Image-Based Quantitative Assessment of Acute Radiation-Induced Changes in Glioblastoma Multiforme

Study Protocol & Statistical Analysis Plan

NCT03168919

March 14, 2016

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Protocol UAB: **[Image-Based Quantitative Assessment of Acute Radiation-Induced Changes in Glioblastoma Multiforme]**

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Version 1.3; Date: 3/14/2016

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1.0 INTRODUCTION AND STUDY RATIONALE

Glioblastoma multiforme (GBM) is an extremely heterogeneous tumor with a rapidly fatal outcome with a median overall survival of 14.3 months from diagnosis. In spite of intense basic science research and numerous clinical trials, no therapy has emerged in the last decade that has prolonged the overall survival of GBM patients. Newly diagnosed GBM is currently treated with maximally safe resection followed by radiation therapy (RT) with concurrent temozolomide therapy followed by temozolomide maintenance therapy. Standard of care RT is prescribed to the tumor volume determined by magnetic resonance imaging (MRI) obtained before or immediately after tumor resection. No tumor physiologic information or adaptive tumor changes during RT are currently incorporated into the radiation treatment plan. Standard fractionation (46 Gy in 23 fractions) RT is delivered to the peritumoral abnormal FLAIR volume followed by a sequential boost (14 Gy in 7 fractions) to the T1 contrast-enhancing tumor volume. This approach disregards the biological complexity and intratumoral heterogeneity of this neoplasm in terms of angiogenesis, cellularity, and even the methylation status of the promoter of the O6-methylguanine-DNA-methyltransferase (MGMT) gene, which has been shown to be predictive biomarker of response to conventional RT. In addition, the prescribed radiation dose is delivered to the tumor and peritumoral areas over 6 weeks without monitoring response of the tumor during this treatment period. As a consequence, opportunities to readjust radiation dose, for example during the boost phase, to more radio-resistant areas in order to potentially prolong RT response of this extremely heterogeneous tumor are missed. Additionally, if the tumor becomes smaller in volume in response to RT, unnecessarily larger dose is given to potentially normal brain areas.

The study is designed to assess the time course and magnitude of dynamic changes of morphology and distinct physiologic properties of GBM during and after completion of hypo-fractionated RT. MRIs obtained during therapy will be used to quantify during therapy patterns of dynamic changes of distinct physiological properties in response to RT. MRI obtained at completion will be used to quantify total effect of radiation to the tumor. Combined with follow-up evaluations of site of recurrence, progression time and overall survival, this study can establish quantitative imaging based biomarker of favorable treatment response and treatment resistance. These quantitative imaging biomarkers can be utilized to adopt a more scientific, quantitative tumor physiology based dynamic adaptive radiation planning compared to the current practice of pre-treatment anatomy-based fixed dose RT. This approach of adaptive radiation can potentially allow dynamic readjustment of the dose to the relatively less responding tumor areas with simultaneous integrated boost, readjusting the target volumes or opt for an alternative radiation techniques and use of radiosensitizers as 6-week long RT progresses. In addition, our proposed

quantitative techniques can identify tumors poorly responding to RT that can allow institution of early additional or alternative therapy rather than waiting the tumors to recur, the current clinical standard. In addition to significant impact in planning RT, these quantitative imaging biomarkers can be used to predict response to RT, progression time, site of recurrence and overall survival.

As no imaging is performed during the 6 weeks long course of hypo-fractionated RT of GBM, imaging changes of the contrast enhancing tumor volume and peri-enhancing abnormal FLAIR volume related to RT are currently not known. There is no documentation of progressive change in tumor volume as therapy progresses. Neither is it known if there are progressive changes of tumor physiology with continued RT. It is also not known if the entire enhancing tumor volume and the entire non-enhancing tumor volume of this extremely heterogeneous tumor responds similarly to the prescribed radiation dose. We want to evaluate the morphologic as well as physiologic changes in response to radiation therapy in combination with temozolomide as described below:

Morphologic changes: There are progressive changes of the size as RT progresses in cancers of other organ. As for example, in head and neck cancers with hypo-fractionated RT causes reductions of 51% in the clinical target volume and 48% in the planning target volume after a partial course (45-Gy dose) of RT.[1] However, we expect that there may be actual worsening of both the enhancing volume and the peritumoral abnormal FLAIR volume secondary to radiation induced release of cytokines and vasodilation.[2]

Post contrast 3DT1 weighted sequence and 3d FLAIR sequence is routinely used to calculate different target volumes and can be used to quantify morphologic changes of the enhancing tumor volume and peri-enhancing abnormal FLAIR volume as therapy progresses.

Physiologic changes:
Diffusion-weighted MRI (DWI) is an MRI method that produces in vivo images of biologic tissues based on the detection of a change in the random motion of water protons.[3,4] Free and random diffusion of water is impeded by cell membrane that is an integral part of the cellular architecture.[5] Apparent diffusion coefficient (ADC) map, one of the many different parameters calculated from diffusion imaging, represents a measure of average water diffusion for each voxel.[6] As tumor cellularity increases with increasing grade of a tumor, the impeding effect of cell membranes on water diffusion is expected to increase, resulting in lower total diffusion in a given voxel and thus decreasing the ADC value. An inverse correlation exists between tumor cellularity and ADC value.[7] Lower ADC value has been associated with poor survival independent of the WHO grading status of the astrocytomas.[5] During routine follow-up of GBM patients after completion of RT, an ADC value of

≤1.09 $10^{-3}mm^2s^{-1}$ has been shown to differentiate radiation related changes from recurrent tumor.[8]

Perfusion MRI is a technique that can provide physiologic information about vascular endothelial proliferation, vascular density, and angiogenesis.[9] Vascular proliferation is an important factor in the biology of GBM, and degree of new blood vessel formation increases with higher grades of tumor. Since DSC perfusion MRI can assess new vessel formation, it has been successfully used to assess grading of the astrocytomas[10] and prediction of survival. In fact higher normalized CBV, a parameter generated from DSC perfusion MRI correlates with survival regardless of WHO grading status of the tumor. Tumors with a high initial relative CBV have more rapid progression than those who have gliomas with a low relative CBV.[11] Fractional tumor burden can be calculated from the perfusion MRI that can reliably differentiate radiation related changes from residual tumor.[12]

MR spectroscopy is a powerful tool in in investigating tumor biology.[13] Specifically, elevation in choline (Cho) with depression of N-acetylaspartate (NAA) is a reliable indicator of tumor. Metabolite ratios of Cho/creatine (Cr), NAA/Cr, and Cho/NAA are useful in grading tumors and predicting tumor malignancy.[14] In addition, there is direct correlation between Cho level and cellular proliferative index.[15,16] MR spectroscopy has also been used to differentiate radiation related changes from recurrent tumor in the post-treatment setting. CNI >1.8 has been shown to reliable differentiate tumor from radiation related changes with sensitivity of 84%, specificity of 75% and diagnostic accuracy of 81%.[17]

2.0 OBJECTIVES

Primary:

Determine the time course and magnitude of MRI measures of enhancing volume, (with 3DT1), non-enhancing abnormal FLAIR volume (with 3D FLAIR), tumor cellularity (with ADC), tumor angiogenesis (with nCBV), and chemical environment of enhancing and non-enhancing tumor during the course of hypofractionated RT.

Secondary:

Determine whether multi-parametric imaging assessment during RT and immediately after completion of RT can predict time to recurrence, site of recurrence and overall survival.

Determine whether imaging changes in tumor with methylated MGMT promoter region differs from tumors with unmethylated MGMT promoter region.

3.0 STUDY DESIGN OVERVIEW

3.1 Study Design

Patient source: Once the study is funded, consecutive 20 patients with newly diagnosed GBM patients scheduled to have RT will be included in the study with the following inclusion and exclusion criteria. The patients will be screened from the radiation oncology department schedule.

Patient selection

***Inclusion criteria**:*

1) Newly diagnosed primary GBM, based on pathology confirmation;
2) At least 8 cm^3 of residual enhancing tumor after surgery (As seen on immediate postoperative scan);
3) Scheduled to receive standard fractionated RT with concomitant temozolomide therapy;
4) Karnofsky Performance Score > 60.

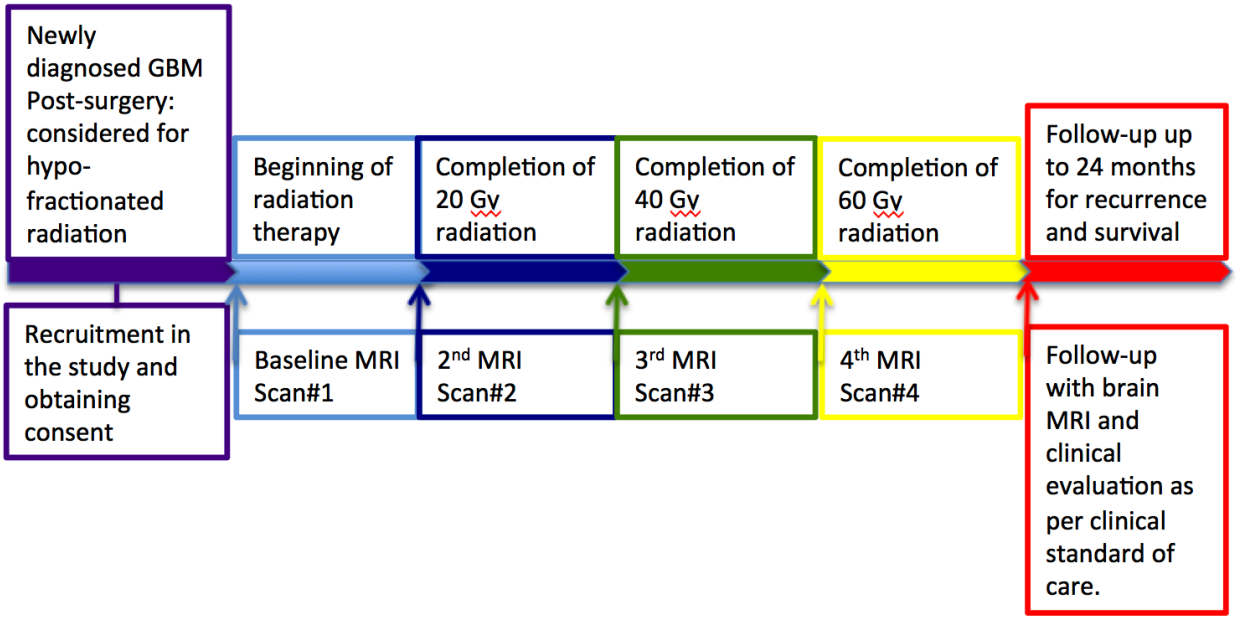
***Exclusion criteria**:*

1) Scheduled to receive investigational chemotherapy, immunotherapy, or any other investigational agents;
2) Placement of GLIADEL® wafer in the resection cavity;
3) Significant amount of hemorrhage within the resection cavity (seen on immediate post-operative scan);
4) A large peritumoral infraction related to surgery (identified by new confluent diffusion restriction);
5) Not suitable to undergo MRI or use the MRI contrast agent (GFR<30 mL/min/1.73 m^2); or the patient has known anaphylactic reaction to gadolinium based contrast agents.
6) Presence of serious systemic illness, including: uncontrolled infection, uncontrolled malignancy, significant renal disease, or psychiatric/social situations, which might impact the survival endpoint of the study or limit compliance with study requirements.

Patient Accrual Process: Once eligibility criteria have been assessed and the informed consent is obtained, participants will undergo a screening process to further ensure eligibility. Screening prior to registration will comprise a review of pathology reports, postoperative MR images, operative reports, and medical history; general physical and neurologic exams; routine blood work; and urine pregnancy test for women of childbearing age.

Radiation Treatment: RT will be prescribed as per the discretion of the treating radiation oncologist as per the University of Alabama at Birmingham Department of Radiation Oncology treatment protocol in combination with temozolomide. The target volumes will be contoured in the conventional way based on the pre-surgery standard of care MRI in combination with the immediate post surgery MRI obtained within 1-2 days after surgery. Enhancing volume will be treated with 60 gray and non-enhancing tumor volume will be treated with 46 Gy with daily 2 Gy hypo-fractionated regimes. The margin of the abnormal FLAIR volume will be decided at the discretion of the prescribing radiation oncologist. Multiple MRIs obtained, as a part of this research project will not be used in clinical decision-making. ***Precisely, there will be no change in the prescribed radiation dose, or the radiation planning based on the MRIs obtained during treatment.*** The patient will be taken care of and followed as per the department protocol.

Figure 1 – Study Design



Timing of acquisitions: The following MRI sequences will be obtained at the baseline (scan#1) (the previous day or on the day of starting RT) after completion of 20 Gy (scan#2), after completion of 40 Gy (scan#3) and after completion of 60 Gy (scan#4). All the following imaging sequences will be with a 3T Siemens MRI system (Siemens Prisma, Siemens AG, Erlangen, Germany).

Imaging parameters:

Precontrast 3DT1 sequence*:* 3D spoiled gradient echo T1 weighted sequence will be performed using the standard parameters.

3D FLAIR sequence: A 3D FLAIR imaging will be obtained in the sagittal plane using the standard parameters.

Diffusion Imaging: Routine diffusion weighted imaging will be obtained with B1000 using three diffusion gradients in the three orthogonal planes using standard parameters.

Echoplanar spectroscopy imaging: We will obtain point resolved echo-planar spectroscopy using a 20 channel head coil with TE/TR 70/1550 ms with acquisition time of 16 minutes as described by Ding et al.[18] After correction for oversampling in the readout spatial and spectral dimensions, the resultant images were equivalent to 50 x 50 voxels in-plane and 18 slices, over a field-of-view of 280 x 280 x 180 mm, with selection of a slab of 140 mm covering the whole brain. Subsequent to spatial smoothing, the effective voxel volume will be approximately 1 ml. The acquisition will also include a second dataset obtained in an interleaved manner without water suppression and using excitation and gradient-echo observation. This data will provide a water reference signal with identical spatial parameters.

Dynamic contrast enhanced susceptibility weighted perfusion imaging: The dynamic contrast enhanced T1 weighted perfusion imaging will be obtained first to use as proposing. The dynamic contrast enhanced susceptibility weighted perfusion imaging will be obtained during the bolus injection of additional 0.05 mmol/kg Prohance at 4ml/s, followed by a 20 ml saline flush with the following parameters: TR/TE in ms: 1900/40; 192 x 128 acquisition matrix, flip angle of 72°, slice thickness 3 mm, total of 20-24 slices. A total of 60 dynamics will be obtained.

Postcontrast 3DT1 sequence: 3D spoiled gradient echo T1 weighted sequence (exactly similar to the precontrast 3DT1 weighted sequence will be obtained after the 2 perfusion sequences will be performed using the standard parameters.

Image archiving:
All the acquired raw images will be permanently saved in the Department of Radiology PACS system. The post-processed images will be saved in the primary investigator's office computer, which has access only the primary investigator, rather than to the PACS because clinical significance of the processed images is not known at the current time. Additionally, post-processing of the raw data takes significant amount of time and the end result may not be available before completion of radiation therapy.

Patient follow-up
All the patients will be followed up and treated as per the standard of care. Patients will return for clinical evaluation and standard of care imaging approximately 4 weeks from

the completion of the radiation therapy. After that, all the patients will be treated with standard of care maintenance temozolomide therapy and will return every 2 months for clinical evaluation and standard of care imaging. The follow up has been detailed below in the section 6.3.

3.2 Endpoints

Primary:
The primary endpoint for this analysis is based on CNI, which is a continuous variable and will be calculated MR spectroscopy from the 4 scans during radiation. We want to demonstrate how change in CNI correlates with time to progression and overall survival. CNI of GBM is typically very high due to high cellularity. As tumor cellularity is expected to decline with continued radiation therapy, we expect that the CNI will decrease over time as radiation therapy progresses. Pattern of change of CNI from baseline to the end of radiation therapy is expected to correlate time progression and overall survival. The patients will be followed up for 24 months from the diagnosis or until death for the progression data and the overall survival data.

Secondary:
Secondary endpoints include change of volume, change of ADC, and change of CBV. These endpoints including the primary endpoints are anticipated to change simultaneously in response to radiation. Change of tumor volume will be measured using 3D T1 sequence, Change of ADC, a continuous variable, is calculated from diffusion imaging, change of CBV, also a continuous variable, is measured from perfusion MRI. We want to demonstrate how changes in these parameters correlate with time to progression and overall survival.

Exploratory:
The exploratory objectives of this study are to examine the time to first recurrence, evaluate the location of the recurrence, as well as the overall survival. Time to first recurrence is defined as the time from diagnosis to the date of the first documented tumor recurrence. The location of the recurrence will be assessed on the follow-up MRI. Overall survival is defined as the time between the date of diagnosis and the date of death due to any cause. Any subjects who have not died will be censored at the last known alive date.

3.3 Study Duration and Dates

Study Inclusion: 9 months
Study duration: 3 years

3.4 Safety and Efficacy Monitoring

We will perform 4 MRIs during the course of the radiation therapy as a part of this research study. Each of these 4 research MRIs possesses no additional risk compared to the standard MRI techniques. Each MRI will be obtained with gadolinium based contrast administration approved for clinical usage. We will use gadoteridol (Prohance, Bracco Diagnostics Inc. NJ)

As described above, risks associated with the research MRIs are no different from the risks associated with a routine clinical MRI. If any adverse event occurs, the patient will be excluded from the study for the subject's best interest. Additionally, the subject may withdraw himself/herself his/her consent at any point of time. All the standard MRI safety precaution will be taken before the image acquisition. As we do not anticipate any adverse event related to the research sequences, no specific precaution related to this study is needed. We will closely monitor the patient during the scanning. Appropriate patient care will be taken should there any adverse event.

This study is aimed to quantify response of standard clinical treatment using advanced MRI technique and has NO therapeutic component. The change seen on the imaging during therapy will not be used for any clinical decision making as we do not know the clinical significance of these changes.

The included subjects will be monitored for potential side effects of contrast injection as per clinical standards. Any side effects, which has been described in <1% of patients, will be managed as per clinical standards.

In addition, gadolinium contrast agents have been associated with nephrogenic systemic fibrosis in patients with severe renal functions. We will not include any subject in this study with chronic severe kidney disease (GFR<30 mL/min/1.73 m^2) or acute kidney injury.

3.4.1 Independent Data Monitoring Committee

NA

3.5 Ethical Considerations

This study will be conducted in accordance with Good Clinical Practice, the Declaration of Helsinki, and 21CFRPart50 – Protection of Human Patients, Part 56 –Institutional Review Boards, and the other applicable local ethical and legal requirements.

The Ethics Review Committee/Institutional Review Board (IRB) must be constituted according to Code of Federal Regulations (CFR).

The IRB will be informed by the principal Investigator of all subsequent protocol amendments and of serious or unexpected AEs occurring during the study, which are likely to affect the safety of the patients or the conduct of the study.

3.6 Informed Consent

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented in this study. A written informed consent will be obtained in accordance with 21CFR50.25 and 21CFR50.27 before the protocol – specified procedures are carried out. Patients, their relatives, guardians or, if necessary, legal representatives must be given ample opportunity to inquire about details of the study.

The consent form generated by the Principal Investigator must be approved (along with the protocol) by the applicable IRB. Consent forms must be in a language fully comprehensible to the prospective patient. The consent form should be signed and dated by the patient or the patient's legally authorized representative, and the investigator. Each patient's signed informed consent form must be kept on file by the investigator for possible inspection by Regulatory Authorities and sponsor (UAB).

3.7 Confidentiality

Patient names will not be supplied in the sponsor data. Only the patient number and patient initials will be recorded in the CRF, and if the patient name appears on any other document (e.g., laboratory report), it must be obliterated on the copy of the document to be supplied by the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. The patients will be informed that representative of the sponsor, independent IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The investigator will maintain a personal patient identification list (patient numbers with the corresponding patient names) to enable records to be identified.

4.0 SELECTION OF PATIENTS

4.1 Number of Patients

Approximately 20 patients should be enrolled and treated in this study. It is planned to recruit this sample in 9 months.

4.2 Inclusion Criteria

See above.

4.3 Exclusion Criteria

See above.

4.4 Patients of Reproductive Potential

The patient must not be pregnant or breast-feeding at enrollment in the study. Absence of pregnancy must be demonstrated by serum or urine testing prior to radiation therapy.

Female patients of child bearing potential (i.e., ovulating, premenopausal, not surgical sterile) must use a medically accepted contraceptive regimen. Male patients must agree to use a medically approved method of contraception.

If a female patient becomes pregnant during the study, the subject will be withdrawn from the study.

5.0 STUDY TREATMENTS

This is an observational study attempting quantification of imaging changes related to STANDARD radiation treatment given with temozolomide. *No investigational therapy will be given to the study subjects.*

6.0 STUDY PROCEDURES AND SCHEDULE

6.1 Screening Phase

Each potential patient will be examined before the start of the study to determine their eligibility for participation by the investigators. Vital signs and complete blood count will be obtained during radiation treatment as per the clinical standard of care. Female patients of childbearing age will be tested for serum beta-hCG.

6.2 Study Days

The following tests/procedures will be completed prior to each MRI:

(1) Vital signs: The research coordinator will take the vital signs before the MRI.

6.3 Follow-up

The final intervention as a part of this research study is to acquire the 4th MRI, which we plan to obtain at the completion of the current clinical standard of care radiation therapy. No therapy is part of this study. However, all included subjects will be followed-up for 24 months from the time of diagnosis. During the follow-up period each patient will be imaged and evaluated according to clinical standard of care. We will document the followings:

Progression: We will assess time to progression as well as site progression.

Progression will be determined by the RANO[19] criteria as described in the Table 1:

Criteria for Determining First Progression Depending on Time From Initial Chemoradiotherapy

First Progression	Definition
Progressive disease < 12 weeks after completion of chemoradiotherapy	Progression can only be defined using diagnostic imaging if there is new enhancement outside of the radiation field (beyond the high-dose region or 80% isodose line) or if there is unequivocal evidence of viable tumor on histopathologic sampling (eg, solid tumor areas [ie, > 70% tumor cell nuclei in areas], high or progressive increase in MIB-1 proliferation index compared with prior biopsy, or evidence for histologic progression or increased anaplasia in tumor). Note: Given the difficulty of differentiating true progression from pseudoprogression, clinical decline alone, in the absence of radiographic or histologic confirmation of progression, will not be sufficient for definition of progressive disease in the first 12 weeks after completion of concurrent chemoradiotherapy.
Progressive disease ≥ 12 weeks after chemoradiotherapy completion	• New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids. • Increase by ≥ 25% in the sum of the products of perpendicular diameters between the first postradiotherapy scan, or a subsequent scan with smaller tumor size, and the scan at 12 weeks or later on stable or increasing doses of corticosteroids. • Clinical deterioration not attributable to concurrent medication

	or comorbid conditions is sufficient to declare progression on current treatment but not for entry onto a clinical trial for recurrence. • For patients receiving antiangiogenic therapy, significant increase in T2/FLAIR nonenhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not be a result of comorbid events (eg, effects of radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).

Abbreviation: FLAIR, fluid-attenuated inversion recovery.

Time to progression and the site of recurrence will be documented.

Survival: After the first recurrence, the study will ONLY document the survival time. No other patient parameter will be recorded for this study.

6.4 Enrollment and Randomization Procedures

Eligible patients will be enrolled into the study through the UAB Department of Radiology Research Administration office (Phone: (205) 934-4080). After the patient is scheduled to have the radiation therapy, the investigators will evaluate for inclusion and exclusion criteria. When a subject meets the inclusion and exclusion criteria, one of the investigators will communicate with the potential subjects describing the study. If a subject is interested, a consent form will be sent the patient using UAB Patient Portal. The research coordinator from the Radiology Research Administration office will consent the subjects.

This study is not a RANDOMIZED study.

7.0 EFFICACY AND SAFETY

No investigational drug will be administered as a part of this trial. We are not measuring any efficacy of any treatment modality either as a part of this research study.

8.0 STATISTICAL METHODS

Based on the number of subjects (n=20), our statistical analysis will be largely descriptive and explanatory. Descriptive summary statistics tabulations will be generated for each time point. Means, standard deviations, median, and interquartile ranges will be generated for continuous variables, and frequency and percentages will be generated for categorical variables. For the primary outcome variable, in order to evaluate the within-subjects changes over time we will assess the pair-wise differences between time points using paired t-test, and may also perform repeated measures analysis of variance (ANOVA) on the continuous outcomes and account for the correlative nature of the repeated measurements with the caution and understanding of the limited sample size.

The Kaplan-Meier approach will be used to produce survival curves as well as the median and corresponding 95% confidence limits for time to first recurrence as well as overall survival. Subjects who have not had an event will be censored at the last known date available.

The location of recurrence will be reviewed and classified to then be presented in terms of frequencies and percentages to determine if there can be any dichotomization scheme created. Based on the potential dichotomization, we may use chi-square tests and logistic regression to examine how other factors may be potentially associated with the site of recurrence.

Distributional assumptions of the statistical analysis will be verified, and if the assumptions are not met, then non-parametric analogs will be considered. Statistical significance will be determined based on the two-sided level of significance at the 0.05 level. All analyses will be performed using statistical analysis software SAS version 9.4.

8.1 Sample size

There were no formal sample size calculations performed for this study, however based on the anticipated number of subjects (n=20), if we assume the largest changes in volume to be between the first and fourth time points, and consider the paired difference in means with correlation ranging from weak to moderate (rho=0.1 – 0.6) we anticipate having 66-88% power to detect statistically significant differences at the 0.05 two sided level of significance.

9.0 PROTOCOL AMENDMENTS AND LEGAL ASPECTS

9.1. Protocol Amendments

Once the study has started, amendments should be made only in exceptional cases. The changes then become part of the clinical study protocol.

9.2 Approval of the Clinical Study Protocol and Amendments

Before the start of the study, the clinical study protocol, informed consent document, and any other appropriate documents will be submitted to the IRB with a cover letter for a form listing the documents submitted, their dates of issue, and the site (or region or area of jurisdiction, as applicable) for which approval is sought. If applicable, the documents will also be submitted to the authorities, in accordance with local legal requirements.

9.3 Closure of the study

The study must be closed at the site on completion. Furthermore, the sponsor or the investigator has the right to close this study site at any time. As far as possible, premature closure should occur after mutual consultation. Depending on local legislation, it may be necessary to inform IRB and the regulatory authorities when the study site is closed.

Study materials must be returned, disposed of or retained as directed by the sponsor.

9.4 Record Retention

The investigator must obtain approval in writing from the sponsor before destruction of any records.

Essential documents should be retained until at least 5 years after the closure of the study.

Essential documents include:

- Signed informed consent documents for all patients.
- Patient identification code list and enrollment log.
- Record of all communications between the investigator and the IRB.
- Record of all communication between the investigator and the Principal Investigator or Designee.
- List of subinvestigators and other appropriately qualified person to whom the investigator has delegated significant trial-related duties, together with their roles in the study and their signatures.

- Copies of CRFs and of documentation of corrections for all patients.
- All other source documents (patient medical records, laboratory records, etc.).

9.5 Financial Disclosure

No financial disclosure.

10.0 STUDY MONITORING AND AUDITING

This is an investigator-initiated study.

11.0 DOCUMENTATION AND USE OF STUDY FINDINGS

11.1 Documentation of Study Findings

All study findings will be documented in an excel file and will be saved in a password protected computer at the principal investigator's office.

11.2 Use of Study Findings

The study findings will be used as preliminary data for application of extramural grant and will be also be presented in appropriate national meetings. Manuscripts will also be written for scientific publications.

12.0 DECLARATION OF SPONSOR AND INVESTIGATOR

12.1 Declaration of Sponsor

This is an investigator-initiated study.

12.2 Declaration of Investigator

I confirm that I have read the above protocol. I understand it, and I will work according to the principles of GCP as described in 21 CFR parts 50, 54, 56, and 312 and according to applicable local requirements.

Investigator

Date: 1/26/16 Signature: _____

Name (block letters): Asim K Bag. MD

13.0 REFERENCES

1. Geets X, Tomsej M, Lee JA, et al. Adaptive biological image-guided IMRT with anatomic and functional imaging in pharyngo-laryngeal tumors: impact on target volume delineation and dose distribution using helical tomotherapy. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 2007;85:105-15.
2. Walker AJ, Ruzevick J, Malayeri AA, et al. Postradiation imaging changes in the CNS: how can we differentiate between treatment effect and disease progression? Future Oncol 2014;10:1277-97.
3. Bammer R, Stollberger R, Augustin M, et al. Diffusion-weighted imaging with navigated interleaved echo-planar imaging and a conventional gradient system. Radiology 1999;211:799-806.
4. Yamasaki F, Kurisu K, Satoh K, et al. Apparent diffusion coefficient of human brain tumors at MR imaging. Radiology 2005;235:985-91.
5. Zulfiqar M, Yousem DM, Lai H. ADC values and prognosis of malignant astrocytomas: does lower ADC predict a worse prognosis independent of grade of tumor?--a meta-analysis. AJR American journal of roentgenology 2013;200:624-9.
6. Maier SE, Sun Y, Mulkern RV. Diffusion imaging of brain tumors. NMR in biomedicine 2010;23:849-64.
7. Server A, Kulle B, Maehlen J, et al. Quantitative apparent diffusion coefficients in the characterization of brain tumors and associated peritumoral edema. Acta Radiol 2009;50:682-9.
8. Kim HS, Goh MJ, Kim N, Choi CG, Kim SJ, Kim JH. Which combination of MR imaging modalities is best for predicting recurrent glioblastoma? Study of diagnostic accuracy and reproducibility. Radiology 2014;273:831-43.
9. Cha S. Update on brain tumor imaging: from anatomy to physiology. AJNR Am J Neuroradiol 2006;27:475-87.
10. Lev MH, Ozsunar Y, Henson JW, et al. Glial tumor grading and outcome prediction using dynamic spin-echo MR susceptibility mapping compared with conventional contrast-enhanced MR: confounding effect of elevated rCBV of oligodendrogliomas [corrected]. AJNR American journal of neuroradiology 2004;25:214-21.
11. Law M, Young RJ, Babb JS, et al. Gliomas: predicting time to progression or survival with cerebral blood volume measurements at dynamic susceptibility-weighted contrast-enhanced perfusion MR imaging. Radiology 2008;247:490-8.
12. Hu LS, Eschbacher JM, Heiserman JE, et al. Reevaluating the imaging definition of tumor progression: perfusion MRI quantifies recurrent glioblastoma tumor fraction, pseudoprogression, and radiation necrosis to predict survival. Neuro Oncol 2012;14:919-30.
13. Law M, Yang S, Wang H, et al. Glioma grading: sensitivity, specificity, and predictive values of perfusion MR imaging and proton MR spectroscopic imaging compared with conventional MR imaging. AJNR Am J Neuroradiol 2003;24:1989-98.
14. Moller-Hartmann W, Herminghaus S, Krings T, et al. Clinical application of proton magnetic resonance spectroscopy in the diagnosis of intracranial mass lesions. Neuroradiology 2002;44:371-81.

15. Tamiya T, Kinoshita K, Ono Y, Matsumoto K, Furuta T, Ohmoto T. Proton magnetic resonance spectroscopy reflects cellular proliferative activity in astrocytomas. Neuroradiology 2000;42:333-8.
16. Shimizu H, Kumabe T, Shirane R, Yoshimoto T. Correlation between choline level measured by proton MR spectroscopy and Ki-67 labeling index in gliomas. AJNR American journal of neuroradiology 2000;21:659-65.
17. Anbarloui MR, Ghodsi SM, Khoshnevisan A, et al. Accuracy of magnetic resonance spectroscopy in distinction between radiation necrosis and recurrence of brain tumors. Iran J Neurol 2015;14:29-34.
18. Ding XQ, Maudsley AA, Sabati M, Sheriff S, Dellani PR, Lanfermann H. Reproducibility and reliability of short-TE whole-brain MR spectroscopic imaging of human brain at 3T. Magn Reson Med 2015;73:921-8.
19. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:1963-72.