) in brain

tumor patients. Although direct effects on the vasculature may also affect perfusion, the precise effects involved remain poorly understood. The effects upon FDG uptake are similarly poorly understood, although the effects on glucose metabolism may be less dramatic than the effects on the vasculature. We believe that adding these patients to an exploratory endpoint will help us improve our understanding of the imaging effects of anti-angiogenic therapy and develop better imaging tools to determine tumor progression.

Exploratory Endpoint:

1. To assess the utility of PET and MRI perfusion studies in predicting tumor progression in patients with malignant gliomas receiving anti-angiogenic therapy (such as bevacizumab). Due to direct drug effects upon the vasculature, these tumors often show little or no enhancement despite clinical worsening and increasing non-enhancing abnormalities. Therefore, we often cannot rely on changes in size of the contrast enhancing mass lesion as usual. The role of advanced imaging in determining progression in lesions that do not show increased enhancement has not been defined. In an exploratory fashion, we will enroll patients with malignant gliomas receiving anti-angiogenic therapy with suspected progression and increased nonenhancing lesions. Analysis will be similar to that for patients under the Primary Endpoint.

3.3.1 Exploratory use of advanced diffusion imaging

Restriction spectrum imaging (RSI) is an advanced diffusion technique that models diffusion signal by incorporating tissue parameters such as the size, shape and orientation fo water diffusion. RSI offers the ability to separate the diffusion components according to the different tissue types, potentially improving tumor margin conspicuity. By maximizing the diffusion signal from within tumor cells while minimizing diffusion signal from edema, RSI may be useful in evaluating tumor progression and in particular patients treated with anti-angiogenic agents such as bevacizumab[56].

3.4 Benefits

The proposed study will make a significant contribution to our understanding of the optimal imaging evaluation of neoplasms treated with brain radiation therapy. Determining the presence of tumor progression can directly impact patient therapy. If the data indicates that MRI perfusion and/or PET/CT (alone or in combination) can accurately predict the presence of radiation injury or tumor progression, then potential treatment options could be pursued at an earlier point in the patient's course and thereby potentially improve patient survival. This information will likely also be very useful for neurosurgical planning to maximize the timing and resection of lesions that require surgical management. The nonsurgical treatments for these two entities are different, as tumors may benefit from additional chemotherapy and radiation therapy, whereas radiation injury will be worsened by additional radiation therapy and likely not helped by chemotherapy.

In addition, if the results indicate that MRI perfusion is comparable to or superior to PET/CT, several patient and systemic advantages may emerge. First, nearly all patients with a history of radiation therapy to the brain undergo MRI scans for surveillance after treatment, whereas PET/CT is reserved as a problem-solving tool. Therefore, MRI perfusion is more convenient for patients, as it may be obtained at the time of their routine MRI scan, and does not require a separate appointment. MRI perfusion requires 5 minutes to acquire, but does not require any additional intravenous contrast. The 3D workstation charge associated with MRI perfusion is much less than the charges associated with PET/CT. In addition, MRI has no radiation risk, in contrast to the very small but present risk with PET/CT.

By designing this as a prospective study, we will minimize the potential biases and variability of a retrospective analysis. Both MRI perfusion and FDG PET/CT scans are part of the standard of care for patients with indeterminate lesions after treatment. The purpose of this study is to prospectively enroll these patients in order to standardize the scanning intervals and formalize the imaging, evaluation and follow up of these patients. What we learn may improve our understanding of radiation injury and thus benefit society and future brain tumor patients.

This research project will help develop the career of the P.I., who has published several papers in peer-reviewed journals about the advanced MRI technique in evaluating malignant gliomas. MRI perfusion is regarded as a very important tool in the diagnosis, prognosis and management of brain tumors and treatment complications. Although the older T2* technique has been used for >10 years, there is still avid interest and research into the optimal imaging parameters, reconstruction algorithms, processing techniques and clinical relevance. This is particularly true for the newer T1 perfusion technique proposed in this protocol. The P.I. has given invited talks at national meetings and built a reputation as an innovative clinical investigator with abundant experience in advanced MRI techniques. The P.I. anticipates that the data collected from this project will be an essential part of a subsequent R21 quick trial or similar grant to the NIH.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

Accrual: In 2011, a total of 825 patients were treated at MSKCC for a brain tumor with surgery, radiation therapy (n=286+) and/or chemotherapy (Caisis database, accessed 3/1/2012). In 2010 and 2011, a total of 86 patients had suspected radiation injury and underwent surgical resection of their lesions (outcomes consisted of “recurrent/residual tumor with treatment effect” and “non-cancer” diagnoses at histopathology). The ratio of nonsurgical vs. surgical treatment for patients with suspected radiation necrosis at MSKCC is estimated at 2-3:1, based on our recent study[36] on suspected pseudoprogression (early radiation injury in high grade glioma patients) and observed patterns from imaging studies and multidisciplinary tumor board discussions. Extrapolating from the 86 patients with suspected radiation injury and subsequent histopathology, we estimate that approximately 258-344 patients over 2 years undergoing brain tumor treatment at MSKCC may have a concern of radiation injury. Based on accrual rates for similar protocols at MSKCC, and our accrual rate thus far (75 patients over 2 years), the estimated accrual for this study is 2-4 patients per month. We plan to enroll approximately 35 patients per year, for a total of 150 patients over a 4 year period. We will continue to follow up until death or a year after the patient’s last MRI if he/she is lost to follow up.

The study will prospectively enroll patients who have increasing size and/or enhancement of brain lesion(s) after brain radiation therapy for a neoplasm (either primary or metastatic), where it is unclear if a lesion represents radiation injury or progressive tumor. The procedures and the minimal risks of MRI perfusion and PET/CT are the same regardless of the situation (tumor type or radiation plan) in which they are applied. We apply identical processing procedures to analyze the data regardless of the original tumor type or radiation therapy regimen, and when applicable apply identical imaging metric thresholds. Radiation injury may occur after stereotactic radiosurgery, partial brain

radiation, or less commonly after whole brain radiation therapy. The most important factors for developing radiation injury are radiation dose, schedule and fractionation. We will for the purposes of this protocol assume that the subject population is homogeneous (given a common radiation injury mechanism and imaging manifestations) and recruit to a single subject cohort.

In addition to studying all enrolled patients as a single cohort, we will at the end of the study examine specific subcohorts as described in the following specific clinical scenarios. This will help us to determine if there are any differences in our techniques and measurements between groups.

Specific clinical scenarios when PET/CT and MRI perfusion may be clinically necessary to examine indeterminate lesions:

1) Gliomas after radiation therapy with increased or new enhancing lesions

2) Gliomas after radiation therapy in patients receiving bevacizumab or other anti-angiogenic chemotherapy with increased or new enhancing and/or non-enhancing lesions

3) Metastases after radiation therapy with increased or new enhancing lesions

4) Metastases after radiation therapy who may be receiving bevacizumab or other anti-angiogenic chemotherapy for systemic or brain disease

This is a nonrandomized study in which each patient will receive the standard clinical care (in the form of surgery, radiation treatment and/or chemotherapy) as per the treating physician(s). Upon enrollment, the patients will undergo both MRI perfusion and PET/CT imaging, preferably on the same day to maximize patient convenience and reduce potential changes between scans, but at most within 12 weeks of one another. The two scans will be performed within 12 weeks after the indeterminate lesion is initially identified (unless the screening scan is a DCE MRI perfusion, in which case it will serve as the protocol MRI intervention as well). The neuroradiologist reading the MRI and nuclear medicine physician reading the PET/CT will be blinded to each other's interpretation and the patient's treatment protocol. Based on both the standard of practice at our institution and existing guidelines in the literature, the nuclear medicine physician and neuroradiologist will independently predict the most likely outcome for each lesion: radiation injury vs. tumor progression. Only the results of the first MRI perfusion and FDG PET/CT will be used for data analysis (some patients may require repeat scans as part of their standard of care, as per their treating physician).

Patient outcome will be determined by a neuro-oncologist using standard histopathologic or clinical and imaging follow-up criteria (excluding any subsequent MRI perfusion or FDG PET/CT data). Unless the patient requires surgery as part of the standard of care for their brain tumor, the diagnosis will be established by follow up. Patients will remain on study until the completion of either the MRI perfusion or PET/CT that are within 12 weeks of each other. After one of these scans, the patient will have no active interventions and will be off study. Follow up will occur approximately every 2-3 months as per the treating physician and the standard of care (most gliomas are followed every 2 months, most metastases every 3 months). Follow up is expected to take 6 months or less in most patients, although some patients with slowly growing or otherwise confusing lesions may require longer follow up.

4.2 Intervention

The MRI perfusion and PET/CT scans will be obtained within 12 weeks of each other. These scans are part of the standard of care for patients with brain tumors and uncertain tumor response or progression after treatment. Although every effort will be made to perform both MRI perfusion and PET/CT on the same day or during the same week, some patients may experience longer intervals between scans due to scheduling conflicts. The disease in question (radiation injury vs. tumor progression) may change slightly during this interval (e.g., the lesion may grow or shrink slightly), but no large changes are expected between the two scans. The patients may continue existing treatments in the interval between scans (e.g., steroids, chemotherapy), but the two scans must be performed before any change or new treatment occurs. Fusion images of MRI and PET/CT will not be reviewed by the neuroradiologist interpreting the MRI perfusion nor the nuclear medicine radiologist interpreting the PET/CT.

4.2.1 MRI perfusion:

Dynamic contrast enhanced (DCE) T1 perfusion will be performed per standard of care using (1) an axial 3D spoiled gradient-recalled acquisition in the steady state (SPGR) sequence with these or similar parameters: TR/TE=8.3/1.5; flip angle 25°; matrix 256x128x128; slice thickness 5 mm; field-of-view 24 cm; acquisition time 10 seconds per volume of 12-18 slices; acquired 40 times, once before and 39 times immediately after intravenous bolus administration of a single dose of contrast material; - or - (2) an axial 3D differential subsampling with Cartesian ordering (DISCO) sequence with these or similar parameters: TR/TE=4.5/1.8; matrix 288x160; slice thickness 4 mm; field-of-view 24 cm; acquisition time 10 seconds per volume of 35-40 slices; acquired 40 times, once before and 39 times immediately after intravenous bolus administration of a single dose of contrast material. Both sequences will require approximately 5 minutes of scanning time. SPGR MRI perfusion does not allow whole brain coverage due the dynamic nature of the contrast injection. If multiple lesions exist, then the lesion(s) of clinical concern (e.g., the lesion(s) that is growing) or the largest lesion(s) will be interrogated and as many other lesions as possible will be included in the perfusion volume. Although patients commonly receive treatment for multiple lesions, in our experience most (>90%) patients only have growth of one lesion whereas the other lesions remain stable. Only existing previously irradiated lesions at the time of enrollment will be considered target lesions for this study. The DISCO sequence is the updated, FDA approved sequence from GE Healthcare that is capable of whole brain coverage. DISCO has been adopted as the standard of care for MRI perfusion, where available on selected MRI scanners. Patients will receive DISCO perfusion if it is available, or the standard SPGR perfusion.

The data will be transferred to an off-line workstation running Windows platform for analysis using commercially available or research perfusion software packages, such as: NordicICE by NordicNeuroLab, which is a commercially available, FDA approved product. The software package performs pharmacokinetic modeling to analyze DCE T1 perfusion data.

The methods of analysis will include: region-of-interest (ROI) measurements of the lesion and normal brain will be performed. ROIs will be placed in the lesion while avoiding areas of hemorrhage, calcification, and cystic/necrotic change. This is the standard way of measuring lesions in clinical practice. Multiple small ROIs will be placed, and the maximum recorded, a technique that has been described as providing the best reproducibility.[37, 38] Recorded metrics will include: plasma volume (Vp) and volume transfer coefficient between plasma and extravascular extracellular space (K^{trans}). We may also use volume of interest (VOI) measurements of the entire lesion.

Additional postprocessing: Feature-based analyses will be performed on an investigational basis. These are not routinely performed as part of the standard of care, and therefore the results will be only for research and not included in the MRI report. The DCE maps will be processed using in-house routines developed in C++ and Matlab for extracting image-based textures and Gabor edge features to characterize each lesion in terms of topological and textural factors. In-house developed machine learning techniques may be applied to automatically find correlations between image features and lesion outcomes.

4.2.2. PET/CT: Patients will fast except for water ± medications (as per their clinical physician) for at least 6 hrs prior to this study. The patient is seated comfortably in the injection room and venous access is established. The patency of the venous access is checked with normal saline prior to the administration of the FDG.

Blood glucose is checked by finger stick. If blood glucose is >60 and <200mg/dL the study continues as planned. If the blood sugar is between 200 and 300mg/dL, then a standard insulin administration protocol is used to bring the blood glucose below 200. If blood glucose is > 300mg/dL, the study is rescheduled. If the blood glucose is <60, juice is given orally and the study is rescheduled.

10 mCi of Fluorine-18 fluorodeoxyglucose (FDG) is injected IV. The patient remains seated quietly in the injection room for 60 minutes. The patient is then positioned on a GE Discovery STE PET/CT. A scout view of the head is acquired at 30 mA, 120 kVp. Once the scanning field is determined from the scout, a spiral CT is acquired using a full helical acquisition at 1 sec/rotation, 30 mA, 140 kV, 5.0 mm slice thickness, 4.5 mm interval. Immediately upon completion of the CT, acquisition of a 10-minute 3D brain PET scan is performed. CT and PET data are re-reconstructed using a 30 cm FOV. CT, PET, and hybrid images are evaluated in transaxial, coronal, and sagittal planes. The standardized uptake value (SUV) of the lesion (SUV_{lesion}), normal brain (SUV_{normal}) and SUV_{ratio} ($SUV_{lesion}/SUV_{normal}$) will be calculated using standard ROI tools. PET/CT allows whole brain coverage. Measurements of each lesion will be performed and each lesion evaluated independently for radiation injury vs. tumor progression.

Additional postprocessing: Feature-based analyses will be performed on an investigational basis. These are not routinely performed as part of the standard of care, and therefore the results will be only for research and not included in the PET report (as compared to SUV, which are standard of care and reported to the clinician). The PET scans will be processed using CERR (Computational Environment for Radiotherapy Research) and/or in-house routines developed in C++ and Matlab for extracting image-based textures and Gabor edge features to characterize each lesion in terms of topological and textural factors. In-house developed machine learning techniques may be applied to automatically find correlations between image features and lesion outcomes. We may also investigate novel analysis methods in addition to SUV by using other correction factors for normal tissue selection and ratio calculations.

4.2.3 Advanced diffusion imaging (Optional): RSI acquisition will require approximately 4-5 min, similar to the time required for the standard of care DTI acquisition. The only thing noticeable by the patient will be the extra scan time required to acquire DSI. The patient will be requested to remain motionless during this scan.

RSI will be acquired using a single-shot gradient spin-echo echo-planar sequence with these or similar parameters: 6-15 directions, TR=96 msec, TE=17 msec, matrix 128x128, slice thickness 3 mm, b-values 0-4000 s/mm2. Total of 40-45 direct axial slices with no interslice gap will be obtained to cover the entire brain. The sequence will require 4-5 min.

Post-processing will be performed using software that will be provided to MSKCC by GE Global Research. The acquired data will be analyzed on an offline workstation, after the RSI data have been acquired and after the patient has left the MRI suite.

At the discretion of PI the RSI sequence may be repeated at SOC FDG or other radiotracer imaging carried out while the patient is still on study, if deemed clinically necessary.

5.0 CRITERIA FOR SUBJECT ELIGIBILITY.

5.1 Subject Inclusion Criteria

- Pathological or clinical/radiological diagnosis of a neoplasm, either primary (e.g., malignant glioma) or secondary (metastasis from systemic malignancy) with a history of brain radiation therapy
- Completed fractionated radiation therapy (to 60 Gy for high grade gliomas) or stereotactic radiosurgery or hypofractionated radiation therapy (e.g. for brain metastases, anaplastic meningiomas), without or with concurrent chemotherapy
- New or increased enhancing brain lesion(s) OR nonenhancing brain lesion(s) if receiving anti-angiogenic therapy, which is considered indeterminate for tumor progression vs. radiation injury by the neuroradiologist or clinician
- Patient and/or guardian is able to provide written informed consent prior to study registration
- Age ≥18 years old

5.2 Subject Exclusion Criteria

- Claustrophobia
- Known allergic reaction to Gd-DTPA
- Any contraindication to gadolinium intravenous contrast as per standard Department of Radiology contrast guidelines
- Any contraindication to MRI (e.g., pacemaker, aneurysm clip, tissue expander).
- Pregnant or nursing female
- Unable to cooperate for MRI and/or PET/CT

6.0 RECRUITMENT PLAN

Patients for this study will be referred by members from the Departments of Radiation Oncology and Neurology. Patients will typically have a CT or MRI scan showing an increasing indeterminate lesion. All adult patients ≥18 years are eligible for participation regardless of sex or race. Every effort will be made to encourage eligible women and minorities to enroll in this study. Prior to study entry, the study staff will explain to each potential subject the research objectives, risks and benefits of study participation, alternative treatments available, and the subjects' rights and responsibilities. If the patient agrees to participate in the study, informed consent will be obtained by a consenting individual on the study. All patients must sign written informed consent prior to being registered on this protocol.

7.0 ASSESSMENT/EVALUATION PLAN

7.1 Imaging evaluation

The maximal abnormalities on the MRI perfusion scans and PET/CT scans will be recorded according to standard ROI and volume of interest (VOI) measurements:

FDG PET/CT:
1. SUV_{lesion} (SUV=standardized uptake value)
2. SUV_{normal}
3. $SUV_{ratio} = SUV_{lesion}/SUV_{normal}$
4. PET texture factors: contrast, homogeneity, entropy, skewness, kurtosis
5. PET topological factors: eccentricity, solidity, and extent

(SUV=standardized uptake value)

MRI Perfusion:
1. V_P (plasma volume)
2. K^{trans} (volume transfer coefficient between plasma and extravascular extracellular space)

RSI Diffusion:
1. RSI Celluarity

Either PET/CT or MRI perfusion may be performed first, but both studies should be completed within 12 weeks of each other. The PET/CT will be interpreted by a study nuclear medicine radiologist while blinded to the MRI perfusion results. The MRI perfusion will be interpreted by a study neuroradiologist while blinded to the PET/CT results. Each MRI perfusion metric and each FDG PET/CT metric will be independently tested to predict the diagnosis of radiation injury vs. tumor progression. In addition to independently testing the utility of MRI perfusion and PET/CT in determining radiation injury, we will also examine if combining both techniques will perform better than either one alone.

The protocol proposes using the current standard of practice for diagnosing radiation injury vs. worsening tumor, in terms of histopathology (when available) or clinical and radiological follow up. We will also perform a subcohort analysis to determine if there is any difference between patients diagnosed by histopathology (anticipated at approximately 15-25%) vs. patients diagnosed by follow up.

Clinical and radiological follow up constitute the current standard of practice when histopathology is not available, either due to lesion location (e.g., deep in brain, in brainstem, or in eloquent motor or language areas) or due to patient comorbidities. The table below summarizes several recent papers from the Radiology and Neuro-oncology literature examining the issue of radiation injury. Of the seven cited papers, only one included only pathology proven patients. The other six papers used follow up for diagnosis in 26.7-92.6% of all patients. Our previous paper[36] was near the middle of this range, with follow up implemented in 69.9% and using a 6 month minimum follow up time. Although there is no clearly defined clinical and radiological criteria to determine radiation injury, the 6 month minimum follow up approach is more conservative than the 3 months minimum follow up time used by other authors.[20, 47, 48]

Table. Representative imaging papers studying radiation injury.

Study	# Patients (# lesions)	Tumor type	Technique	# Diagnosis by follow up (minimum follow up)	# Diagnosis by histo-pathology
Barajas et al[20]	27 (30)	Metastases	MR perfusion	8 (3 months)	22
Mitsuya et al[47]	27 (28)	Metastases	MR perfusion	25 (3 months)	2
Narang et al[48]	29 (29)	Gliomas (n=24), metastases (n=5)	MR perfusion	9 (3 months)	20
Amin et al[49]	24 (24)	High grade gliomas	DMSA SPECT, MR spectroscopy	19 (not described)	5
Mullins et al[50]	27 (>39)	High grade gliomas	Conventional MRI	0 (N/A)	27
Langleben et al[51]	72 (not described)	High grade gliomas	PET/CT	61 (not described)	11
Young et al[36]	93 (not described)	High grade gliomas	Conventional MRI	65 (6 months)	28

7.2 Histopathologic Diagnosis

The diagnosis of radiation injury versus tumor progression will be made using histopathologic findings if resection is determined to be clinically necessary by the treating physician. If pathology indicates a mixed population of tumor cells and treatment effects, then the predominant cell population will be used to determine the patient outcome. If the diagnosis remains unclear based on pathology, then the lesion will be placed in a third category of uncertain, and excluded from statistical analysis. Based on the retrospective results presented in Section 3.2, we anticipate 15-25% (n=13-21) of the patients will undergo surgical resection or biopsy of their increasing lesions.

Based on clinical experience, we anticipate that <10% of these 13-21 patients will have indeterminate pathology results. Resection of the indeterminate lesion in itself may have therapeutic value for both radiation injury and tumor progression. Therefore, we not anticipate that we will be able to use clinical and radiological follow up for these patients with uncertain diagnosis after indeterminate pathology.

7.3 Clinical and Radiological Diagnosis

If tissue sampling is clinically not warranted, then the lesion outcome will be determined after clinical and imaging follow up by the neuro-oncologist and/or radiation oncologist, while blinded to the MRI perfusion and PET/CT results. The accuracy of these non-pathologic diagnoses is expected to be maximized by the follow up process, in which determinations may be made up to 6 months (or more) after presentation of the lesion(s). In many cases, following lesions to determine their natural history is expected to provide the best characterization of the underlying biology. These assessments to determine lesion(s) outcome will be performed approximately every 2 months during the study. Blinding will be performed by a third party (e.g., research study assistant). The results of both MRI perfusion and PET/CT are available in the electronic medical record as individual Radiology reports, and may be mentioned in the clinical notes of the treating physician. The chart records and official radiology reports will be saved and/or printed with redaction of these results, by deleting and/or blacking-out the relevant sentences. The blinding party will also pull up the patient's scans on a computer workstation without the MRI perfusion images and without the PET/CT images. Only the regular MRI images would be reviewed to examine changes in size of the lesion(s).
We will adopt the updated response criteria proposed by the Response Assessment in Neuro-Oncology Working Group[52] shown in the table, with some modifications as below.

Table 4. Summary of the Proposed RANO Response Criteria

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.
*Progression occurs when this criterion is present.
†Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

Specifically, to be classified as radiation injury, the lesion must spontaneously stabilize (stable disease) or decrease (partial response or complete response) on subsequent scans over a minimum of 6 months without new chemotherapy. Lesions that continue to increase (fulfilling criteria for progressive disease) will be considered tumor. If the lesion is stable for <6 months and therefore does not meet above criteria for radiation injury, it will be placed in a third category of uncertain and may be excluded from statistical analysis, unless additional follow up beyond the 6 months shows stability (radiation injury) or enlargement (worsening tumor). Therefore, the designation of uncertain may be only temporary, as we will continue to follow the lesion and the patient whenever possible. If a lesion has an uncertain diagnosis despite continued follow up at the end of the study, it will also be placed in the uncertain category and may be excluded from statistical analysis.

Patients with metastatic disease who have enhancing lesions and receive chemotherapy for systemic disease may be determined to have radiation necrosis if: 1) the systemic chemotherapy does not change during the 6 month follow up period to establish radiation necrosis; and 2) the systemic chemotherapy has no known efficacy for CNS disease. If the lesion diagnosis is uncertain after follow up for reason, including interval changes in systemic chemotherapy or additional radiation therapy for new lesions (unrelated to the initially treated lesion that is being followed) as per their treating physician and the

standard of care, then the treated lesion will be placed in the third category of uncertain and excluded from statistical analysis.

7.4 Multiple lesions

If a patient has multiple lesions, only the enlarging or otherwise worrisome lesion(s) will be evaluated. MRI perfusion (that has incomplete brain coverage) will be performed through this dominant lesion and as many of the other lesions of interest as feasible. Only a minority of patients are expected to have multiple lesions of concern, as supported by published papers summarized in the Table shown in Section 7.1.

Patients may get a variety of radiation therapy doses. Sometimes patients receive whole brain radiation therapy followed by a boost or stereotactic radiosurgery to one dominant lesion or postoperative cavity. Therefore, we cannot assume that all lesions in a single patient will behave in a similar fashion and represent a single process. In patients with multiple irradiated lesions, usually the lesions respond in unison (ideally, they all decrease in size and indicate a positive treatment response). Sometimes, one or two or more lesions may increase in size and become concerning for radiation injury vs. tumor progression. This presents several possible scenarios in a single patient: one or more lesions may be due to radiation injury and the others due to worsening tumor; or they are all due to radiation injury; or they are all due to worsening tumor. Although clinically a bottom line result may be concluded as progression vs. no progression, for the purpose of possibly initiating systemic chemotherapy or whole brain radiation therapy, this oversimplifies a problem in which each lesion represents an unknown that is not necessarily related to the other. If there are only two lesions, and they both increase, it is possible that they represent the same or different process. The outcome for multiple lesions within a single patient may be dependent and statistical analyses based on dependent samples will be employed. There is no plan to aggregate imaging metrics of multiple lesions into a single metric for an entire patient.

If several lesions all increase in size, then that would suggest treatment failure and tumor progression (especially if new lesions also appear). This patient would not be enrolled into this protocol because there is no or little doubt about the diagnosis of the worsening lesion(s). Note that patients with >3 lesions from metastatic disease usually receive whole brain radiation therapy, which is less likely to cause radiation injury than the higher dose partial brain and stereotactic radiosurgery techniques.

The potential problem of multiple lesions will be avoided with the new DISCO sequence.

8.0 TOXICITIES/SIDE EFFECTS

PET/CT of the Brain: FDG PET/CT is a routine medical imaging procedure proven to be safe and noninvasive. Risks and side effects related to the IV catheter may include discomforting pain at the site of injection, bleeding, and bruising.

Radiation safety: The whole body dose from 370MBq (10 mCi) of FDG is 0.43rem (4.3mSv). A low milliampere CT scan of the brain, done as part of the hybrid FDG

PET/CT, has a dose of 0.2 rem (2.0 mSv). The total dose from the FDG PET/CT examination is 0.63 rem (6.3 mSv), which is well below the annual radiation dose limit of 50 mSv, and within typical doses for imaging cancer patients. Pregnant and/or breast-feeding women will not be included in this study.

MRI of the Brain: MRI is a proven safe and noninvasive technique that entails no radiation risk. MRI involves magnetic and radiofrequency energy. Therefore, subjects who have implanted metal devices, such as pacemakers, certain aneurysm clips, or shrapnel or metal in the eye are at risk, and will not be scanned. There are no known risks or adverse effects resulting directly from exposure to magnetic fields and radiofrequency energy used in this study.

Some people feel claustrophobic in the MRI scanner. If the subject is unable to tolerate being in the scanner, the technologist can stop the scan immediately at any time. Other rare risks of MRI include neurostimulation effects, such as muscle twitches and tingling sensations, due to the rapid switching of magnetic fields, and a slight increase in body temperature that may occur in the presence of radio frequency energy. These are very unlikely under current operational guidelines.

Risks and side effects related to the IV catheter are similar to those for PET/CT, and may include discomforting pain at the site of injection, bleeding, and bruising. Gd-DTPA is a very safe IV contrast agent, with severe adverse reactions reported in 1/350,000-450,000 cases and death in 1/10,000,000 cases. More common side effects such as headaches, hives, nausea/vomiting usually quickly and spontaneously resolve. Medications and intravenous fluids may be given at the discretion of the radiologist if necessary to treat these symptoms. Severe anaphylactic reactions are extremely rare; if this should occur, institution wide protocols for treating contrast reactions, supporting the airway and cardiovascular system would be implemented. Nephrogenic systemic fibrosis (NSF) is a rare, fatal disease in patients with severe renal failure requiring prolonged hemodialysis that receive multiple doses of gadolinium. NSF is thought to reflect a cumulative, toxic effect of gadolinium in these patients with severe renal failure. We will conservatively exclude patients with known renal failure defined as serum creatinine >2.0 from this study. Only the standard dose of gadolinium contrast (0.1 mmol/kg) is given for each MRI – this represents the dose that the patient would have received even if he or she had not participated in this study. In other words, no additional Gd-DTPA is necessary to perform the proposed MRI perfusion sequence. The MRI perfusion sequence is FDA approved and readily available on all commercial MRI machines. The patient will need to stay in the scanner for approximately 5 extra minutes to acquire the perfusion sequence.

The patient will be allowed to withdraw from the study if desired. If only PET or MRI perfusion is acquired, the data may be used for subanalysis of the acquired technique. If no PET or MRI perfusion sequence is acquired, then the patient will be removed from the study as described under Section 10 (Criteria for removal from study, Refusal of patient to continue treatment and/or observations). In this situation, efforts will be made to accrue an additional patient.

9.0 PRIMARY OUTCOMES

We will use the PET/CT and MRI perfusion to independently predict whether or not an increased enhancing lesion represents radiation injury or tumor progression. The diagnosis will be established by a neuro-oncologist and/or a radiation oncologist according to pathology results if available, or by clinical and radiological follow up after a minimum of 6 months. If diagnosis is rendered by follow up, then the clinician will review available chart records and imaging studies after blinding of the PET/CT and MRI perfusion results by a third party (e.g., research assistant). Based on clinical practice, we anticipate that approximately 18 patients will undergo repeat surgery and have pathological evidence of the diagnosis.

10.0 CRITERIA FOR REMOVAL FROM STUDY

1. Inability to undergo MRI (e.g., pacemaker, claustrophobia) or PET/CT scan
2. Found to be ineligible for the protocol as described in the section on Criteria for Patient/Subject Eligibility
3. Patient and/or guardian requests to withdraw from study for any reason
4. Referring physician requests to withdraw from study for any reason, including believing that it would be in the patient's best interest
5. Refusal of patient to continue treatment and/or observations
6. Loss to follow-up
7. Patient death, unless an autopsy is performed to render a diagnosis of the indeterminate brain lesion

11.0 BIOSTATISTICS

The purpose of this nonrandomized study is to test the predictive value of PET/CT and MRI perfusion in distinguishing radiation injury from tumor progression.

11.1 Entire cohort analyses

Despite the heterogeneity of potential tumor types, radiation injury has a common mechanism in all patients: blood brain barrier disruption, inflammation, gadolinium contrast leakage into the interstitial space, and enhancement. In most clinical scenarios, we expect lower MRI perfusion and lower FDG PET/CT measurements for radiation injury regardless of the original tumor type. When considering the target accrual of 150 patients with indeterminate lesions, we anticipate that tumor progression will occur more commonly (approximately 90 patients) than radiation injury (approximately 42 patients) or uncertain diagnoses (approximately 8 patients). We anticipate that only 20-30% (28-42 patients) will require the entire 6 month follow up period to establish their diagnosis. Some patients may require >6 months and be followed up to 1 year to establish their diagnosis. Loss to follow up or death prior to determining a definitive diagnosis is expected to be uncommon (<5 patients). Unless an autopsy is performed to render a diagnosis for the brain lesion, death will be a criterion for removal from the study as described in Section 10.

We will perform one-sided two-sample t-tests (because previous analysis showed that MRI perfusion measurements are reasonably symmetric, have a small range and are usually lower in radiation injury than in tumor progression). To approximately assess the power of the analysis, we focus on the one-sided two sample t-test with the type I error

(claiming a difference while there is not) rate of 5%. With 150 patients over 2 years and assuming the effect size is 0.5 (meaning that the difference is half of the pooled standard deviation), type II error (failure to detect a difference that actually exists) rate is about 10%. If the effect size is 0.7, the type II error rate is about 1%. Here we used generic mean/standard deviation ratios because multiple measurements will be examined.

An interim analysis is also planned at half of the expected total. An O'Brien-Fleming boundary will be used to ensure the type I error rate is not inflated. Specifically, this requires the p-value must be <0.0088 at the interim analysis to stop the trial early and declare statistical significance and <0.0467 at the final analysis to declare statistical significance. Moreover, if the interim analysis p-value is >0.50 then we will stop the trial and declare futility. Under null hypothesis (that tumor and necrosis cannot be differentiated by the new imaging method) the early stopping probability is 0.5 for declaring futility and is 0.0088 for declaring significance. Under the alternative hypothesis with the effect size of 0.7, the early stopping probability is 0.002 for declaring futility and is 0.69 for declaring significance.

To quantify predictive power of the MRI perfusion package, receiver operating characteristic (ROC) analyses will be used.

For the analysis of PET/CT, similar approaches will be used. To compare the predictive power of various MRI perfusion measurements and PET/CT measurements, ROC curves will be drawn, and the area under the curve (AUC) will be computed and compared among MRI perfusion and PET/CT measurements. Bootstrap confidence intervals will be constructed to establish confidence levels and the difference between the two AUCs will be calculated and examined whether it is significantly different from 0. Due to the multiple comparisons needed, a false discovery rate (FDR) approach will be employed to control for the family-wise error rate. The positive FDR approach will yield new quantities called q-values for making statistical inferences.[53, 54] The purpose of implementing an FDR approach is to adjust the p-values from multiple tests (due to multiple MRI perfusion and PET/CT parameters to be examined).

For the exploratory objective to examine increasing non-enhancing masses, similar analyses will be done. We estimate 5-10 patients with recurrent tumors may receive anti-angiogenic therapy. If the numbers are small, we will summarize the results descriptively and suggest future research directions. If the numbers are sufficient, we will perform similar sensitivity, specificity, ROC and AUC analyses.

To further consider the variation among radiation doses, fractionations and schedules (which may be employed as per the standard of care for the specific tumor type in each patient), a more complicated ANCOVA (analysis of variance with covariates) will be used with the above parameters specified as covariates. If the numbers are small, we will summarize the results descriptively and suggest future research directions. We estimate that 15-25% (21-35 patients of the total) will undergo repeat surgical resection or biopsy to determine the diagnosis. A separate subset analysis will examine for potential differences between lesions diagnosed by pathology vs. lesions diagnosed by clinical and radiological follow up.

Most of the time, the two imaging methods (i.e., MRI perfusion and PET/CT) are expected to yield concordant results (>80%). Therefore, it is meaningful to compare the predictive power of various MRI perfusion measurements and PET/CT measurements. Due to the multiple comparisons needed, an FDR approach will be employed to control for the family-wise error rate. We may further construct a decision rule by combining results from both techniques in the hope of identifying an even more powerful predictive tool, although its feasibility would depend on the exact number of discordant cases and the individual

predictive power (if both MRI perfusion and PET/CT have sufficient predictive power and there are too few discordant cases then the combination may not show significant improvement).

As mentioned above, an interim analysis will be performed after 1 year or accrual of 48 (1/2 of expected total) subjects. Statistical tools may be adjusted at that time point if necessary to account for higher or lower than expected accruals within subgroups. For example, if many more than expected patients have uncertain outcomes or death before diagnosis (without autopsy), then we will may increase the target accrual to compensate.

11.2 Subcohort analyses

The patients will also be grouped according to the following clinical scenarios:

1) Gliomas after radiation therapy with increased or new enhancing lesions

2) Gliomas after radiation therapy in patients receiving bevacizumab or other anti-angiogenic chemotherapy with increased or new enhancing and/or non-enhancing lesions

3) Metastases after radiation therapy with increased or new enhancing lesions

4) Metastases after radiation therapy who may be receiving bevacizumab or other anti-angiogenic chemotherapy for systemic or brain disease

Although we anticipate similar changes in MRI perfusion and PET/CT for radiation injury (smaller values) vs. tumor (larger values) regardless of the clinical scenario, it is possible for different results to occur in gliomas vs. metastases, or initially treated vs. recurrent disease. Each scenario encompasses similar tumor types that are expected to receive similar standardized radiation therapy plans (in terms of dose, fractionation and schedule). For each subcohort of patients, we will determine the efficacy of MRI perfusion and PET/CT in predicting radiation injury vs. tumor progression in an exploratory fashion.

11.3 Multiple lesions

Cluster analysis will be performed to take into account the correlation among lesions from the same patient. For this generalized estimating equation (GEE) techniques will be used to estimate regression parameters. Moreover, subcohort regression analysis will be performed after the whole set analysis is done in order to preliminarily explore possible heterogeneity among the subcohorts. We acknowledge that the small subset sample sizes may not allow us to reach statistical significance, and thus nonsignificant results would not necessarily prove homogeneity, in which case we will report the summary statistics and point out future research directions.

11.4 RSI Cellularity

RSI cellularity indices will be measured. Similar t-tests and ROC analysis will be performed. If insufficient data is acquired for this exploratory aim, only summary statistics will be provided with suggestions for future study.

12.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

12.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent

Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

12.2 Randomization

No randomization will occur in this study.

13.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

13.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

13.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: http://www.cancer.gov/clinicaltrials/conducting/dsm-guidelines/page1.

The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at:

http://inside2/clinresearch/Documents/MSKCC%20Data%20and%20Safety%20Monitoring%20Plans.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the

activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

14.0 PROTECTION OF HUMAN SUBJECTS

Risks: There are no expected additional risks to the patients who participate in this study. Patients will receive the current standard of care for their disease. MRI and PET/CT are considered minimal risk procedures. MRI and PET/CT are standard diagnostic tests utilized in the diagnosis and management of patients with brain tumors. No additional risk will be incurred from magnetic and/or radiofrequency exposure to acquire the FDA approved MRI perfusion sequence. Patients may experience pain and/or discomfort related to the IV catheter, but they would commonly require IV contrast for their routine scan anyway.

Costs: The patient and/or patient's insurance will be responsible for all charges, which are part of the standard of care. No financial reimbursement or other financial incentive will be provided for patients to enroll in this study.

Benefits: We do not expect patients to derive any clinical benefit from this clinical trial. We hope that in the future, knowledge from this trial will help better evaluate treatment response in subsequent patients with brain tumors.

All patients will sign informed consents and have all their questions fully addressed before enrolling in this study. During the informed consent process, it will be made clear to the potential patient that participation is entirely voluntary, and will not impact the care that they receive at MSKCC. Potential patients will be advised of alternatives to the proposed study, including not participating in the study. All the data will be held confidential, maintained in a password protected electronic database, and comply with all HIPAA guidelines.

14.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

14.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death

- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the participant's condition
    - Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form

• If the SAE is an Unanticipated Problem

For IND/IDE protocols: The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

15.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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17.0 APPENDICES

Definition of feature-based analysis descriptors.
Texture[55]:

- Contrast is a statistical measure of the range of intensity over an input image, calculated as the distance moment of the image matrix.
- Homeogeneity is a statistical measure of point-to-point variability within an input image, calculated as the angular second-moment of the image matrix.
- Entropy is a statistical measure of randomness that can be used to characterize the texture of the input image.
- Skewness is a measure of the asymmetry of the data around the sample mean. If skewness is negative, the data are spread out more to the left of the mean than to the right. If skewness is positive, the data are spread out more to the right. The skewness of the normal distribution (or any perfectly symmetric distribution) is zero.
- Kurtosis is a measure of how outlier-prone a distribution is. The kurtosis of the normal distribution is 3. Distributions that are more outlier-prone than the normal distribution have kurtosis greater than 3; distributions that are less outlier-prone have kurtosis less than 3.

Shape[31]:

- Eccentricity is a measure of non-circularity defined in terms of the ratio of the minor axis to the major axis of the best-fitted ellipsoid to the defined structure of interest.
- Solidity is a measurement of convexity and is defined as the proportion of pixels in the convex hull that are also in the region. The convex hull is defined as the smallest convex polygon that can contain the region.Extent is similar to solidity except that it represents the proportion of pixels in the bounding box that are also in the region. The bounding box is defined as the smallest rectangle containing the region.