Pilot study of spectroscopic MRI-guided, dose-escalated radiation therapy for newly-diagnosed glioblastoma (RAD 3383-17)

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TABLE OF CONTENTS

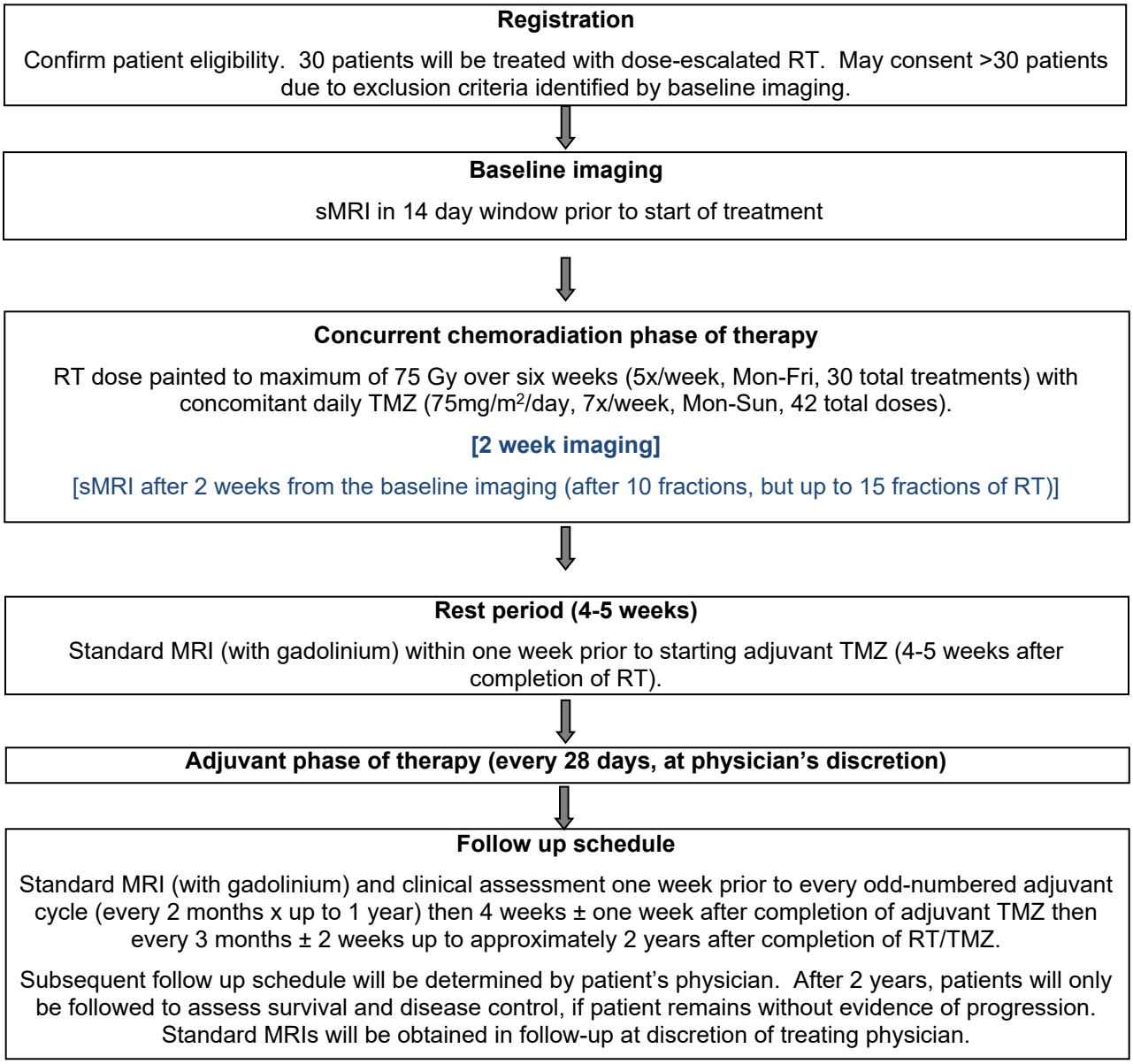
ABSTRACT

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. The current standard of care is maximal safe resection followed by radiation therapy (RT) with concurrent and adjuvant temozolomide (TMZ) chemotherapy. Despite this aggressive management, GBMs invariably progress resulting in significant morbidity and mortality for patients. Overall, the single most effective adjuvant treatment for this disease is RT. Patients are generally treated with focal radiation to a dose of 60 Gy to the highest risk regions and a lower dose (45-54 Gy) to lower risk regions. However, despite this therapy, tumor will generally regrow within the high dose volume suggesting that GBMs are relatively radioresistant and that increasing radiation doses may be beneficial for patients with this tumor. However, dose escalation studies that treat beyond the 60 Gy standard have not been shown, to date, to improve patient outcomes. It is important to note that most of these dose escalation studies were conducted in the pre-temozolomide era. Interestingly, one study utilizing proton therapy with doses up to 90 cobalt gray equivalent (CGE) did change the pattern of recurrence from within the high dose volume to the margins of this region, particularly where doses fall below 70 CGE. This result suggests that current MRI-based approaches for defining tumor volume/margin may not fully identify all regions at highest risk for recurrence and that better identification of the high risk regions will now allow realization of the benefits of dose escalation in this disease. Indiscriminate increases in treatment margin may allow delivery of dose-escalated radiation to the needed region(s) at the cost of irradiating wide areas of the brain with these higher doses significantly increasing the likelihood of unacceptable morbidities. Thus, improved definition of the regions at highest risk for recurrence will allow dose escalation to be targeted to a more limited volume that would be better tolerated by patients.

Currently, MRI using T1-weighted (T_{1w}) post-contrast and T_2/FLAIR sequences is used to define treatment volumes for radiation planning; however, these sequences may not identify all regions in the brain at highest risk for tumor recurrence. Proton magnetic resonance spectroscopic imaging (MRSI) is an alternative modality that is able to map certain metabolite levels within the brain. This may allow for more accurate characterization of regions with significant tumor involvement than would be achievable with conventional MRIs. Several reports demonstrate that a high choline (Cho) to N-acetyl apartate (NAA) ratio (Cho/NAA) correlates with areas of high tumor cell density and, after treatment, areas of tumor recurrence. However, MRSI is not widely used in clinical practice due to relatively low resolution, long acquisition time, and inefficient data analysis and visualization in commercially available packages. The first two limitations have now been overcome with state-of-the-art technology utilizing EPSI (echo planar spectroscopic imaging) with GRAPPA (parallel imaging) for the spectroscopic imaging. High resolution, three dimensional (3D), whole brain metabolite maps can now be obtained in relatively short (12-15 minute) scan times and in a form that can be registered with other conventional MR images by utilizing an advanced analysis and visualization tool. We have now termed this advanced imaging modality spectroscopic MRI (sMRI). Based on a recently completed early phase clinical study sponsored by NCI, “combining high resolution MRSI with 5-ALA to improve complete resection in GBM surgery” (NCI R21 CA186169; Emory IRB00051663), we learned that sMRI Cho/NAA showed significant correlations with tumor cell density in histological results ($\rho = 0.82$, $p < 0.001$). Based on early data from the control arm of on-going clinical study, “Quantitative MRSI to predict early response to histone deacetylase inhibitor therapy in new GBM management” (NCIU01 CA172027; Emory IRB00055973) and an institutional study “MRSI in the treatment planning and assessment of glioblastoma” (Emory IRB0006545), sMRI metabolic abnormalities predated contrast-enhancement at sites of tumor recurrence and exhibited an inverse relationship with progression-free survival (Cordova et al. This Neuro-Oncology article is enclosed in Appendix).

We believe that the combination of sMRI and conventional MRI scans can be used to guide radiation therapy target definition and dose-escalated treatment planning, significantly improving outcomes for patients with this highly aggressive brain tumor.

STUDY SCHEMA



1.0 INTRODUCTION AND BACKGROUND

Glioblastomas (GBMs) are aggressive primary brain tumors of astrocytic origin. Approximately ten thousand new cases are diagnosed each year in the U.S., making it the most common primary malignant brain tumor in adults. While radiation therapy (RT) has long been used in the treatment of GBMs and will delay their progression, it generally will not control these tumors long term. Incremental progress has been made in the management of GBMs, most recently with the addition of temozolomide (TMZ) chemotherapy to RT; however, outcomes remain poor, with a median survival of only 14-15 months [1, 2]. Recurrence is largely due to the inherently infiltrative nature of GBM, with tumor cells migrating to regions distant from the central contrast-enhancing regions. Because of this, regions of nonenhancing signal abnormality on fluid-attenuated inversion recovery (FLAIR) or T2-weighted (T_{2w}) images are covered in a typical RT treatment plan, albeit with moderate doses. However, T_{2w}/FLAIR is not tumor-specific due to difficulties in differentiating nonenhancing tumor from other causes of increased FLAIR or T2 signal (e.g. radiation effects, ischemic injury, edema, and infection) [3, 4]. T1-weighted -contrast-enhancing (T_{1w}-CE) MRI displays the leaky neovasculature associated with these tumors, indicating well-perfused tumor lesions with excellent oxygen and chemotherapeutic drug delivery. Despite the constant effort of tumor cells to recruit new blood vessels (neoangiogenesis), there is a significant gradient of oxygen. Hypoxia occurs in tumors 100 μm away from the blood supply, and tends to be widespread in GBMs. The viable hypoxic cells existing in solid tumors are associated with the failure of radiation and certain chemotherapy regimens [5]. Obviously, IV contrast agents cannot effectively reach all the tumor cells beyond the contrast-enhancing border; therefore, it is unreasonable to use conventional T_{1w}-CE MRI alone as the basis for RT treatment planning. Newer imaging methods are desperately needed to identify the actively proliferating tumor beyond the T_{1w}-CE area. This could potentially make a significant impact on improving tumor control.

Based on retrospective analysis of successive Brain Tumor Study Group trials, a fractionated dose of 60 Gy was determined to be optimal for GBMs [6]. A typical RT dosing regimen for the treatment of GBM involves treating a wide volume, including nonenhancing T_{2w}/FLAIR-hyperintense regions with margin, to a moderate dose (45-54 Gy), followed by a boost to the resection cavity plus any residual T_{1w}-CE abnormality with margin to a higher dose (60 Gy). A study using proton therapy for GBM found that patients not only tolerated doses up to 90 cobalt-gray equivalent (CGE), but also started to display a change in the pattern of recurrence with nearly all failures in regions receiving < 70 CGE [7]. Of note, TMZ was not used for this study. This result suggests that tumoricidal doses (in the 70+ Gy range) may have finally been achieved, with marginal failures now being due to growth of disease that had infiltrated into brain surrounding the initial contrast-enhancing portion of the tumor. This study also suggests that better definition of the brain volume most at risk for tumor recurrence may allow better targeting of these regions with higher doses leading to improved local control and overall patient outcomes. Combining RT dose escalation with concurrent/adjuvant TMZ may produce more toxicities, especially since the incidence of pseudoprogression after concurrent RT/TMZ is significantly higher than after RT alone. Pseudoprogression is the phenomenon where an early increase in volume of contrast enhancement is seen following treatment with RT/TMZ (usually within 3 months post-RT). This effect is indistinguishable from early true tumor progression by standard MR imaging, and is generally only distinguished by serial imaging over time, when this change either stabilizes/improves (pseudoprogression) or continues to worsen (true progression) without alterations in the therapy. Brandes et al. have reported that the overall incidence of pseudoprogression is on the order of 30% in patients treated with the Stupp regimen [8]; therefore, it follows that TMZ may also alter tolerance to dose-escalated RT. This concern has been somewhat allayed by Tsien et al. with their report that TMZ could be safely paired with dose-escalated intensity modulated RT [9]. Of note, no late grade III or greater CNS toxicities were seen with doses up to 75 Gy, and for doses above 75 Gy (7 pts at 78 Gy and 9 pts at 81 Gy), only 3 patients developed ≥ grade III late CNS toxicities. Thus, it is appears that dose-escalated RT may be safely paired with TMZ. Since previous results also suggest that very high radiation doses (70+ Gy range) shift the pattern of recurrence to the margins of the high dose irradiation zone [7], there is now a greater premium on defining further areas that may be at increased risk of recurrence that can potentially be boosted by these higher doses.

Currently, GBMs are imaged with conventional MRI sequences, including T_{2w}, FLAIR, and pre- and post-contrast T_{1w} sequences, and some advanced MRI techniques, including diffusion-weighted imaging (DWI), and dynamic susceptibility contrast (DSC) and/or dynamic contrast-enhanced (DCE) perfusion-weighted imaging (PWI). DWI and PWI MRIs have been used to follow response of GBMs to therapy, but have not proven particularly useful for RT planning [10-12]. Similarly, diffusion tensor imaging (DTI), a MR technique that describes the movement of water molecules using metrics, such as mean diffusivity (MD), and fractional anisotropy (FA), which represent the magnitude and directionality of water diffusion, respectively [13, 14], did not have value for predicting recurrences outside the high dose irradiated regions of brain when analyzing a cohort of GBM patients treated at the University of Pennsylvania (Dr. Verma, personal communications). Overall, the most common advanced MRI techniques have proven disappointing at identifying high risk regions that can be better targeted with radiation.

Proton magnetic resonance spectroscopic imaging (MRSI), which can characterize regions of brain based on levels of various metabolites and other substances, is a candidate imaging modality for defining high risk regions that are not identified by standard MRI. Metabolites that can be evaluated include choline (Cho), a peak reflecting cell membrane synthesis that is elevated in highly proliferating, non-necrotic gliomas; creatine (Cr), an energy metabolite; and N-acetyl aspartate (NAA), a healthy neuronal biomarker. Early studies established that the MR spectra of GBMs differ significantly from normal brain, with increased levels of Cho, and decreased levels of NAA [14, 15]. MRSI has even been evaluated as a guide for RT planning when registered with the conventional MRI scans and treatment-planning CT scans [16-19]. Park et al. correlated the pattern of recurrence after RT with pre-treatment MRSI findings and noted that 8 of 9 patients with a growing enhancing lesion post-RT had recurrence in regions with high Cho/NAA [20]. Stadlbauer et al. demonstrated in gliomas that MRSI-derived Cho/NAA ratios frequently identified regions at higher risk of tumor beyond the T_{2w} signal abnormalities, and concluded that MRSI may be useful for delineating infiltrating nonenhancing tumor (beyond contrast enhancing tumor), which has clear implications for therapeutic planning [21, 22]. These previous studies have shown that MRSI can help identify GBMs, and potentially provide guidance for RT management. However, with poor resolution and limited field-of-views among a host of other difficulties, it has not been possible to exploit current clinical implementations of MRSI for use in RT treatment planning.

We have been using an advanced spectroscopic technique we have termed spectroscopic MRI (sMRI) which combines advanced technologies, such as 3D echo-planar spectroscopic imaging (EPSI), parallel acquisition (GRAPPA), and elliptical k-space encoding, with a 32-channel head coil. This acquisition obtains metabolite maps over approximately 65% of the brain coverage with high resolution in 15 minutes. This sMRI sequence was developed 10 years ago by Dr. Andrew Maudsley (University of Miami) and has been adapted in multiple clinical studies by numerous investigators in the world. We have been using sMRI in various clinical studies at Emory. Based on a recently completed early phase clinical study sponsored by NCI, “combining high resolution MRSI with 5-ALA to improve complete resection in GBM surgery” (IRB00051663), we learned that sMRI Cho/NAA showed significant correlations with tumor cell density in histological results ($\rho = 0.82$, $p < 0.001$). Based on early data from the control arm of on-going clinical study, “Quantitative MRSI to predict early response to SAHA therapy in new GBM management” (IRB00055973) and an institutional study “MRSI in the treatment planning and assessment of glioblastoma” (IRB0006545), sMRI Cho/NAA metabolite ratio map before RT treatment initiation matched well with contrast-enhancement at sites of tumor recurrence and exhibited an inverse relationship with progression-free survival (Cordova et al. This Neuro-Oncology article is enclosed in Appendix). Here, we will use our high-resolution, volumetric sMRI combined with standard MRIs that are routinely performed in the clinic for focal radiotherapy planning with escalated doses to select regions for the potential benefit of newly-diagnosed GBM patients.

2.0 OBJECTIVES

Our main goal is to establish the use of sMRI in addition to standard contrasted MRIs to guide dose-escalated radiation therapy for newly-diagnosed GBM patients. We have demonstrated that sMRI

has reached a level of technical development and validation where it is now feasible to use it for mapping regions of brain at high risk for tumor recurrence that may not have otherwise been appreciated by contrast-enhanced MRIs. In addition, the safety of this dose-escalated treatment approach will need to be demonstrated before it can be more widely adopted. Finally, we will also assess the efficacy of selectively targeting RT dose escalation with sMRI guidance in comparison to historical controls.

2.1 Primary Objectives

2.1.1 To determine the feasibility of using sMRI to guide dose-escalated RT for newly-diagnosed GBMs.

2.1.2 To determine the safety of using sMRI to guide dose-escalated RT for newly-diagnosed GBMs.

2.2 Secondary Objectives

2.2.1 To determine whether the progression free survival at 1 year with sMRI-guided, dose-escalated RT is improved for newly-diagnosed GBMs.

2.3 Exploratory Objectives

2.3.1 To determine whether sMRI-guided, dose-escalated RT increases the overall survival of patients with newly-diagnosed GBMs.

2.3.2 To determine whether sMRI data obtained after initiation of therapy (at 2 weeks after RT/TMZ start and prior to cycle 1 and 5 of adjuvant TMZ) will provide early evidence of GBM progression not seen on standard MRIs.

2.3.3 To determine whether performance on neurocognitive and quality-of-life (QOL) assessments in newly-diagnosed GBM patients treated with sMRI-guided, dose-escalated RT differ from historical controls.

3.0 PATIENT ELIGIBILTY

3.1 Minorities and Women: Subjects will be approximately representative of the demographics of the patient population at Emory University, Johns Hopkins, and University of Miami. This study is designed to include women and minorities, but is not designed to measure differences of intervention effects. While males and females will be recruited with no preference to gender, and based on the results of previous studies, we expect 50% of our accrual to be female. No exclusion to this study will be based on race. Minorities will actively be recruited to participate. However, based on previous enrollment, we expect about 27% of subjects to be minorities.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	3	+	3	=	6
Not Hispanic or Latino	12	+	12	=	24
Ethnic Category: Total of all subjects	15	+	15	=	30
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	2	+	2	=	4
Black or African American	3	+	3	=	6

Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	10	+	10	=	20
Racial Category: Total of all subjects	15	+	15	=	30
	(A1 = A2)		(B1 = B2)		(C1 = C2)

Accrual Rate: 1 pts/month/site

Total Expected Accrual: 30 Min 43 Max

Projected Start Date of Study: July 1, 2017

3.2 Subject Enrollment

Subjects will be recruited from newly-diagnosed GBM patients referred by neurosurgery, neuro-oncology or radiation oncology. Initial screening will be conducted/reviewed by clinical investigators and/or their trained designee (e.g. research nurse, research coordinator, etc.). Cases will be identified from the neurosurgery, neuro-oncology and radiation oncology outpatient clinics and neurosurgery inpatient service.

3.3 Patient Selection - Inclusion Criteria:

3.3.1 Patients must have a newly-diagnosed glioblastoma or gliosarcoma that has been confirmed pathologically by a board-certified neuropathologist.

3.3.2 Patients must be ≥ 18 years of age.

3.3.3 Patients must be able to have MRI scans.

3.3.4 Patient must have the following lab values ≤ 14 days prior to registration:

- WBC ≥ 3,000/μL
- ANC ≥ 1,500/μL
- platelet count of ≥ 75,000/μL
- hemoglobin ≥ 9.0 gm/dL (transfusion is allowed to reach minimum level)
- SGOT ≤ 2.0x UNL
- bilirubin ≤ 2 x UNL
- creatinine ≤ 1.5 mg/dL

3.3.5 Patients must have a life expectancy of ≥ 12 weeks.

3.3.6 Patients must have a Karnofsky Performance Status (KPS) ≥ 60.

3.3.7 Patients who are women of childbearing potential must have a negative pregnancy test documented ≤ 14 days prior to registration. This is not specific to dose escalation and is mandatory for standard care for patients being treated with radiation therapy. The cost of this test will be covered by standard of care.

3.3.8 Patients must be able to understand and provide written informed consent.

3.3.9 Both men and women, and members of all races and ethnic groups are eligible for this trial. Subjects will be approximately representative of the demographics of the referral base for the participating institutions.

3.3.10 Patient must be able to swallow capsules.

3.3.11 Patients must be willing to forego other cytotoxic and non-cytotoxic therapies against the tumor while being treated on this protocol.

3.4 Patient Selection - Exclusion Criteria:

3.4.1 Patients with pacemakers, aneurysm clips, neurostimulators, cochlear implants, metal in ocular structures, history of being a steel worker, or other incompatible implants which makes MRI safety an issue are excluded.

3.4.2 Patients that have any significant medical illnesses that in the investigator's opinion cannot be adequately controlled with appropriate therapy or would compromise the patient's ability to tolerate this therapy are excluded.

3.4.3 Patients with a history of any other invasive cancer (except non-melanoma skin cancer and excluding carcinoma in-situ), unless in complete remission and off of all therapy for that disease for ≥ 3 years, are ineligible.

3.4.4 Patients with an active infection or serious intercurrent medical illness are ineligible.

3.4.5 Patients receiving any other investigational agents are excluded.

3.4.6 Patients who have received prior cytotoxic, non-cytotoxic or experimental drug therapies for brain tumor are excluded.

3.4.7 Patients with a history of prior cranial radiation are ineligible.

3.4.8 Patients may not be enrolled on any other therapeutic trial for which they are receiving an anti-tumor therapy.

3.4.9 Patients with GBMs located in the following anatomical regions known to have magnetic susceptibility or poor signal will be excluded: mesial temporal lobe, orbitofrontal cortex, prefrontal cortex, medial frontal gyrus, brainstem, and cerebellum.

3.4.10 The maximum radiation target volume for 75 Gy is 65 cc (per NRG Oncology Guide). Patient may be excluded after the first sMRI scan if the 75 Gy volume is greater than 65 cc [we anticipate that contrast-enhancing tumor volume (residual tumor volume following tumor resection) would be less than 20 cc].

4.0 PATIENT SAFETY

4.1 Risks associated with dose-escalated RT (research-related). The risks of dose-escalated RT are expected to be similar to the risks of standard dose RT for newly-diagnosed GBMs although the incidence may be slightly increased. Incidence of serious toxicities will be carefully monitored in treated patients and is a primary objective of this study. Potential toxicities are listed below:

Acute

Expected adverse events include hair loss, fatigue, and erythema or soreness of the scalp. Potential acute toxicities include nausea and vomiting as well as temporary aggravation of brain tumor symptoms such as headaches, seizures, and weakness. Reactions in the ear canals and on the ear should be observed and treated symptomatically; these reactions could result in short-term hearing impairment. Dry mouth or altered taste has been occasionally reported.

Early Delayed

Possible early delayed radiation effects include lethargy and transient worsening of existing neurological deficits occurring 1-3 months after radiotherapy treatment.

Late Delayed

Possible late delayed effects of radiotherapy include radiation necrosis, leukoencephalopathy, endocrine dysfunction, and radiation-induced neoplasms. In addition, neurocognitive deficits, which could lead to mental slowing and behavioral change, are possible. Permanent hearing impairment and visual damage are rare. Cataracts can be encountered.

4.2 Risks associated with temozolomide (standard care). The risks related to the use of temozolomide are outlined in the information below regarding this drug.

4.3 Risks associated with sMRI (research-related). The risks of undergoing sMRIs are the same as those with conventional MRI. Movement or heating of metallic implants is a potential risk, and so subjects will be screened to exclude people with metallic implants, fragments, or pacemakers. Some individuals experience claustrophobic reactions in the scanner. Subjects will be informed of this prior to the study, but because it is difficult to predict who will have such a reaction, this is not a specific exclusion criterion. Any subject experiencing claustrophobia or discomfort during the study will be removed from the scanner immediately. There is no invasive component to this study and so discomfort, bruising, or infection is not a risk. Of note, there is NO injection of contrast agent.

There may be additional risks associated with scanning at 3T, which are addressed here:

1. Effect of the static field. The FDA has approved, for routine clinical use, scanners up to 4.0 T. The scanners that we will be using are FDA approved. However, the sMRI software package being used is experimental and has been provided through a master research agreement with the University of Miami. The software has been used for 10 years in numerous clinical studies all over the world. The 3D whole brain MR spectroscopic imaging sequence (EPSI/GRAPPA) and the analysis program MIDAS (Metabolic Imaging Data Analysis System) was developed by Dr. Andrew Maudsley (scientific PI of U of Miami) in 2006 [31-42]. The scan will be done in a 3T Siemens MR scanner (TIM/TRIO, Prisma, Skyra) or equivalent. Metabolite maps and their ratio maps will be calculated. Due to some degree of variability of Cho/NAA ratio between subjects, we will use the signal from each patient's contralateral normal-appearing white matter for normalization. The sMRI measurement has been validated in several previous studies including a recent Neuro-Oncology article (enclosed in Appendix). Dr. Shim (Emory) has used sMRI to guide tumor resection in GBM patients (NCI R21 CA186169; Emory IRB00051663), and has four on-going clinical studies (Emory IRB00055973, IRB0006545, IRB00073702, and IRB00086047) using sMRI. Dr. Maudsley ran several clinical studies (e.g., Miami IRB20020513, IRB20130481, IRB20020513, IRB20020787, IRB20090847, IRB20061005) with hundreds of human subjects (normal volunteers and patients). In addition, sMRI has been used worldwide by numerous investigators. Since 2016, we began to use a web-based sMRI clinical interface to extract information from MIDAS to visualize the metabolite maps for easier clinical workflow of the MIDAS results for busy clinicians. In addition, this web-based tool allows efficient data sharing among imaging scientists and clinicians for consultation and storage of de-identified data sets. sMRI studies will be obtained at the schedule outlined in section 7.1. There is no conclusive evidence for irreversible or hazardous bioeffects to acute, short-term exposures of humans up to 3.0 T. Studies have indicated some mild side-effects at 7.0 T, including nausea, vertigo, and metallic taste when moving into or out of the scanner. However, there is no evidence that this is either irreversible or harmful. If subjects experience unusual sensations, they will be withdrawn.

2. Effect of the gradient field. MRI operates by rapidly changing small additional fields, called gradients. By Faraday's induction law, a changing magnetic field will induce electrical currents in any conductor. Thus, rapid cycling of the gradient field can induce peripheral nerve stimulation. However, this is not substantially different at higher magnetic fields since the gradients are separate from the main magnet. If subjects experience peripheral nerve stimulation, e.g. tingling or twitching, they will be withdrawn.

3. Effect of the RF electromagnetic field. The fundamental principle of MRI is that protons are excited by sending in an RF pulse at their resonant frequency for the magnetic field. The FDA provides guidelines for the safe use of MR systems, which includes specific recommendations for how much RF power is safe. The "specific absorption rate", or SAR is the mass normalized rate at which RF power is coupled to biologic tissue and is typically indicated in units of watts per kilogram (W/kg) (NRCP, 1986). The FDA provides recommendations for two alternative safe levels of exposure to RF radiation during MR

procedures, primarily to control the risk of systematic thermal overload (heating) and local thermal injury. These are (FDA, 1988):

a. The exposure to RF energy below the level of concern is an SAR of 0.4 W/kg or less averaged over the body, and 8.0 W/kg or less spatial peak in any 1 g of tissue, and 3.2 W/kg or less average over the head; or

b. The exposure to RF energy that is sufficient to produce a core temperature increase of 1^{o}C and localized heating to no greater extent than 38^{o}C in the head, 39^{o}C in the trunk, and 40^{o}C in the extremities, except for patients with impaired systemic blood flow and/or perspiration.

We will adhere to the recommendations for the head, which is also monitored by Siemens' built in monitor.

5.0 PHARMACEUTICAL INFORMATION

5.1 Temozolomide (NSC# 362856) (Standard Care)

5.1.1 Chemical Name: 3.4-Dihydro-3-methyl-4-oxoimidazo-[5,1-d]-1,2,3,5-tetrazin-8-carboxamide. (Former name includes 8 carbamoyl-3-methylimidazo-5,1-d] 1.2.3.5-tetrazin-4-(3H)-one.

5.1.2 Other Names: Temodar

5.1.3 Mechanism of Action: Temozolomide is not directly active but undergoes rapid non-enzymatic conversion at physiologic pH to the reactive compound MTIC. The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O6 and N7 positions at guanine.

5.1.4 Pharmacokinetics: Temozolomide is rapidly and completely absorbed after oral administration; peak plasma concentrations occur in 1 hour. Food reduces the rate and extent of temozolomide absorption. Mean peak plasma concentration and AUC decreased by 32% and 9%, respectively, and Tmax increased 2-fold (from 1.1 to 2.25 hours) when temozolomide was administered after a modified high-fat breakfast. Temozolomide is rapidly eliminated with a mean elimination half-life of 1.8 hours and exhibits linear kinetics over the therapeutic dosing range. Temozolomide has a mean apparent volume of distribution of 0.4 L/kg (%CV=13%). It is weakly bound to human plasma proteins; the mean percent bound of drug-related total radioactivity is 15%.

5.1.5 Metabolism and Elimination: Temozolomide is spontaneously hydrolyzed at physiologic pH to the active species MTIC and to temozolomide acid metabolite. MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P450 enzymes play only a minor role in the metabolism of temozolomide and MTIC. Relative to the AUC of temozolomide, the exposure to MTIC and AIC is 2.4% and 23%, respectively. Approximately 38% of the administered temozolomide total radioactive dose is recovered over 7 days: 37% in urine and 0.8% in feces. The majority of the recovery of radioactivity in urine is as unchanged temozolomide (5.6%), AIC (12%), temozolomide acid metabolite (2.3%), and unidentified polar metabolite(s) (17%). Overall clearance of temozolomide is ~5.5 L/hr/m.

5.1.6 Pharmaceutical Data: Temozolomide (Temodar®) is supplied in white, opaque, preservative free, 2-piece, hard gelatin capsules in the following p.o. dosage strengths: 5 mg, 20 mg, 100 mg, and 250 mg. Capsules should not be opened or chewed. If capsules are accidentally opened or damaged, inhalation or contact with the skin should be avoided. Each capsule contains drug substance in combination with lactose, anhydrous NF, colloidal silicon dioxide NF, sodium starch glycolate NF, tartaric acid

NF, and stearic acid NF. The capsule shells contain gelatin NF, titanium dioxide USP, and sodium lautyl sulfate NF.

5.1.7 Storage and Stability: Temozolomide should be stored at room temperature. The capsules are packaged in 30 cc 28 mm-48-Type I amber glass bottles (30 capsules/bottle) and should be stored between 2 and 30 degrees Centigrade. Capsules are stable for at least 30 months when stored in amber glass bottles at this temperature.

5.1.8 Route of Administration: Temozolomide should be taken by mouth after fasting from solid food for 2 hours.

5.1.9 Drug Source: Temozolomide has been approved by the FDA for refractory anaplastic astrocytomas and newly-diagnosed glioblastoma. Patients or third party payers must pay for the Temozolomide. If the patient is unable to pay for the drug, he/she may seek assistance through Schering-Plough Pharmaceutical's Commitment to Care Program.

5.1.10 Adverse Reactions: The most common reactions to temozolomide include nausea, vomiting, headache, fatigue and hematologic effects. These events are usually mild to moderate. Nausea and vomiting is usually readily controlled with antiemetics. Myelosuppression (thrombocytopenia and neutropenia) is the dose-limiting side effect. It usually occurs within the first few cycles of therapy and is not cumulative. In prior studies, myelosuppression occurred late in the treatment cycle and returned to normal, on average, within 14 days of nadir counts. Other less common side effects may include somnolence or insomnia, anorexia, constipation, diarrhea, weight loss, abdominal pain, rash, pruritis, anxiety, depression, pain when swallowing, hyperglycemia, epistaxis, empyema, pulmonary edema, respiratory insufficiency, respiratory arrest, cardiac arrest, toxic hepatitis, liver or kidney abnormalities and/or breast tumors.

<u>The information provided in the consent includes the following:</u>

Likely side effects:

- Nausea and vomiting, especially on the first day of each cycle. It may be necessary to use medication to prevent this.
- Constipation
- Loss of appetite
- Lowering of your blood counts, which may result in low white blood cells, platelets, and red blood cells. If you have very low white blood cells, you are at a higher risk for infections. Lung infections have occurred in patients receiving daily treatment with temozolomide combined with radiation and steroids (e.g., dexamethasone). To prevent this, your doctor may ask you to take preventative medication during this time. Apart from this, if you develop fever it may be necessary to treat you with antibiotics. Low platelets may result in a bleeding tendency, if necessary this can be treated with platelet transfusions. Low red blood cells can also be treated with transfusions.
- Fatigue, lethargy, insomnia, weakness
- Headache
- Hair loss and rash

Less Likely side effects:

- Kidney problems and high blood sugar
- Abnormal liver tests and diarrhea

- Recently, cases of hepatic injury, including fatal hepatic failure, have been observed in patients enrolled in clinical studies utilizing the agent temozolomide. In addition, it was noted that liver toxicity may occur several weeks or more after initiation of treatment or after temozolomide discontinuation. Refer to the package insert for additional information on adverse events observed to date.

A tabular summary of side effects follows:

	Concomitant Phase RT Alone (n=285)				Concomitant Phase RT+TMZ (n=288)*				Maintenance Phase TNZ (n=224)			
	All		Grade ≥ 3		All		Grade ≥ 3		All		Grade ≥ 3	
Subjects Reporting any Adverse Reaction	**258**	**(91)**	**74**	**(26)**	**266**	**(92)**	**80**	**(28)**	**206**	**(92)**	**82**	**(37)**
Body as a Whole - General Disorders												
Anorexia	25	(9)	1	(< 1)	56	(19)	2	(1)	61	(27)	3	(1)
Dizziness	10	(4)	0		12	(4)	2	(1)	12	(5)	0	
Fatigue	139	(49)	15	(5)	156	(54)	19	(7)	137	(61)	20	(9)
Headache	49	(17)	11	(4)	56	(19)	5	(2)	51	(23)	9	(4)
Weakness	9	(3)	3	(1)	10	(3)	5	(2)	16	(7)	4	(2)
Central and Peripheral Nervous System Disorders												
Confusion	12	(4)	6	(2)	11	(4)	4	(1)	12	(5)	4	(2)
Convulsions	20	(7)	9	(3)	17	(6)	10	(3)	25	(11)	7	(3)
Memory Impairment	12	(4)	1	(< 1)	8	(3)	1	(< 1)	16	(7)	2	(1)
Disorders of the Eye												
Vision Blurred	25	(9)	4	(1)	26	(9)	2	(1)	17	(8)	0	
Disorders of the Immune System												
Allergic Reaction	7	(2)	1	(< 1)	13	(5)	0		6	(3)	0	
Gastrointestinal System Disorders												
Abdominal Pain	2	(1)	0		7	(2)	1	(< 1)	11	(5)	1	(< 1)
Constipation	18	(6)	0		53	(18)	3	(1)	49	(22)	0	

Diarrhea	9	(3)	0		18	(6)	0		23	(10)	2	(1)
Nausea	45	(16)	1	(< 1)	105	(36)	2	(1)	110	(49)	3	(1)
Stomatitis	14	(5)	1	(< 1)	19	(7)	0		20	(9)	3	(1)
Vomiting	16	(6)	1	(< 1)	57	(20)	1	(< 1)	66	(29)	4	(2)
Injury and Poisoning												
Radiation Injury NOS	11	(4)	1	(< 1)	20	(7)	0		5	(2)	0	
Musculoskeletal System Disorders												
Arthralgia	2	(1)	0		7	(2)	1	(< 1)	14	(6)	0	
Platelet, Bleeding and Clotting Disorders	3	(1)	0		11	(4)	8	(3)	19	(8)	8	(4)
Thrombocytopenia Psychiatric Disorders												
Insomnia	9	(3)	1	(< 1)	14	(5)	0		9	(4)	0	
Respiratory System Disorders												
Coughing	3	(1)	0		15	(5)	2	(1)	19	(8)	1	(< 1)
Dyspnea	9	(3)	4	(1)	11	(4)	5	(2)	12	(5)	1	(< 1)
Skin and Subcutaneous Tissue Disorders												
Alopecia	179	(63)	0		199	(69)	0		124	(55)	0	
Dry Skin	6	(2)	0		7	(2)	0		11	(5)	1	(< 1)
Erythema	15	(5)	0		14	(5)	0		2	(1)	0	
Pruritus	4	(1)	0		11	(4)	0		11	(5)	0	
Rash	42	(15)	0		56	(19)	3	(1)	29	(13)	3	(1)
Special Senses Other, Disorders												
Taste Perversion	6	(2)	0		18	(6)	0		11	(5)	0	

*One patient who was randomized to RT only arm received RT+temozolomide.
RT+TMZ=radiotherapy plus temozolomide; NOS=not otherwise specified.
Note: Grade 5 (fatal) adverse reactions are included in the Grade ≥ 3 column.

Source: http://www.rxlist.com/temodar-drug.htm

Please refer to the package insert for additional information.

5.1.12 Summary of Background: Temozolomide (TMZ, Temodar®) is an orally administered alkylating agent with activity against malignant gliomas. It is a prodrug that

spontaneously converts at physiologic pH to the active alkylating agent 5-(3-methyltriazen-1-yl)imidazole-4-carboximide (MTIC) under physiologic conditions. The cytotoxicity of temozolomide is principally mediated through methylation of DNA at the O6 position of guanine (Investigational Brochure-Temozolomide).

5.1.13 Effects of Temozolomide on Glioma Cells: The alkylating agents temozolomide and BCNU are mainstays of adjuvant therapy for malignant glioma. Temozolomide functions primarily as an alkylating agent inducing methylation of the O-6 residues of guanine, generating mismatch pairing and leading to futile DNA mismatch repair possibly with strand breaks [22]. The biologic consequences of these molecular effects of the agent include alterations in the cell cycle kinetics and induction of apoptosis. These effects have been demonstrated in a variety of malignant cells particularly in hematopoietic malignancies in vitro and are believed to be the mechanisms of action of the agent in humans as well. Studies focusing on the effects of this agent against gliomas are fewer but have shown that mechanisms similar to those for hematogenous malignancies are operative in this tumor type as well. The cell cycle changes induced by temozolomide are of particular relevance to the rationale for this trial proposal. In vitro studies have shown that temozolomide induces a prolonged G2/M arrest associated with an increase in p53 and p21 protein levels [23]. The cells showed decreased proliferation by a clonogenic assay and exhibited features of senescence in cells with wild type p53; in cells with deficient p53, a transient G2/M arrest was followed by induction of apoptosis possibly by mitotic catastrophe. The induction of G2/M arrest in glioma cells has been associated with Chk1 activation and inhibitory phosphorylation of cdc25c and cdc2 inactivating the cyclin B1/cdc2 complex necessary for progression of the cell cycle from G2 to M phase [23]. Pharmacologic inhibition of Chk1 or of the p38MAPK pathways sensitized glioma cells to temozolomide-induced apoptosis. These data suggest the biologic effects of temozolomide are related to the cell cycle changes and apoptosis induced by this agent.

5.1.14 Temozolomide Clinical Experience:

5.1.14.1 Adult Phase I/II Studies of Temozolomide: As a result of the encouraging preclinical data, a phase I trial with temozolomide was conducted by the Cancer Research Campaign (CRC) Phase I-II Clinical Trials Unit in the United Kingdom (UK). This trial was conducted in two phases. The first 51 patients were treated with temozolomide administered either orally or intravenously, using a single-dose schedule. The subsequent 133 patients received a 5-day oral schedule. Cycles of temozolomide were repeated every 28 days. The total doses administered over 5 days were 750, 900, 1000, and 1200 mg/m2. Dose-limiting toxicity was myelosuppression and the maximum tolerated dose (MTD) was defined at 1000 mg/m2. Only one course was administered at 1200 mg/m2 and resulted in Grade 4 myelotoxicity. The nadir for hematologic parameters of neutropenia or thrombocytopenia usually occurred at 21 days or later following the first dose of each cycle with recovery to at least Grade 1 by 28 days. Overall, Grades 1 to 4 anemia, leukopenia and thrombocytopenia occurred in 17%, 12% and 9% of the evaluable courses, respectively. Grade 3 and 4 thrombocytopenia and leukopenia occurred in 4% and 3% of the evaluable courses, respectively, and were primarily seen at doses >1000 mg/m2. Mild to moderate (Grades 1 and 2) nausea and vomiting were reported in 28% of the evaluable courses at doses up to 750 mg/m2, inclusive. These events were usually limited to day 1 and were readily controlled with standard antiemetics. At higher doses (>750-1200 mg/m2), Grades 3 and 4 nausea and vomiting were seen in 9% of the evaluable courses. The percentages of adverse events reported included: constipation (10%), headache (5%), rash (4%), renal disorders (3%), elevated hepatic enzymes (3%), alopecia

(2%), diarrhea (2%), itching and burning (<1%), lethargy (<1%), altered consciousness (<1%), and esophagitis (<1%).

Based on this study, the recommended starting dose of temozolomide for Phase II trials was 150 mg/m2/day, orally, once a day for 5 days (total dose 750 mg/m2) for the first cycle with subsequent dose escalation to 200 mg/m2/day, once a day for 5 days (total dose 1000 mg/m2) in the absence of myelotoxicity. The CRC subsequently conducted three Phase II trials of temozolomide in patients with documented progression who had either high-grade glioma, advanced malignant melanoma, or low-grade non-Hodgkin's lymphoma (Investigator's Brochure -Temozolomide. Schering Plough) [24]. Activity in these tumor types was reported in all three studies.

5.1.14.2 Temozolomide for Recurrent Malignant Gliomas: A randomized trial was conducted to compare the efficacy of temozolomide with procarbazine in patients with GBM at first relapse [25]. Patients were randomized to temozolomide administered orally once daily for 5 days at a starting dose of either 200 mg/m2/day (no prior chemotherapy) or 150 mg/m2/day (prior chemotherapy) every 28 days, or procarbazine 150 mg/m2/day for 28 days every 56 days. Temozolomide performed better than procarbazine in terms of progression free survival (PFS), overall survival and objective response to treatment, although the differences were modest. The 6-months progression free survival (6M-PFS) was 21% in the temozolomide group compared to 8% in the procarbazine group (p=0.008). In comparison, the 6M-PFS for GBM patients in 8 negative phase II trials from MD Anderson Cancer Center was only 15% (Wong et al, 1999). Median PFS was 2.89 months with temozolomide compared to 1.97 months with procarbazine. The median overall survival for the intent-to-treat population of temozolomide recipients with GBM (7.34 months) was longer than for procarbazine recipients (5.82 months). 5.4% of TMZ patients and 5.3% of procarbazine patients had a PR, while 40.2% of temozolomide patients and 27.4% of procarbazine patients had SD. The overall response rate (PR + SD) was 45.6% in the temozolomide group and 32.7% in the procarbazine group [25]. The most common grade 3 or 4 toxicities in the temozolomide group were headache (10%), thrombocytopenia (7%), neutropenia (4%), fatigue (3%), vomiting (4%) and nausea (4%). Quality of life was significantly better in the temozolomide group.

In another trial, the efficacy of temozolomide for AA at first relapse was evaluated in a phase II study consisting of 162 patients [24]. Temozolomide was administered once daily for 5 days every 28 days at a starting dose of either 200 mg/m^2/day in patients who had received no prior chemotherapy or 150 mg/m^2/day in patients who had received prior chemotherapy. 8% of patients experienced a CR, 27% had a PR and 26% had SD, producing an overall response rate of 61%. 6M-PFS was 46% compared to 31% from the MD Anderson database of 8 negative phase II trials for recurrent AA [26]. The MTP was 5.4 months and the median survival was 14.6 months. Both overall response and maintenance of progression-free status were associated with health-related quality-of-life benefits, independent of steroid use [27]. Based on these results, temozolomide was approved by the FDA for the treatment of recurrent AA in 1999. Over the past few years TMZ has become the treatment of choice for patients with recurrent malignant gliomas. However, the responses tend to be modest and short-lived. Several different schedules have been evaluated but none have been clearly superior to the standard regimen [28]. There is significant interest in combining temozolomide with other agents to increase its effectiveness.

5.1.14.3 Temozolomide for Newly Diagnosed Glioblastomas: Based on preclinical evidence that temozolomide has additive activity when combined with radiation therapy (RT) [29], Stupp et al., conducted a phase II study in which 64 patients with newly-diagnosed GBM were treated with temozolomide 75 mg/m2/d x 6 weeks with concomitant fractionated RT (60 Gy; 2 Gy x 5d/wk for 6 weeks) followed by temozolomide adjuvant therapy (200 mg/m2/d x 5 days every 28 days for 6 cycles) [30]. This regimen was well-tolerated and the median survival of 16 months was significantly better than the historic median survival of GBM of 9-12 months. As a result of these promising results, the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) conducted a randomized phase III trial (EORTC Study 26981) of 573 patients comparing this regimen of concomitant and adjuvant temozolomide and radiation therapy to radiation therapy alone in patients with newly-diagnosed GBM [1, 2]. This study showed that the combination of temozolomide with radiation therapy was well-tolerated and resulted in a survival benefit. The median survival of patients treated with temozolomide + radiation therapy was increased compared to radiation therapy alone (14.6 mo v.12.1 mo; p< .0001). In addition, patients receiving temozolomide with radiation therapy had a significantly higher percentage of patients surviving at two years (26%) than patients receiving radiation therapy alone (10%). This study conclusively demonstrated for the first time that adjuvant chemotherapy is of benefit in patients with GBM.

6.0 REGISTRATION PROCEDURES

6.1 Patient registration

6.1.1 Pre-registration: Emory: Pre-registration must be completed and sent to the Central Subject Registrar and to the Office of Clinical Research. After receipt of the pre-registration form and confirmation of a valid, signed informed consent form/HIPAA authorization form, the Winship Clinical Trials office will assign a patient study number.

Participating site(s): After each subject signs consent, the Central Subject Registration form is to be completed and sent to Winship Clinical Trial Office within 48 hours of consent. This form, along with the valid, signed informed consent form/HIPAA authorization form, is to be faxed or emailed to Winship's Central Subject Registrar per instructions on the form. The Winship Clinical Trials office will assign a patient study number to these patients.

6.1.2 Patient study number assignment/confidentiality: Subjects will be assigned a coded designation according to the site at which the subject signs consent. (QTR01, the site initials, and enrollment number entered on study will be assigned QTR01EM001). Actual names, contact information and relationship to this code will be kept secure in the Winship Clinical Research Office.

6.1.3 Registration: Participating site(s): The Eligibility checklist is to be printed from OnCore and verified by 2 people, of which one must be a clinical investigator or co-investigator. The completed and signed eligibility checklist along with all redacted supporting source documentation must be submitted to the Winship Multi-site Coordinator or designee (fax 404-778-5033) within 14 days after pre-registration but no later than 2 business days from scheduled treatment visit. Eligibility will be confirmed by a clinical principal or co-investigator and the Multi-site Coordinator or designee within 2 business days of receipt of all eligibility documentation and confirmation will be sent to the participating site along with cohort assignment, if subject meets criteria. Once eligibility is confirmed, then patient will be registered and scheduled for appropriate appointments.

6.2 Investigator registration/requirements: Prior to recruitment of subjects to this trial,

investigators must complete training as required per institutional IRB for participation on clinical trials along with protocol specific training. Each investigator must be registered (FDA Form 1572 with original signature, current CV, Supplemental Investigator Data Form with signature, and Financial Disclosure Form with original signature) and maintain an "active" registration status.

6.3 Site registration/requirements

6.3.1 IRB: IRB approval for this study will be obtained and maintained for the duration of the study treatment and follow up times. Documentation of initial and continued IRB approval will be submitted to the Winship Clinical Research Office.

6.3.2 Radiation Therapy: All institutions participating on this trial will need to be credentialed to use IMRT on the study. Previous credentialing for cooperative group and/or consortium trials (eg. RTOG/NRG, ABTC, etc.) is acceptable.

6.3.3 Neurocognitive certification: All study staff that will be administering neurocognitive testing will need to be certified as specified in section 7.2.1. Previous certification for administering these tests on other studies is acceptable after submission of documentation to the Winship Clinical Research Office.

7.0 STUDY EVALUATIONS

7.1 Summary of Active Monitoring Schedule

Tests and procedures	Pre-reg	≤21 days prior to start of therapy	Chemoradiation (RT+TMZ, 6 weeks)						Adjuvant TMZ Cycles 1-12[9] ≤7 days before each cycle	After completion of adjuvant TMZ
			1	2	3	4	5	6		
Path diagnosis	X									
H&P, weight, KPS		X		X[7]		X[7]		X[7]	X	X[12]
Height		X								
Neuro exam		X		X[7]		X[7]		X[7]	X	X[12]
Adverse event assessment		X	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X	X[12]
Rad Onc consult	X[3]									
Neuro Onc consult	X[3]									
CBC w/diff		X	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X	
CMP[1]		X	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X	
MRI w/contrast[2]		X							X[10]	X[12]
sMRI (Research)		X			X[8]					
Steroid/anti-Sz/chemo med documentation		X	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	X	
Neurocognitive and QOL assessments (Research)		X							X[11]	X[11]
Pregnancy test		X[4]								

[1] Comprehensive Metabolic Panel (CMP) - Sodium, potassium, bicarbonate, chloride, BUN, creatinine, calcium, glucose, total bilirubin, SGOT (AST), SGPT (ALT), alkaline phosphatase, albumin, total protein (Standard care). The frequency of CMP will be up to the treating physician's discretion.

[2] Standard diagnostic brain MRI w/ & w/o contrast.

[3] Either Rad Onc or Neuro Onc consultation needs to be completed at pre-registration. The other consultation can be completed ≤ 21 days before initiation of therapy. (Standard care)

[4] To be completed only in women of childbearing potential. Test obtained ≤ 21 days prior to registration. May use a test result obtained for other clinically warranted indications if the test date is within the above time window. (Standard care)

[5] To be assessed during chemoradiation. (Standard care)

[6] Document steroids, anti-seizure, and temozolomide meds weekly during cycle 1. (Standard care)

[7] To be performed every other week starting on week 2 of chemoradiation. (Standard care)

[8] sMRI should be performed during the 3rd week of chemoradiation (after 10 fractions up to 15 fractions, or prior to receiving the 16th fraction). (Research)

[9] TMZ cycles will be determined at treating physician's discretion. (Standard care)

[10] Standard diagnostic brain MRI w/ & w/o contrast to be completed ≤ 7 days prior to starting each odd number cycles. (Standard care)

[11] To be performed every 6 months (± 1 month) after pre-cycle 2 assessment until progression (will attempt be obtain final assessment only if date of progression is > 3 months from previous assessment) or until approximately 2 years after pre-cycle 2 assessment (maximum of 4 additional assessments). (Research activity)

[12] Follow-ups and standard diagnostic brain MRI w/ & w/o contrast to be completed 1 month (± 2 weeks) after completion of adjuvant TMZ chemotherapy and then every 3 months (± 2 weeks) (until approximately 2 years after RT). Subsequent followups/MRIs will be determined by treating physician. (Standard care)

7.2 Neurocognitive Testing (Research)

7.2.1 Certification: All members of the study team that will be administering the neurocognitive tests will need to be certified. Previous certification in the prior two years is acceptable. If necessary, refresher certification will be provided to any study personnel that are uncertain about test administration even if they have undergone certification within the past two years. This certification process will be under the direction of Dr. Drenna Waldrop-Valverde (404-712-9487).

7.2.2 Timing of Assessments: Evaluations will be performed ≤ 21 days before starting chemo radiation (may complete as late as day 1 of RT/TMZ), ≤ 7 days prior to initiation of cycle 1 of adjuvant TMZ, and every 6 months (± 1 month) thereafter for approximately 2 years after RT completion. (See Table in section 7.1) In case of disease progression within 2 years from cycle 1, patients will be re-tested for the final time only if previous assessment was > 3 months from date of progression.

7.2.3 Tests to be administered: Hopkins Verbal Learning Test – Revised (HVLT-R), Controlled Oral Word Association test from the Multilingual Aphasia Examination (COWA), Trail Making Test A and B, and Recall and Recognition of Word List encoded from the HVLT-R.

7.3 Quality-of-Life (QOL) Testing (Research): These questionnaires have been validated in the brain tumor population and used in other large, prospective studies for this patient population, providing a good potential comparison group for patients on this study. Subjects can opt out of this assessment if they so choose.

7.3.1 Timing of Assessments: Evaluations will be performed ≤ 21 days before starting chemo radiation (may complete as late as day 1 of RT/TMZ), ≤ 7 days prior to initiation of cycle 1 of adjuvant TMZ, and every 6 months (± 1 month) thereafter for

approximately 2 years after RT completion. (See Table in section 7.1) In case of disease progression within 2 years from cycle 1, patients will be re-tested for the final time only if previous assessment was > 3 months from date of progression.

7.3.2 QOL Questionnaires: EORTC Quality of Life Questionnaire-Core 30/Brain Cancer Module-20 (EORTCQLQ30/BN20); M. D. Anderson Symptom Inventory Brain Tumor Module (MDASI-BT)

8.0 TREATMENT PLAN

8.1 Radiation Therapy

8.1.1 Timing of Radiotherapy: RT must begin ≤ 50 days from resection. Daily oral temozolomide will start together with the first day of radiation. RT may start on M-Th. Temozolomide is given every day during course RT (M-Su) up to a total of 42 doses (may halt as early as 40 doses if RT is completed prior to 42 dose course).

8.1.2 Certification: IMRT or VMAT technique will be used for all cases. To utilize IMRT or VMAT on this study, proper credentials are required. Previous certification by the Radiological Physics Center (RPC) for use of IMRT or VMAT on another protocol is acceptable.

8.1.3 Radiation Energy: Minimum photon energy is 6 MV with IMRT or VMAT plans designed and verified by simulation. Minimum SSD or SAD is 80 cm.

8.1.4 Target Volume Definitions

8.1.4.1 ICRU Definitions: ICRU terminology defines the gross tumor volume (GTV) and clinical tumor volume (CTV) as the extent of gross and microscopic disease, respectively. The planning target volume (PTV) is geometrically, not anatomically, defined and is an expansion of the CTV intended to account for uncertainty in patient positioning.

8.1.4.2 GTV1: This volume encompasses the edema and T2 hyper-intensity surrounding the main tumor mass as estimated from the T2-weighted or FLAIR postoperative MRI scan. By definition, this volume will also include all of GTV2 and GTV3.

8.1.4.3 GTV2: This volume encompasses the post-operative resection cavity (as seen on both T1 post-contrast and T2 sequences) plus any contrast enhancing volume or mass that is considered likely to represent residual enhancing tumor. Fusion of the pre-operative T1-weighted, contrast enhanced MRI is also encouraged to allow comparison with RT planning MRI to ensure inclusion of the pre-operative localized contrast-enhancement or mass within the GTV2. GTV2 may be modified to account for anatomic changes post-operatively to respect the anatomic structures within which the tumor was initially bound. For example, if a large tumor with mass effect has been resected, anatomically distant brain parenchyma may post-operatively be within the fusion-generated GTV2. GTV2 may be modified to exclude geographically distant brain parenchyma in this situation. By definition, this volume will also include all of GTV3.

8.1.4.4 GTV3: This volume will encompass the volume of Cho/NAA ratio ≥ 2x contralateral normal brain from the pre-RT sMRI scan plus any T1 contrast enhancing residual seen on the post-operative and pre-RT diagnostic brain MRIs. <u>This can be modified at discretion of treating physician(s) with consultation with other investigator(s)</u>, as needed.

8.1.4.5 CTV1: This volume includes GTV1 plus a 5 mm margin (at discretion of treating radiation oncologist). CTV1 may be anatomically confined, for

example, to within the calvarium. However, high grade gliomas can invade most intracranial structures and should not be confined by the tentorium or an arbitrary midline. This expansion can be excluded from the brainstem and optic chiasm/nerves at the treating physician's discretion if there is not felt to be risk of extension into these regions. By definition, this volume will completely encompass CTV2 and CTV3

8.1.4.6 CTV2: This volume includes GTV2 plus a 5 mm margin cropped to be completely encompassed by CTV1. By definition, this volume will also completely encompass CTV3.

8.1.4.7 CTV3: This volume includes GTV3 with no margin cropped to be completely encompassed by CTV1 and CTV2.

8.1.4.8 PTV1, PTV2 and PTV3: These volumes are uniform 3 mm expansions of CTV1, CTV2 and CTV3, respectively, to account for set-up uncertainty. It is not confined anatomically, with the exception of cases that would otherwise exceed normal tissue tolerance (see below for normal tissue dose constraints).

8.1.5 Prescription Isodose: The prescription isodose surface (100% dose curve) shall encompass a minimum of 95% of the PTV. In addition, the entire PTV shall be encompassed by the 95% isodose surface. The dose uniformity guidelines below must be met for all PTVs.

8.1.6 Tissue Heterogeneity: Calculations to take into account the effect of tissue heterogeneities are required.

8.1.7 Target Dose

8.1.7.1 Prescription dose for IMRT or VMAT techniques: Patients will be treated with a simultaneous in-field boost plan. PTV1 will receive 5010 cGy in 30 fractions of 167 cGy per day. PTV2 will simultaneously receive 6000 cGy in 30 fractions of 200 cGy per day. PTV3 will simultaneously receive 7500 cGy in 30 fractions of 250 cGy per day.

8.1.8 Dose Uniformity: The entire PTV shall be encompassed within the 95% isodose surface as evaluated by dose volume histogram. Does plan should try to achieve 99% of PTVs receive at least 95% of prescription dose unless coverage needs to be compromised to meet OAR constraints. In addition, no part of PTV3 shall receive more than 110% of the prescription dose (8250 cGy) and ≤ 10% of PTV3 shall receive more 105% of the prescription dose (7875 cGy).

8.1.9 Time Considerations: Patients will receive one treatment per day, five days per week. All fields will be treated each day. At least two fractions must be given during the first week of treatment.

8.1.10 Simulation: A conventional CT or MRI simulator will be used for patient simulation. The patient shall be treated in the supine position with a head-holding device that is transparent to x-rays (thermoplast masks, bite-block, etc.) to ensure adequate immobilization and reproducible daily setup of the treatment position.

8.1.11 Organs at Risk (OARs): When possible, without shielding GTV/CTV/PTV, the listed OARs should not receive more than the following doses:

brainstem ≤ 6000 cGy (95% of volume ≤ 5400 cGy)

optic nerves and optic chiasm ≤ 5500 cGy (99% of volume ≤ 5400 cGy)

retina ≤ 4000 cGy

cervical spinal cord ≤ 5000 cGy

lens ≤ 500 cGy

No more than 1% or 1 cc of unspecified tissue outside of the PTV should receive > 110% of the prescribed dose. Planning organ at risk volumes (PRVs) are recommended such that the spinal cord should have 3-dimensional expansion by 5 mm while the optic nerves, optic chiasm, and brainstem should have a 3-dimensional expansion by at least 1 mm. The dose constraints to the PRVs will be the same as for their respective OARs as outlined above. Higher priority OARs (higher priority than PTV coverage) include: brainstem, cervical spinal cord, optic nerves and optic chiasm as defined above. In these cases, PTV coverage will be compromised to keep OARs dosing within the above constraints. Lower priority OARs (lower priority than PTV coverage) include: parotids (entire volume) ≤ 10 Gy; oral cavity/lips/nasal cavity mean dose ≤ 20 Gy and < 1% receiving > 40 Gy; inner/middle ear mean dose ≤ 30 Gy and < 1% receiving > 50 Gy; and lens/retina as defined above. For example (for the lower priority OARs), typically 100% of the parotids should receive ≤ 10 Gy, but tumor coverage is of higher priority leading to cases where this dose may exceed 10 Gy.

8.1.12 IMRT or VMAT Plan Verification: The monitor units used for the IMRT or VMAT plan must be independently checked prior to the patient's first treatment. Measurements in a QA phantom can suffice for a check as long as the plan's fluence distribution can be re-computed for a phantom geometry.

8.1.14 Verification of treatment set-up: Orthogonal pair of reference films is sufficient for the initial and subsequent ports. While setup checks using orthogonal pairs are required at least weekly, use of daily kV OBI equipment to check and reset the isocenter is encouraged.

8.2 Treatment schedule

Concurrent RT/TMZ (~42 days + 4-6 week break)

Agent	Dose level	Route	Schedule
RT	75 Gy	Ext. beam	1x/day on M-F for 30 txs, may start M-Th
TMZ	75 mg/m^2	Oral	1x/day, 7 days/week on M-Su during RT[1]
4-5 week treatment break after completion of RT[2]			

Adjuvant TMZ, Cycles 1-12 (28 days) at the discretion of the treating physician (Standard)

[1] TMZ will start with 1st RT treatment. If RT is extended due to holidays or unplanned breaks, up to 7 additional doses of TMZ can be given (up to 49 days). While a typical TMZ course will involve 42 days, the last dose will be on the last day of RT. This may result in as few as 40 days of TMZ if the first day of RT falls on a Monday and there are no breaks resulting in completion of RT on Friday of the 6th week of therapy.

[2] Rest period may extend longer depending on dose limiting toxicity.

8.2.1 Administration of temozolomide (Standard care): TMZ dosing will be based on actual body weight. The smallest TMZ capsules are 5 mg. Therefore, patient doses of TMZ will be rounded to the nearest interval of 5 mg. TMZ capsules should be taken preferably in the morning for the concurrent RT/TMZ course with up to 200 mL of water on an empty stomach one hour before or two hours after food. Evening/night dosing of TMZ right before bedtime can be used during adjuvant TMZ therapy (cycles 1-12). If possible, TMZ should be taken 2-3 hours before RT but the timing of the dose is left to the discretion of the treating physician. Since TMZ may cause nausea, an

appropriate anti-emetic (e.g., 1 mg dose of granisetron or 4-8 mg of ondansetron) should be given once a day before the TMZ. On days when patients do not have radiation, they should continue to take temozolomide in the morning on an empty stomach at approximately the same time as radiation days.

8.2.2 Pneumocystis jirovecii pneumonia [PJP (PCP)] prophylaxis: Since low dose TMZ is immunosuppressive and associated with an increased risk of PJP, all subjects must receive prophylaxis during RT with concomitant TMZ. Prophylaxis must be continued for at least two weeks after the completion of RT. The choice of prophylactic medications will be left to the discretion of the treating physician. Options include atovaquone and dapsone. Based on NABTC experience, trimethoprim/sulfamethoxazole (Bactrim) appears to contribute to myelosuppression in patients on chemotherapy. Thus, this agent is not preferred. It may be used in patients who cannot tolerate other options listed above, although the final decision will be left to the discretion of the treating physician. The decision to continue PJP prophylaxis beyond the time points noted is left to the discretion of the treating physician. Subjects who continue to demonstrate lymphopenia after the continuous daily dosing of TMZ has been discontinued should be considered for ongoing prophylaxis.

8.2.3 Treating physician: Subjects will be treated as outpatients. All treatments are prescribed/given by study physicians. During RT, subjects will be evaluated on a weekly basis. During the adjuvant TMZ phase, subjects will be evaluated on a q28 day basis.

9.0 DOSAGE MODIFICATION BASED ON ADVERSE EVENTS

Follow modifications based information given below until individual treatment tolerance can be ascertained. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. If an adverse event is not covered below, doses may be reduced or held at the discretion of the treating physician for the subject's safety. Dose adjustments for hematological adverse event are based on the blood counts obtained in preparation for the day of treatment.

9.1 Radiation therapy: While interruption due to acute radiation adverse events is unlikely given the type of radiation planned, individual reasons, such as major worsening of neurological or mental status or any other medical condition may preclude the continuation of radiotherapy. Resuming RT in this setting will be at the discretion of the treating physician. For example, cranial irradiation can be withheld for CNS adverse events or toxicity ≥ grade 3 attributable to radiotherapy. The overall time of interruption and overall time of radiotherapy must be recorded. If radiotherapy is interrupted, actions regarding dosing on concomitant temozolomide are described below:

- If administration of TMZ is interrupted, RT will proceed normally and no catch up days of TMZ will be given after end of RT.
- If RT extends beyond 42 days because of delays, TMZ may be extended to a maximum of 49 days (at discretion of treating physician). If RT is still not completed, additional TMZ beyond 49 days is not permitted.
- If RT is stopped for an adverse event felt clearly related to radiation and not TMZ, concomitant TMZ should be continued as per protocol unless PD occurs.

9.2 Concomitant treatment: If the attribution of the adverse event can be clearly linked to TMZ, these dose modifications should be followed.

9.2.1 Dose modification criteria for TMZ during RT (chemoradiation phase): Concurrent TMZ dose for cycle 1 is 75 mg/m2 given every day (including weekends) over the RT course (up to a maximum of 49 days). Maximum dose reduction is 50% of the starting dose. If significant toxicity is still experienced at 50% of the starting dose, TMZ will be

discontinued.

Use Common Terminology Criteria for Adverse Events (CTCAE) v4.0 unless otherwise specified		
Dose modification criteria for TMZ (during concurrent RT/TMZ phase)		
CTCAE Category	**Adverse Event**	**Dosage Change**
Blood/bone marrow	Grade 2 - ANC<1500-1000 or PLTS<100,000-50,000	Continue RT but omit TMZ until ANC≥1500 and PLTS≥100,000, ↓TMZ dose by 15%.
	Grade 3 - ANC<1000-500 or PLTS<50,000-25,000	Continue RT but omit TMZ until ANC≥1500 and PLTS≥100,000, ↓TMZ dose by 25%.
	Grade 4 - ANC<500 or PLTS<25,000	Hold RT and TMZ. Resume RT when ANC ≥500 & PLTS≥25,000 and TMZ when ANC≥ 1500 & PLTS≥100,000, ↓TMZ dose by 50%.
Gastrointestinal	Grade 2 - Nausea/vomiting on optimal anti-emetics	TMZ may be held until recovery to grade 0-1 at physician's discretion.
	Grade 3 - Nausea/vomiting on optimal anti-emetics	Omit TMZ until grade 0-2 (or ≤1 grade of baseline), ↓TMZ dose by 25%.
	Grade 4 - Nausea/vomiting on optimal anti-emetics	Omit TMZ until grade 0-2 (or ≤1 grade of baseline), ↓TMZ dose by 50%.
Non-hematologic Other	Grade 2	TMZ may be held until recovery to grade 0-1, ↓TMZ dose by 15% at physician's discretion.
	Grade 3	Omit TMZ until grade 0-2 (or ≤1 grade of starting value for pre-existing abnormalities), ↓TMZ dose by 25%.
	Grade 4	Omit TMZ until grade 0-2 (or ≤1 grade of starting value for pre-existing abnormalities), ↓TMZ dose by 50%.
Development of alopecia does not require dose modification		

9.3 Adjuvant treatment: If the attribution of the adverse event can be clearly linked to TMZ, these dose modifications should be followed.

During adjuvant therapy only, patients who require resection for radiation necrosis without evidence of recurrent tumor may continue to receive protocol therapy as long as they are not off study drug for >6 consecutive weeks.

9.3.1 Dose modification criteria for adjuvant TMZ after RT (cycles 1-12): Adjuvant TMZ dose for cycle 1 is 150 mg/m^2 given days 1-5 of 28 day cycle. This dose is used even if TMZ dose reductions or treatment delays were necessary during concurrent RT/TMZ

as long as other criteria at time of starting cycle 1 do not preclude beginning adjuvant TMZ therapy. If tolerated, subsequent cycles will increase dose to 200 mg/m^2 given days 1-5 of 28 day cycles. Maximum dose reduction is 50% of the starting dose (75 mg/m^2 if dose reduction required after cycle 1 or 100 mg/m^2 if dose reduction required after cycle 2 or later). If significant toxicity is still experienced at 50% of the starting dose, TMZ will be discontinued.

Use Common Terminology Criteria for Adverse Events (CTCAE) v4.0 unless otherwise specified		
Dose modification criteria for TMZ at time of retreatment		
CTCAE Category	**Adverse Event**	**Dosage Change**
Blood/bone marrow	ANC<1500 or PLTS<100,000	Hold TMZ until ANC≥1500 and PLTS≥100,000, ↓TMZ dose based on nadirs (see table below based on interim AEs). If no recovery for > 4 weeks then discontinue TMZ.
Non-hematologic Other	Grade 3/4	Hold TMZ until recovery to grade 0-1, ↓TMZ based on interim AEs (see table below). If no recovery for > 4 weeks then discontinue TMZ.

Use Common Terminology Criteria for Adverse Events (CTCAE) v4.0 unless otherwise specified		
Dose modification criteria for TMZ based on interim adverse events		
CTCAE Category	**Adverse Event**	**Dosage Change**
Blood/bone marrow	Grade 3 - ANC<1000-500 or PLTS<50,000-25,000	↓TMZ dose by 25%. (Hold TMZ based on above table's criteria)
	Grade 4 - ANC<500 or PLTS<25,000	↓TMZ dose by 50%. (Hold TMZ based on above table's criteria)
Gastrointestinal	Grade 3 - Nausea/vomiting on optimal anti-emetics	↓TMZ dose by 25%.
	Grade 4 - Nausea/vomiting on optimal anti-emetics	↓TMZ dose by 50%.
Non-hematologic Other	Grade 3	↓TMZ dose by 25%. (Hold TMZ based on above table's criteria)
	Grade 4	↓TMZ dose by 50%. (Hold TMZ based on above table's criteria)

10.0 ANCILLARY TREATMENT/SUPPORTIVE CARE

10.1 Antiemetics: Since TMZ may cause nausea, it is suggested that an appropriate antiemetic (e.g., 1 mg dose of granisetron, or 4 mg of ondansetron, or other) be given one hour before

TMZ. As an alternative, antiemetic may be used on the first 3 days of concomitant RT/TMZ and the first 3 days of adjuvant TMZ, with any further need determined by the treating physician based on perception of clinical need. Additional symptoms will be managed as per standard antiemetic guidelines.

10.2 Antidiarrheals: Use of loperamide (Imodium) for treatment-induced diarrhea is strongly encouraged. Treat diarrhea promptly with appropriate supportive care, including loperamide. Instruct patients to begin taking loperamide at the first signs of: 1) poorly formed or loose stool, 2) occurrence of more bowel movements than usual in one day, or 3) unusually high volume of stool. Loperamide should be taken in the following manner: 4 mg at first onset of diarrhea, then 2 mg after each unformed stool. Daily dose should not exceed 16 mg/day. Loperamide should not be taken prophylactically. Advise patients to drink plenty of clear fluids to help prevent dehydration caused by diarrhea. Avoid loperamide if there is the presence of blood or mucus in the stool or if diarrhea is accompanied by fever.

10.3 Supportive care: Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications (i.e., antidiarrheals, analgesics, antiemetics) received from the first day of treatment until 30 days after the final dose will be recorded on the concomitant medication form. Adequate hydration should be maintained in the setting of dysgeusia (popsicles and Gatorade have been found to be useful by some investigators).

10.4 Colony stimulating factors: These factors should not be used prophylactically to prevent granulocytopenia. Growth factors are not permitted to induce elevations in neutrophil count for the purposes of (1) administration of TMZ on the scheduled dosing interval, or (2) allowing treatment with TMZ at a higher dose, or (3) avoiding interruption of the treatment during concomitant radiotherapy. The therapeutic use of colony stimulating factors will be based on clinical judgment and may be appropriate in patients with a documented infection in the presence of severe granulocytopenia (granulocyte count less than 500/mm^3). Use of colony stimulating factors in clinically indicated situations should be consistent with product labeling and recorded on the concomitant medication form. The use of growth factors should follow published guidelines of the American Society of Clinical Oncology 2006 Update of Recommendations for the Use of White Blood Cell Growth Factors: An Evidence-Based Clinical Practice Guideline. J Clin Oncol 24(19): 1-19 AND American Society of Clinical Oncology/American Society of Hematology 2007 Clinical Practice Guideline Update on the Use of Epoetin and Darbepoetin J Clin Oncol 25(34): 1-17. (See www.asco.org web site)

10.5 Erythropoietin: RBC support with erythropoietin can be used at the physician's discretion.

10.6 Pneumocystis jirovecii pneumonia (PJP [PCP]) prophylaxis: Pneumocystis jirovecii pneumonia has developed in patients when taking concomitant TMZ and steroids. This is of concern especially for lymphopenic patients. Prophylactic treatment is required for the concomitant RT/temozolomide of the study (cycle 1). See section 8.2.3 for details.

10.7 Surgery: If neurosurgical management is required for reasons not due to tumor progression, these procedures must be documented, including the indications for surgery, the surgical operative note and pathology report.

10.8 Corticosteroid treatment: Dosages and evaluation date of dexamethasone use must be recorded on concomitant steroid, anticonvulsant, and antiemetic medication form. Corticosteroids should be used in as low a dose as possible. At progression of disease, corticosteroid choice and dosages are at the discretion of the patient's physician.

10.9 Treatment of fatigue: Patients experiencing profound fatigue may use medications such as modafinil at the discretion of the treating physician.

11.0 TREATMENT EVALUATION

11.1 Objectives – (see section 2.1-3)

11.2 Imaging assessment

11.2.1 Conventional MRI scans (Standard care): Diagnostic MRI scans with and without contrast will be utilized to assess for response.

11.2.2 sMRI (Research, 2 scans at week 0 (before treatment) and week 2): 3-D whole brain MR spectroscopic imaging sequence (EPSI/GRAPPA) and the analysis program MIDAS (Metabolic Imaging Data Analysis System) were developed by Dr. Andrew Maudsley (scientific PI of U of Miami) in 2006 [31-42]. The scan will be done in 3T Siemens MR scanner (TIM/TRIO, Prisma, Skyra) or equivalent. Metabolite maps and their ratio maps will be calculated in MIDAS. Due to some degree of variability of Cho/NAA ratio between subjects, we will use the signal from contralateral normal white matter for normalization. We will use a web-based sMRI clinical interface to extract information from MIDAS to visualize the metabolite maps for efficient clinical workflow of the MIDAS results for busy clinicians and non-MRS experts. In addition, this web-based tool allows effective data sharing among imaging scientists and clinicians for consultation and storage of de-identified data sets (this sMRI clinical interface has been used at Emory (NCI U01 CA172027 & NCI R21 NS100244; Emory IRB00055973). sMRI studies will be obtained at the schedule outlined in section 7.1.

11.3 Response criteria

11.3.1 Measurable Disease: Measurable lesions with clearly defined margins by contrast-enhanced MRI scan beyond pseudoprogression period. We anticipate an extended period of pseudoprogression with dose escalation.

11.3.2 Objective Status, To Be Recorded at Each Evaluation: Unless progression is observed, objective status can only be determined when ALL measurable sites and lesions are assessed.

11.3.3 Response Definitions

11.3.3.1 Complete Response (CR): Complete disappearance of all measurable disease in contrast-enhancing MRI. No new lesions. No evidence of non-evaluable disease. All measurable lesions and sites must be assessed using the same techniques as baseline. Patients must be on no steroids.

11.3.3.2 Partial Response (PR): (Measurable disease only) Greater than or equal to 50% decrease under baseline in tumor volume [43] of all measurable lesions. No progression of evaluable disease. No new lesions. All measurable lesions and sites must be assessed using the same techniques as baseline. *The steroid dose at the time of the scan evaluation should be no greater than the maximum dose used in the first 8 weeks from initiation of therapy.*

11.3.3.3 Stable Disease (SD): Does not qualify for CR, PR, or progression. The designation of SD requires a minimum of 8 weeks duration (2 assessment of stable disease 8 weeks apart). All measurable sites must be assessed using the same techniques as baseline. The steroid dose at the time of the scan evaluation should be no greater than the maximum dose used in the first 8 weeks from initiation of therapy.

11.3.3.4 Progressive Disease (PD): 50% increase in the tumor volume of all measurable lesions over previous exam (over baseline if no decrease) using the same techniques as baseline, OR clear appearance of any new lesion/site, OR clear clinical worsening or failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer). NOTE: Increasing contrast enhancing disease in months 1-3 (potentially up to month 6) post-RT may represent pseudoprogression. For the current study, we anticipate that incidence of this phenomenon will be increased.

Because of difficulties in the interpretation of pseudo-progression on MRI, the first three post-RT/TMZ scans should NOT be used to declare progression. Progressive worsening on subsequent imaging studies usually distinguishes true progression from pseudoprogression. In particular, DSC perfusion imaging may be helpful at distinguishing true and pseudoprogression. If the rCBV map does not show evidence of increased perfusion in regions with increasing contrast-enhanced lesion(s), then pseudoprogression is favored and patient may be maintained on study. Subsequent increases up to 6 months post-RT will be evaluated on a case-by-case at a multi-disciplinary tumor board of the respective institution to assist in making a determination regarding true versus pseudoprogression and whether pathologic sampling would be beneficial. The date of progression will be backdated to when the changes were first observed in the case that the patient is later determine to have true progression.

11.3.3.5 Regressive Disease (REGR): (Evaluable disease only) Unequivocal reduction in extent of contrast as compared to baseline-enhancement or a decrease in mass effect as agreed upon independently by primary physician and quality control physicians; no new lesions.

11.3.3.6 Unknown: Progression has not been documented and one or more measurable sites have not been assessed.

11.4 Best Response: This will be calculated from the sequence of objective statuses. For patients with all disease sites assessed every evaluation period, the best response will be defined as the best objective status as measured according to section 11.5.

11.5 Neurological Exam: Although not used for determining response, it is useful to evaluate improvement in the neurologic exam, as compared with the baseline assessment, that should coincide with objective measurement of tumor size.

NEURO EXAM STATUS (compared to pre-treatment exam)	
Better	Must be on stable or decreasing dose of steroids
Same	Failure to qualify for better or worse
Worse	Include patients requiring increasing steroid doses to remain stable

11.6 Performance Status: Patients will be graded according to Karnofsky Performance Status (see Appendix 3).

11.7 Time to Treatment Failure: From date of registration to the date of first observation of PD (as defined in section 11.5), non-reversible neurologic progression or permanently increased steroid requirement (applies to SD only), death due to any cause, or early discontinuation of treatment.

11.8 Overall Survival Time: From date of definitive surgery or biopsy to date of death due to any cause.

11.9 Progression Free Survival Time: From date of definitive surgery or biopsy to date of progression (defined in section 11.3.3.4) or death. Once the real progression is confirmed from the subsequent scans, the first date exhibiting PD will be noted as the PFS time.

12.0 ADVERSE EVENT REPORTING

12.1 Definitions

12.1.1 Adverse Event Definition - An **adverse event** (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (eg, including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the drug, whether or not it is considered to be drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of drug.

12.1.2 Serious Adverse Event Definition - A **serious adverse event** (SAE) is any adverse event, occurring at any dose and regardless of causality that:

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

Clarification should be made between the terms "serious" and "severe" since they ARE NOT synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is NOT the same as "serious," which is based on patient/event outcome or action criteria described above and are usually associated with events that pose a threat to a patient's life or functioning. A severe adverse event does not necessarily need to be considered serious. For example, persistent nausea of several hours duration may be considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild, but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

12.2 Procedures for AE and SAE Reporting

All serious adverse events (SAEs) will be assessed in a timely fashion and, if deemed reportable, SAE reports will be forwarded to its institutional IRB per IRB reporting requirements (e.g., Hopkins events get reported to Hopkins IRB and notify Emory PIs).

Assessing Causality:

Investigators are required to assess whether there is a reasonable possibility that dose escalated RT caused or contributed to an adverse event. The following general guidance may be used.

Yes: if the temporal relationship of the clinical event to dose-escalated RT makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

No: if the temporal relationship of the clinical event to dose-escalated RT makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.

12.3 Attribution

The clinical Principal Investigator (PI) at each site or his designee will document his opinion and any supporting laboratory and clinical information of the potential attribution of the study treatment to any grade 3 or greater toxicity based on the following guidelines:

12.3.1 Unrelated

This category applies to those toxicities that are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.)

12.3.2 Unlikely (*must have any two criteria*)

In general, this category can be considered applicable to those toxicities that are judged to be unrelated to the test treatment. A toxicity may be considered unlikely if or when:

1. It does not follow a reasonable temporal sequence from administration of the test treatment;
2. It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
3. It does not follow a known pattern of response to the test treatment;
4. It does not reappear or worsen when the test treatment is re-administered.

12.3.3 Possible (*must have any two criteria*)

This category applies to those toxicities for which a connection with the test treatment administration appears unlikely but cannot be ruled out with certainty. A toxicity may be considered possibly related if and when:

1. It follows a reasonable temporal sequence from administration of the test treatment;
2. It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
3. It does follow a known pattern of response to the test treatment.

12.3.4 Probable (*must have any two criteria*)

This category applies to those toxicities that are felt with a high degree of certainty to be related to the test treatment. A toxicity may be considered probably related if and when:

1. It follows a reasonable temporal sequence from administration of the test treatment;

2. It could not reasonably be explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
3. It disappears or decreases on cessation or reduction in test treatment. There are important exceptions when a toxicity does not disappear upon discontinuation of the test treatment, yet treatment-relatedness clearly exists (e.g. bone marrow depression, fixed drug eruptions, tardive dyskinesia);
4. It follows a known pattern of response to the test treatment.

12.3.5 Definite (*must have all four criteria*)

This category applies to those toxicities that are felt to be incontrovertibly related to the test treatment. A toxicity may be considered definitely related if and when:

1. It follows a reasonable temporal sequence from administration of the test treatment;
2. It could not reasonably be explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject;
3. It disappears or decreases on cessation or reduction in dose with re-exposure to drug. (Note: this is not to be construed as requiring re-exposure of the subject, however, a category of definitely related can only be used when a recurrence is observed.)
4. It follows a known pattern of response to the test treatment.

The Emory IRB, Johns Hopkins University IRB and University of Miami IRB will be notified of the event happened at each site promptly per each IRB requirements.

Intensity for each adverse event, including any lab abnormality, will be determined by using the NCI CTCAE, version 4.0, as a guideline.

12.4 Monitoring of Adverse Events and Period of Observation

Adverse events, both serious and non-serious, and deaths that occur during the subject's study participation will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

13.0 DATA AND SAFETY MONITORING PLAN (DSMP)

13.1 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

13.2 Winship Cancer Institute Data Safety Monitoring Committee:

The study will also be followed by the Winship Cancer Institute Data Safety Monitoring Committee to allow for local review and confirmation of proper study execution and safety measures.

13.2.1 Patient safety, study efficacy and compliance will be reviewed at the Neuro-oncology Working Group meeting. The Data and Safety Monitoring Committee (DSMC) of the

Winship Cancer Institute will also oversee the conduct of this study (annually or semi-annually – depending on the risk level assigned to the protocol by the Winship Cancer Institute Clinical and Translational Research Committee). The DSMC will review pertinent aspects of study conduct including patient safety, compliance with protocol, data collection and efficacy. The committee will review the charts of 10% of patients enrolled to the study and two of the first 5 patients entered to the study. The Committee reserves the right to conduct additional audits if necessary. The clinical Principal Investigator (PI) at each site or his designee is responsible for notifying the DSMC about the accrual of patients when the first 5 have been entered to the study. The PI or designee will also notify the DSMC of study status within 2 months before the next scheduled review is due.

13.2.2 Procedures to assure data integrity and protocol adherence

13.2.2.1 Imaging and clinical data will be analyzed in a quarterly meeting of investigators, clinical research coordinators and regulatory personnel.

13.2.2.2 Adverse event reporting will utilize NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and is detailed in section 12.0 above.

13.2.2.3 Study Team Oversight: The study progress in terms of enrollment, activity of current patients under active treatment, observed toxicities will be reviewed in the monthly Emory Neuro-oncology Working Group. Here there will be random and selected case report form and chart review. Special and problematic items requiring additional attention will be addressed in separate sessions of the Neuro-oncology Working Group occurring up to weekly including selected study investigators, clinical research coordinators and regulatory personnel.

13.2.2.4 Training of investigators, clinical research coordinators and regulatory personnel at all sites will be performed by one of the principal investigators utilizing the written protocol and a summary of pertinent treatment activities. Completion of the training of investigators, clinical research coordinators and regulatory personnel will be documented on a study training log.

13.2.2.5 External Site Management: Enrollment will occur at Johns Hopkins University and University of Miami

- Monitoring of Study Activity: Monitoring of study activity will occur by monthly review of enrollment activity of current patients under active treatment, observed toxicities by the investigator, clinical research coordinator and regulatory personnel at Johns Hopkins and University of Miami. The results of this review will be summarized and shared and logged monthly in written form (and verbal form when needed) with the Emory investigators.
- Investigator Communication: Verbal investigator communication among three sites will occur and be logged quarterly to discuss the content mentioned above.
- Reporting of Adverse Events Occurring at Johns Hopkins and University of Miami: The criteria and methods of collecting adverse event data outlined in section 12.0 will apply at Johns Hopkins and U of Miami also.
    - Each site Principal Investigators (PI) are responsible for review and assessment of all adverse events at their site as specified in the protocol. AEs, SAEs (initial and follow ups), and UPs will be reported to the each site IRB as per protocol requirements

including reporting SAEs and UPs within 24 hours of the participating site becoming aware of the event.

- Johns Hopkins and University of Miami sites are also responsible for the following:
  - Report events, as applicable, to their IRB.
  - Submit all acknowledgements of reports submitted promptly to their IRB to the Winship multisite coordinator or Emory PIs.
  - Resolve all queries related to the SAE report in a timely manner.
  - Maintain copies of each event reported in the subject's study records.
  - Enter AEs into Oncore® in a timely manner.
  - Enter SAEs and UPs into Oncore® or equivalent (depending on the institution) in the SAE category, in a timely manner.
- Winship multisite coordinator will inform all three sites of adverse events that require expedited reporting to local IRBs.
- Emory Lead investigator will review SAE data summary reports on a regular basis, at least annually
- Emory Lead investigator will submit SAE data summary reports to the Data Safety Monitoring Committee for review as per their guidelines.

13.3 On-site Audits

Regulatory authorities and the IRB may request access to all source documents, data capture records, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support given reasonable notice at all times for these activities.

<u>The following applies for monitoring of Sub-sites:</u>
At the study initiation, the PI, regulatory specialist, and research coordinators will perform a site initiation teleconference. During this teleconference, the Emory team will review the study, enrollment, reporting, and regulatory compliance. The participating site will have internal monitoring meetings. These meetings will include the investigators, the clinical research coordinator and the regulatory affairs coordinator and will meet at least on a monthly basis to review and discuss study data to ensure subject safety. The research coordinators will maintain one spread sheet which will summarize all the patient data for patients actively being treated on the trial as well as a roadmap detailing pending tests/treatments for each individual patient. The spread sheet will be shared with the Emory PIs. Teleconferences will be conducted monthly among the PIs and the research team with the participating sites. The purpose of the meetings is to discuss the enrollment, regulatory updates, monitor toxicities, and evaluate the progress of the trial. The minutes from the teleconference will be maintained in the regulatory binder for the study. In addition, electronic copies will be sent via email to the PIs at each site. Chart reviews will be performed on selected cases from the participating site to confirm that the data collection is accurate.

Winship's Multisite Coordinator will perform remote quarterly monitoring of participating sites' data and will perform an annual on-site monitoring visit as necessary per site enrollment. Monthly

reviews of data in OnCore will be conducted to ensure compliance or identify discrepancies

14.0 STATISTICAL CONSIDERATIONS AND METHODOLOGY

14.1 Primary Objectives

14.1.1 *To determine the feasibility of using sMRI to guide dose-escalated RT for newly-diagnosed GBMs.* Feasibility of this approach will be determined by whether treatment volumes based on sMRI can be co-registered with clinical images and transferred into the radiation treatment execution platform in a seamless manner, so that sMRI information can be efficiently applied to the patient treatment.

14.1.2 *To determine the safety of using sMRI to guide dose-escalated RT for newly-diagnosed GBMs.* The safety of this approach will be confirmed by assessing toxicity potentially attributable to the dose-escalated RT. Toxicity will be determined by CTCAE v4.0 criteria. If > 6 of 30 patients (> 20% of subjects) experience a grade 3 or greater toxicity by 6 months after completion of RT/TMZ that is ruled as at least possibly attributable to the dose-escalated RT, then the safety of this treatment approach will be questioned and require further review by the study team. Interim analyses with stopping rules are described later in this Section.

14.2 Secondary Objectives

14.2.1 *To determine whether the progression free survival at 1 year sMRI-guided, dose-escalated RT increases with newly-diagnosed GBMs.* Subjects on this study will be followed for progression free survival (PFS) which is defined from the time of surgical resection to the time of either radiographic progression or death, whichever occurs first. While PFS actuarial curves will be assessed and compared to historical controls, we are particularly interested in comparing the 1-year PFS rate which, based on the control arm (receiving standard dose RT with TMZ) of recent GBM trials, is approximately 30% in historical cohorts [44-46].

14.3 Exploratory Objectives

14.3.1 *To determine whether sMRI-guided, dose-escalated RT increases the overall survival of patients with newly-diagnosed GBMs.* Subjects on this study will also be followed for overall survival (OS) which is defined from the time of surgical resection to the time of death. The OS actuarial curve and 1-year OS rate will be assessed and compared to historical controls. The 1-year OS rate of control arm patients on recent trials is approximately 66%

14.3.2 *To determine whether sMRI data obtained after initiation of therapy (at 2 weeks after RT/TMZ start and prior to cycle 1 and 5 of adjuvant TMZ) will provide early evidence of GBM progression not seen on standard MRIs.* Changes in sMRI parameters over time will be assessed to determine whether they will be able to predict development of recurrence. Global changes in sMRI parameters including to absolute metabolite concentrations or metabolic ratios will be examined to see if these factors may be an early marker of tumor recurrence. In addition, further assessments will be made on a voxel-wise basis to determine whether individual changes may be predictive of ultimate recurrence within particular voxels.

14.3.3 *To determine whether performance on neurocognitive and quality-of-life (QOL) assessments in newly-diagnosed GBM patients treated with sMRI-guided, dose-escalated RT differ from historical controls.* We will compare performance on neurocognitive and QOL assessments in our 30 subject cohort with performance by historical control cohorts from recent GBM trials (eg. RTOG 0525 and 0825) [45, 46]. Better performance may be attributable to better control of disease while worse performance may imply increased toxicity from the study treatment regimen.

14.3 Accrual rate and numbers: The anticipated rate of accrual will be 2-3 patients/month among three sites. This will allow consenting of 30 patients over a period of 1.5 years.

14.3 Criteria for removal from trial: All reasons for discontinuation of treatment must be documented.

14.3.1 Progression of disease will be hard to determine due to pseudoprogression as defined in section 11.3. The final progression free survival will be determined at year 1, which is beyond pseudoprogression possibility.

14.3.2 Patient may withdraw from study at any time for any reason.

14.4 Adverse event stopping rule: Safety assessments will be performed throughout treatment, at two week intervals during RT and prior to each odd number adjuvant TMZ cycle following completion of RT. Toxicity will be carefully assessed using CTCAE v4.03 criteria, and the rate of radiation necrosis development will be determined. While we expect this regimen to be tolerated based on the previous experience reported by Tsien et al. [9], we will still employ an interim safety analysis with a special stopping rule if neurotoxicity is greater than expected. Because the expected incidence of late grade 3+ neurotoxicity with standard-of-care chemoradiation is ~5%, or 2 of 30 patients in our planned cohort, we will employ the following interim analysis and stopping rule after 10 subjects have completed treatment and have been followed for 6 months after completion of chemoradiation: if more than 2 subjects (out of 10) develop CTCAE v4.03 grade III or greater neurotoxicity (> 20% incidence) judged to be at least possibly due to RT by 6 months from completion of RT/TMZ, study accrual will halt and await assessment of toxicities by the DSMC to review attribution of toxicities before a decision will be made about either permanent closure or re-opening with continuation/completion of study accrual.

14.5 Statistical analysis for survival endpoints: The primary objectives are to determine the feasibility of this escalated protocol and the safety of increased doses in patients with GBM. We anticipate that 30 patients will provide us sufficient data to make these conclusions. Since survival analyses are exploratory objectives of this study, these endpoints are not used to determine accrual numbers for this study. The PFS of patients in this protocol will be compared with historical controls, and that data will be used to estimate the effect size for future power analyses. With a planned accrual of 30 subjects, the following power to detect potential improvements in 1 year PFS is as follows: 1-year PFS rate is expected to be 30-35% based on historical controls [44-46]. Our study will have 82% power to detect an improvement to a rate of 60% at α=0.05 using a two-sided test. Cox's proportional hazard regression model will be applied to model 1-year PFS outcome to test whether subjects treated with escalated RT doses guided by the sMRI demonstrate significantly higher PFS as compared to historical controls receiving standard treatment, controlling for other potential risk factors. Of note, calculations to determine the power to detect a certain PFS effect size based the number of patients we needed to determine feasibility and safety were presented above. However, this study was not actually powered based on PFS improvement.

14.6 Statistical analysis of sMRI scans: This study is mainly using pre-RT sMRI parameters to help define a region at high risk for recurrence that can be treated with higher doses of RT. Exploratory objectives will involve examining changes in absolute metabolite levels and metabolic ratios during RT that may be useful for adaptive radiation therapy (changing dose plan during early phase of RT). sMRI data will be evaluated both on a patient-wise basis and on a voxel-wise basis with utilization of deformable registration technique, as needed, to achieve good matching of maps across serially acquired scans.

14.7 Statistical analysis of neurocognitive function and QOL scores: Linear mixed effects models will be applied to model the repeatedly measured score from each assessment and/or test over time. We hypothesize that changes in individual and/or composite metabolite levels will be predictive of the temporal changing patterns in the scores on the neurocognitive functional tests and QOL assessments. Changes in individual and multiple metabolite levels

within normal brain surrounding the region of the tumor as well as in tumor-bearing regions of brain will be evaluated with scores on these assessments using logistic regression models.

14.8 Interim analysis and stopping rule for efficacy/futility: While there will be stopping rules for toxicity/adverse events (see **Section 14.4**), *there will not be stopping rules for efficacy/futility.* The primary goal of the study is to determine the feasibility and safety of sMRI-guided, dose-escalated RT and TMZ for newly diagnosed GBM patients. At the time we anticipate performing interim analysis for toxicity assessment in the first 10 patients with adequate follow-up, approximately 20 patients will have already initiated therapy allowing up to 10 patients to avoid enrollment if this treatment regimen is determined to be too toxic.

15.0 DATA COLLECTION

15.1 Data submission forms: A copy of the eligibility/registration form will be completed and kept at the Emory Winship Cancer Institute Clinical Research Office.

- The clinical management system being used for this study is The Online Collaborative Research Environment (OnCore). OnCore will be used to record all study related information for all registered patients. All data must be entered no later than 30 days following each visit completion. All queries are to be resolved within 4 weeks of issue.

15.2 Imaging and RT data: Imaging data will be collected with masked patient identification. All diagnostic imaging data sets will be electronically transferred under the study ID to a secure server at Emory University. In addition, patient treatment plans, including isodose clouds and all structure including tumor volumes and OARs under the study ID, will be similarly transferred and stored. For instructions on how to transfer each data set, please contact Edi Schreibmann at 404-778-5667.

15.3 REDCap data: To be able to accurately determine/analyze the progression (date and recurrence patterns) and outcomes/benefits of the new treatment strategy, we need the following information uploaded to the REDCap database set up for this trial. Our REDCap for this study is set up under Study ID, rather than patients' identity. The following documents will be collected to REDCap after masking patient's name or hospital ID number. Those include all reports from neuro-oncologists (to monitor the condition of the patients and any new interventions that are prescribed to the patients) and pathology reports that are generated after surgical interventions. The initial pathology report has genomic/histological information of the tumor subtype while the follow up pathology reports contain the tumor/necrosis proportion of sampling to determine progression/treatment effect. For instructions on how to upload data to the REDCap, please contact Karthik Ramesh at 425-512-3406.

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APPENDIX 1 - Karnofsky Performance Scale

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

APPENDIX 2 - NEUROCOGNITIVE TESTS

NEUROCOGNITIVE TEST INSTRUCTIONS AND ADMINISTRATION PROCEDURES

1. Testing must be completed in one session. Test instructions must be followed verbatim with every patient at every study visit. All tests should be completed in black pen.
2. Tests should be administered in the following order to every patient and at every study visit:

   HVLT-R Part A (Learning Trials); Trail Making Test Part A; Trail Making Test Part B; COWAT; HVLT-R Part B (Delayed Recall); and the HVLT-R Part C (Delayed Recognition).
3. You may fill the delay interval between COWAT and HVLT-R Part B (Delayed Recall) with QOL questionnaires.
4. Patients should not be given copies of their tests to avoid learning the material between test administrations.

1. HOPKINS VERBAL LEARNING TEST - REVISED (HVLT-R)

This test has three parts and six alternate forms:

Part A - Free Recall: Complete the three learning trials first

Part B - Delayed Recall: Complete after a 20 minute delay that includes administration of Trail Making Tests and COWAT as well as QOL assessments and symptom self-report measures, if appropriate

Part C - Delayed Recognition: Complete immediately after Delayed Recall

Part A – Free Recall: Trial 1

Examiner*: "I am going to read a list of words to you. Listen carefully, because when I am through, I'd like you to tell me as many of the words as you can remember. You can tell them to me in any order. Are you ready?"*

- Read the words at the rate of one word every 2 seconds.

***Examiner: "OK. Now tell me as many of those words as you can remember*."**

- Check off the words the patient recalls on the form.
- If a word is said that is not in the list (*for example, "intrusion"*), do not write that word on the form and say nothing to the patient about the word not being on the list.
- There is no time limit for each recall trial. However, if the patient does not produce any words for 10-15 seconds, ask the patient if he/she can remember any more words.
- If not, move on to trial 2. Later, you can record the number of words that were correctly repeated on the summary form.

Part A – Free Recall: Trial 2

Examiner*: "Now we are going to try it again. I am going to read the same list of words to you. Listen carefully, and tell me as many of the words as you can remember, in any order, including the words you told me the first time."*

- Read the words at the rate of one word every 2 seconds.
- Check off the words the patient recalls on the form.
- If a word is said that is not in the list (*for example, "intrusion"*), do not write that word on the form and say nothing to the patient about the word not being on the list.
- There is no time limit for each recall trial. However, if the patient does not produce any words for 10-15 seconds, ask the patient if he/she can remember any more words.
- If not, move on to trial 3. Later, you can record the number of words that were correctly repeated on the summary form.

Part A – Free Recall: Trial 3

Examiner*: "I am going to read the list one more time. As before, I'd like you to tell me as many of the words as you can remember, in any order, including all the words you've already told me."*

- Read the words at the rate of one word every 2 seconds.
- Check off the words the patient recalls on the form.
- If a word is said that is not in the list (*for example, "intrusion"*), do not write that word on the form and say nothing to the patient about the word not being on the list.
- There is no time limit for each recall trial. However, if the patient does not produce any words for 10-15 seconds, ask the patient if he/she can remember any more words.
- Do not tell the respondent that recall of the words will be tested later.
- Record the time on the clock that you *complete* 'Part A – Free Recall' (for example, 10:00 am) on the designated space on the HVLT-R form.

2. TRAIL MAKING TEST [Timed Test]

Part A – Sample*:* The Sample for Part A must be completed/attempted by each patient and every assessment. Place the Sample A worksheet flat on the table, directly in front of the patient *(the bottom of the worksheet should be approximately six inches from the edge of the table).* Give the patient a black pen and say:

Examiner*: "On this page* (point) *are some numbers. Begin at number 1* (point to 1) *and draw a line from 1 to 2* (point to 2), *2 to 3* (point to 3)*, 3 to 4* (point to 4), *and so on, in order, until you reach the end* (point to the circle marked END). *Draw the lines as fast as you can. Ready, begin."*

If the patient completes Sample A correctly and in a manner demonstrating that s/he understands what to do, proceed immediately to Test A. If the patient makes a mistake on Sample A, point out the error and explain it. The following explanations of mistakes serve as illustrations:

- ***"This is where you start (point to number 1)"***
- ***"You skipped this circle (point to the circle omitted)"***

- ***"You should go from number 1 to 2, 2 to 3, and so on, until you reach the circle marked END"***

If it is clear that the patient intended to touch a circle but missed it, do not count it as an omission. Remind the patient, however, to be sure to touch the circles. If the patient still cannot complete Sample A, take his/her hand and guide him/her through the trail using the opposite end of the pen, lightly touching the worksheet to avoid making marks on the copy. Then say:

Examiner*: "Remember, begin at number 1 (*point to 1*) and draw a line from 1 to 2 (*point to 2*), 2 to 3 (*point to 3*), 3 to 4 (*point to 4*) and so on, in order, until you reach the circle marked END (*point*). Do not skip around, but go from one number to the next in proper order. Remember to work as fast as you can. Ready, begin."*

If the patient does not succeed, or it becomes evident that s/he cannot do the task, DISCONTINUE testing and indicate the corresponding reason on the Trail Making Data Sheet. If the patient completes Sample A correctly and appears to understand what to do, proceed immediately to Part A.

Part A – Test: After the patient has completed Sample A, place the Part A test worksheet directly in front of the patient and say:

Examiner*: "Good! Let's try the next one. On this page are numbers from 1 to 25. Do this the same way. Begin at number 1 (*point*) and draw a line from 1 to 2 (*point to 2*), 2 to 3 (*point to 3*), 3 to 4 (*point to 4*) and so on, in order, until you reach the circle marked END (*point*). Do not skip around, but go from one number to the next in proper order. Remember to work as fast as you can. Ready, begin."*

- Start timing as soon as the instruction is given to "begin"
- Watch closely in order to catch any errors as soon as they are made. If the patient makes an error, call it to his/her attention immediately and have him/her proceed from the point the mistake occurred
- The patient must complete the test in 3 minutes or less
- DO NOT STOP TIMING UNTIL HE/SHE REACHES THE CIRCLE MARKED "END"
- If the patient does not complete the test within 3 minutes, terminate the testing. The test can also be discontinued if the patient is extremely confused and is unable to perform the task. Collect the worksheet and complete the Trail Making Data Sheet indicating the reason the test was terminated and the last correct number reached on the test.
- If the patient successfully completes the test, collect the worksheet and record the time to completion on the Trail Making Data Sheet in minutes and seconds. Then say, ***"That's fine. Now we'll try another one."***

Part B – Sample: The Sample for Part B must be completed/attempted by each patient and every assessment. Place the Sample B worksheet flat on the table, directly in front of the patient (the bottom of the worksheet should be approximately six inches from the edge of the table) and say:

Examiner*: "On this page (*point*) are some numbers and letters. Begin at number 1 (*point to 1*) and draw a line from 1 to A (*point*), A to 2 (*point to 2*), 2 to B (*point to B*), B to 3 (*point to 3*), 3 to C (*point to C*) and so on, in order, until you reach the end (*point to the circle marked END*). Remember, first you have a number (*point to 1*), then a letter (*point to A*), then a number (*point to 2*), then a letter (point to B), and so on. Draw the lines as fast as you can. Ready, begin."*

If the patient completes Sample B correctly, and in a manner demonstrating that s/he understands what to do, proceed immediately to Part B. If the patient makes a mistake on Sample B, point out the error and explain it. The following explanations of mistakes serve as illustrations:

- ***"You started with the wrong circle. This is where you start (point to number 1)"***
- ***"You skipped this circle (point to the circle omitted)"***
- ***"You should go from number 1 (*point*) to A (*point*), A to 2 (*point to 2*), 2 to B (*point to B*), B to 3 (*point to 3*) and so on, until you reach the circle marked END (*point*)"***

If it is clear the patient intended to touch a circle but missed it, do not count it as an omission. Remind the patient, however, to be sure to touch the circles. If the patient still cannot complete Sample B, take their hand and guide them through the trail using the opposite end of the pen, lightly touching the worksheet to avoid making marks on the copy. Then say:

Examiner*: "Now you try it. Remember, begin at number 1 (*point to 1*) and draw a line from 1 to A (*point to A*), A to 2 (*point to 2*), 2 to B (*point to B*), B to 3 (*point to 3*) and so on, in order, until you reach the circle marked END (*point*). Ready, begin."*

If the patient does not succeed or it becomes evident that s/he cannot do the task, DISCONTINUE testing and indicate the corresponding reason on the Trail Making Test Data Sheet. If the patient completes Sample A correctly and appears to understand what to do, proceed immediately to Part A.

<u>Part B – Test</u>:

After the patient has completed Sample B, place the Part B Worksheet directly in front of the patient and say:

Examiner*: " Good! Let's try the next one. On this page are both numbers and letters. Do this the same way. Begin at number 1 (*point*) and draw a line from 1 to A (*point to A*), A to 2 (*point to 2*), 2 to B (*point to B*), B to 3 (*point to 3*), 3 to C (*point to C*) and so on, in order, until you reach the circle marked END (*point*). Remember, first you have a number (*point to 1*), then a letter (*point to A*), then a number (*point to 2*), then a letter (*point to B*), and so on. Do not skip around, but go from one circle to the next in the proper order. Draw the lines as fast as you can. Ready, begin."*

- <u>Start timing as soon as the instruction is given to "begin"</u>
- Watch closely in order to catch any errors as soon as they are made. <u>If the patient makes an error, call it to his/her attention immediately and have him/her proceed from the point the mistake occurred - do NOT start from the beginning</u>
- The patient must complete the test in <u>5 minutes</u> or less
- <u>DO NOT STOP TIMING UNTIL HE/SHE REACHES THE CIRCLE MARKED "END"</u>
- Collect the worksheet and record the time to completion on the Trail Making Test Data Sheet in minutes and seconds
- If the patient does not complete the test within <u>5 minutes,</u> terminate the testing. The test can also be discontinued if the patient is extremely confused and is unable to perform the task. Collect the worksheet and complete the Trail Making Test Data Sheet indicating the reason the test was terminated and the last correct number or letter reached on the test.

- At the top of both Sample forms and both Test forms please write: subject ID number, date of evaluation, institution name, name of certified tester, and the certified tester's phone number.

3. CONTROLLED ORAL WORD ASSOCIATION TEST (COWAT) [Timed Test]

This test has three parts (letters) and two alternate forms.

Examiner*: "I am going to say a letter of the alphabet, and I want you to say as quickly as you can all of the words that you can think of that begin with that letter. You may say any words at all, except proper names such as the names of people or places. So you would not say 'Rochester' or 'Robert'. Also, do not use the same word again with a different ending, such as 'Eat,' and 'Eating.'*

"For example, if I say 's,' you could say 'son', 'sit,' 'shoe,' or 'slow.' Can you think of other words beginning with the letter 's'?"

Wait for the patient to give a word. If it is a correct response, say ***"good"***, and ask for another word beginning with the letter "s". If a second appropriate word is given, proceed to the test itself.

If the patient gives an inappropriate word on either occasion, correct the patient, and repeat the instructions. If the patient then succeeds, proceed to the test.

If the patient fails to respond, repeat the instructions. If it becomes clear that the patient does not understand the instructions or cannot associate, stop the procedure, and indicate the reason(s) on the scoring sheet and the Neurocognitive Evaluation Summary Form (CS).

If the patient has succeeded in giving two appropriate words beginning with the demonstration letter, say:

Examiner*: "That is fine. Now I am going to give you another letter and again you say all of the words beginning with that letter that you can think of. Remember, no names of people or places, just ordinary words. Also, if you should draw a blank, I want you to keep on trying until the time limit is up and I say STOP."*

"You will have a minute for each letter. The first letter is '___'" (see scoring sheet).

Allow exactly one minute for each letter

- If the patient discontinues before the end of the time period, encourage him/her to try to think of more words.
- If he/she is silent for 15 seconds, repeat the basic instruction and the letter (e.g., **"Tell me all the words you can think of that begin with a "c"**).
- No extension on the time limit is made in the event that instructions are repeated.
- Continue the evaluation with the remaining two letters, allowing one minute for each.

Recording and Scoring:

- The record sheet provides lines on which the patient's responses can be entered *(e.g., write in the word that is said by the patient)*. Record all patient responses verbatim. If his/her speed of word production is too fast to permit verbatim recording, a "+" should be entered to indicate a correct response.
- Incorrect responses should be struck through with a line and then initial and date in the margin next to the error.
- If the patient provides more responses than there are lines on the record sheet, place check marks in the boxes to indicate correct responses only.
- Count all the correct responses. The number of correct words should be indicated below each column on the recording sheet and on the Neurocognitive Evaluation Summary Form (CS) that is sent to the RTOG.

Comments on scoring:

- Note: It can be helpful for the first several patients and for patients known to be fast with their word production to tape record the session for transcription at a later time.
- The instructions include a specific prohibition against giving proper names or different forms of the same word. Therefore, inflections of the same word *(e.g., eat-eating; mouse-mice; loose-loosely; ran-run-runs)* are not considered correct responses.
- Patients often give both a verb and a word derived from the verb or adjective *(e.g., fun-funny; sad-sadness)*. These are not considered correct responses. On the other hand, if the word refers to a specific object *(e.g., foot-footstool; hang-hanger),* it would be counted as a correct answer.
- Many words have two or more meanings *(e.g., foot; can; catch; hand)*. A repetition of the word is acceptable IF the patient definitely indicates the alternative meaning to you.
- Slang terms are OK if they are in general use.
- Foreign words *(for example, pasta; passé; lasagna)* can be counted as correct if they can be considered part of the lexicon of the relevant language, the criterion being their listing in a standard dictionary of that language. All incorrect and repeated responses MUST be crossed out with one single line, initialed and dated. Additionally, all duplicate entries that have been verified to have different meanings must be marked "ok", initialed and dated. Refer to the descriptions above for guidelines for acceptability. Add the total number of correct responses in each column and input the totals where indicated on the COWAT worksheet.
- If the test is discontinued or omitted, please mark this on the bottom of the test form and indicate the reason on the Neurocognitive Evaluation Summary Form (CS)

4. HOPKINS VERBAL LEARNING TEST - REVISED (HVLT-R)

Part B – Delayed Recall

- ***DO NOT READ THE WORD LIST AGAIN.***
- Record the time on the clock that you *start* 'Part B – Delayed Recall' (for example, 10:20 am) on the designated space on the HVLT-R form.

- Administer 'Part B – Delayed Recall' after completing all Trail Making Tests and the COWAT. There should be at least 20 minutes between 'Part A' and 'Part B' of the HVLT-R. If the time is too short, allow the patients to complete a questionnaire.

Examiner: "*Do you remember that list of words you tried to learn before? Tell me as many of those words as you can remember."*

- Check the box on the corresponding line of the HVLT-R worksheet for each word the patient accurately recalls.
- If a word is said that is not in the list (*for example, "intrusion"*), do not write that word on the form and say nothing to the patient about the word not being on the list.
- There is no time limit for each recall trial. However, if the patient does not produce any words for 10-15 seconds, ask the patient if he/she can remember any more words.
- If not, record the number of words that were correctly recalled on the summary form.

Part C – Delayed Recognition

Examiner*: "Now I'm going to read a longer list of words to you. Some of them are words from the original list, and some are not. After I read each word, I'd like you to say "Yes" if it was on the original list or "No" if it was not. Was [word] on the list?"*

- Read the words from the top of the columns down.
- Check either the "**Y"** (Yes) or "**N"** (No) box next to each word to indicate the patient's response.
- Guessing is allowed.
- If the test is discontinued or omitted, please mark this on the bottom of the test form and indicate the reason on the Neurocognitive Evaluation Summary Form (CS).
- The score for this portion of the HVLT-R is the number of list words (i.e., words that in CAPS) correctly identified ("yes" response) minus the number of non-list words (i.e., words in lower case) incorrectly identified ("yes" response). Therefore, the actual score can range from –12 *(no list words identified and all non-list words identified)* to +12 *(all list words identified and no non-list words identified)*.

APPENDIX 3 – QOL QUESTIONNAIRES

ENGLISH

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ⌊ ⌋⌊ ⌋⌊ ⌋

Your birthdate (Day, Month, Year): ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋

Today's date (Day, Month, Year): 31 ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋⌊ ⌋⌊ ⌋

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4
16.	Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

EORTC QLQ - BN20

Patients sometimes report that they have the following symptoms. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:		Not at All	A Little	Quite a Bit	Very Much
31.	Did you feel uncertain about the future?	1	2	3	4
32.	Did you feel you had setbacks in your condition?	1	2	3	4
33.	Were you concerned about disruption of family life?	1	2	3	4
34.	Did you have headaches?	1	2	3	4
35.	Did your outlook on the future worsen?	1	2	3	4
36.	Did you have double vision?	1	2	3	4
37.	Was your vision blurred?	1	2	3	4
38.	Did you have difficulty reading because of your vision?	1	2	3	4
39.	Did you have seizures?	1	2	3	4
40.	Did you have weakness on one side of your body?	1	2	3	4
41.	Did you have trouble finding the right words to express yourself?	1	2	3	4
42.	Did you have difficulty speaking?	1	2	3	4
43.	Did you have trouble communicating your thoughts?	1	2	3	4
44.	Did you feel drowsy during the daytime?	1	2	3	4
45.	Did you have trouble with your coordination?	1	2	3	4
46.	Did hair loss bother you?	1	2	3	4
47.	Did itching of your skin bother you?	1	2	3	4
48.	Did you have weakness of both legs?	1	2	3	4
49.	Did you feel unsteady on your feet?	1	2	3	4
50.	Did you have trouble controlling your bladder?	1	2	3	4

Date: ☐☐ / ☐☐ / ☐☐
(month) (day) (year)

Subject Initials: ☐☐☐

MD Anderson # : ☐☐☐☐☐☐

Study Name: _____

Protocol #: _____

PI: _____

PDMS # : ☐☐☐☐

M. D. Anderson Symptom Inventory - Brain Tumor (MDASI - BT)

Part I. How severe are your symptoms?

People with cancer frequently have symptoms that are caused by their disease or by their treatment. We ask you to rate how severe the following symptoms have been in the last 24 hours. Please fill in the circle below from 0 (symptom has not been present) to 10 (the symptom was as bad as you can imagine it could be) for each item.

	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As You Can Imagine 10
1. Your **pain** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
2. Your **fatigue (tiredness)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
3. Your **nausea** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
4. Your **disturbed sleep** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
5. Your feeling of being **distressed (upset)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
6. Your **shortness of breath** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
7. Your problem with **remembering things** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
8. Your problem with **lack of appetite** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
9. Your feeling **drowsy (sleepy)** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
10. Your having a **dry mouth** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
11. Your feeling **sad** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
12. Your **vomiting** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
13. Your **numbness or tingling** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
14. Your **weakness** on one side of the body at its WORST?	○	○	○	○	○	○	○	○	○	○	○
15. Your difficulty **understanding** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
16. Your difficulty **speaking** (finding the words) at its WORST?	○	○	○	○	○	○	○	○	○	○	○

Date: ☐☐ / ☐☐ / ☐☐
(month) (day) (year)

Study Name: _____
Protocol #: _____
PI: _____

Subject Initials: _____

MD Anderson # : ☐☐☐☐☐☐ PDMS # : ☐☐☐☐

	Not Present 0	1	2	3	4	5	6	7	8	9	As Bad As You Can Imagine 10
17. Your **seizures** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
18. Your difficulty **concentrating** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
19. Your **vision** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
20. Your change in **appearance** at its WORST?	○	○	○	○	○	○	○	○	○	○	○
21. Your change in **bowel pattern** (diarrhea or constipation) at its WORST?	○	○	○	○	○	○	○	○	○	○	○
22. Your **irritability** at its WORST?	○	○	○	○	○	○	○	○	○	○	○

Part II. How have your symptoms interfered with your quality of life?

Symptoms frequently interfere with how well we perform daily function. How much have your symptoms interfered with the following items in the last 24 hours:

	Did not interfere 0	1	2	3	4	5	6	7	8	9	Interfered Completely 10
23. **General activity?**	○	○	○	○	○	○	○	○	○	○	○
24. **Mood?**	○	○	○	○	○	○	○	○	○	○	○
25. **Work (including work around the house)?**	○	○	○	○	○	○	○	○	○	○	○
26. **Relations with other people?**	○	○	○	○	○	○	○	○	○	○	○
27. **Walking?**	○	○	○	○	○	○	○	○	○	○	○
28. **Enjoyment of life?**	○	○	○	○	○	○	○	○	○	○	○

APPENDIX 4 – Cordova et al. 2016

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Whole-brain spectroscopic MRI biomarkers identify infiltrating margins in glioblastoma patients

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Background. The standard of care for glioblastoma (GBM) is maximal safe resection followed by radiation therapy with chemotherapy. Currently, contrast-enhanced MRI is used to define primary treatment volumes for surgery and radiation therapy. However, enhancement does not identify the tumor entirely, resulting in limited local control. Proton spectroscopic MRI (sMRI), a method reporting endogenous metabolism, may better define the tumor margin. Here, we develop a whole-brain sMRI pipeline and validate sMRI metrics with quantitative measures of tumor infiltration.

Methods. Whole-brain sMRI metabolite maps were coregistered with surgical planning MRI and imported into a neuronavigation system to guide tissue sampling in GBM patients receiving 5-aminolevulinic acid fluorescence-guided surgery. Samples were collected from regions with metabolic abnormalities in a biopsy-like fashion before bulk resection. Tissue fluorescence was measured *ex vivo* using a hand-held spectrometer. Tissue samples were immunostained for Sox2 and analyzed to quantify the density of staining cells using a novel digital pathology image analysis tool. Correlations among sMRI markers, Sox2 density, and ex vivo fluorescence were evaluated.

Results. Spectroscopic MRI biomarkers exhibit significant correlations with Sox2-positive cell density and *ex vivo* fluorescence. The choline to *N*-acetylaspartate ratio showed significant associations with each quantitative marker (Pearson's $\rho = 0.82$, $P < .001$ and $\rho = 0.36$, $P < .0001$, respectively). Clinically, sMRI metabolic abnormalities predated contrast enhancement at sites of tumor recurrence and exhibited an inverse relationship with progression-free survival.

Conclusions. As it identifies tumor infiltration and regions at high risk for recurrence, sMRI could complement conventional MRI to improve local control in GBM patients.

Keywords: 5-aminolevulinic acid, glioblastoma, quantitative histological image analysis, spectroscopic MRI, surgical and radiation therapy planning.

Approximately 15 000 new cases of glioblastoma (GBM; World Health Organization [WHO] grade IV glioma) are diagnosed each year in the United States, making it the most common primary malignant brain tumor in adults.[1] The standard of care for GBM is maximal safe surgical resection followed by radiation therapy (RT) with concurrent and adjuvant temozolomide chemotherapy. Despite such aggressive management, the tumor recurrence rate is high: ~70% within 6 months of RT, and the median overall survival (OS) is 13–15 months.[2] Currently, both surgery and RT are based on T1-weighted contrast-enhanced (T1w-CE) MRI using an intravenous injection of gadolinium-based contrast agents. While

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contrast agent accumulates in regions where GBM tumors have compromised the blood–brain barrier or exhibit leaky neovasculature, they cannot effectively reach infiltrating tumor where perfusion is limited.[3,4] Multiple studies have found infiltrating tumor cells centimeters away from the contrast-enhancing mass.[5] One study found that tissue extracted from a zone 6–14 mm outside of the T1w-CE region was composed of 60%–100% tumor cells.[6] Furthermore, these nonenhancing regions are biologically distinct, with varying oncogenic profiles that appear to influence treatment efficacy and recurrence.[3,7] Due to the remaining infiltrating cancer cells, nearly 80% of patients recur within 2 cm of the original T1w-CE tumor after therapy.[8]

Molecular imaging techniques, including MR spectroscopy and fluorescence microscopy, have shown promise in identifying and directing therapy to tumor-infiltrated regions beyond the T1w-CE lesion.[9] Proton spectroscopy, which includes 2D-chemical shift and 3D MR spectroscopic imaging, is a molecular imaging technique that maps the metabolism of native small molecules to tumor regions in vivo without the need for exogenous tracers.[10,11] Using tumor metabolism, physicians can identify and target regions of significant tumor infiltration beyond contrast diffusion even when edema is present.[10,12–15] Additionally, infiltrating tumor cells can be identified intraoperatively using 5-aminolevulinic acid (5-ALA) fluorescence microscopy.[16] An orally administered pro-drug, 5-ALA is readily metabolized by malignant gliomas to protoporphyrin IX, a molecular species that fluoresces red (600–700 nm) under blue-violet light (400–410 nm). Fluorescence-guided surgery (FGS) with 5-ALA allows for the real-time visualization of tumor-infiltrated tissue with exceptionally high sensitivity, specificity, and positive predictive values.[17] This technique has enabled surgeons to achieve significantly more complete malignant glioma resections compared with conventional methods and, consequently, has become indispensable in neurosurgical oncology departments around the world.[17]

The complementary nature of MR spectroscopy and 5-ALA FGS is clear: spectroscopy allows the identification of tumor-infiltrated tissue via metabolic perturbations *preoperatively*, while FGS provides a method for confirming infiltration and directing the resection of tissue *intraoperatively*. However, the clinical use of spectroscopy has been limited due to various technical pitfalls, including low spatial resolution, limited field of view, and insufficient tools for spectral display and analysis. To overcome these limitations, we have developed an imaging pipeline utilizing state-of-the-art, high-resolution (0.1 cm^3 nominal voxel size) spectroscopic imaging and automated analysis tools to allow the addition of whole-brain metabolic maps to intraoperative neuronavigation.[11] Using this novel whole-brain spectroscopic MRI (sMRI) method, we performed 5-ALA FGS in a cohort of GBM patients with sMRI scans and evaluated the relationships among metabolic markers, *ex vivo* tissue fluorescence, and histological measures of tumor infiltration. We also measured recurrence and survival outcomes in patients on trial. Our aims are to provide quantitative evidence that sMRI noninvasively identifies infiltrating GBM tissue beyond the margin of contrast enhancement and to set forth clinical evidence for the use of sMRI to assist in directing surgery and RT in malignant gliomas.

Materials and Methods

Study Design

The objective of the surgical study was to describe the relationships among sMRI metrics, *ex vivo* fluorescence, and histological markers to test whether sMRI is capable of identifying infiltrating GBM tissue. Patients included in this pilot study ($N = 20$) were part of an institutional review board–approved prospective Phase II 5-ALA FGS trial at Emory University (2011–2014) for patients with malignant glioma. The trial included patients ≥18 years of age with normal bone marrow and normal renal and liver function, with KPS ≥ 60%, and able to provide written informed consent. Patients with deep-seated tumors, receiving experimental therapies before surgery, and with a family history of porphyrias were excluded. Tissue excised in a biopsy-like fashion from metabolically abnormal regions was analyzed as the primary endpoint. Progression-free survival (PFS; in days) was measured as a secondary outcome in those patients who had recurred after the standard of care ($n = 11$) per the updated Response Assessment in Neuro-Oncology (RANO) criteria.[4] Survival data were frozen at August 2015 and the date of recurrence was retrospectively determined by a board-certified neuroradiologist backdating to the earliest known recurrence. Preoperative necrotic, T1w-CE, and T2w-hyperintense tissue along with T1w-CE tissue at recurrence were segmented semi-automatically with a previously described method and confirmed by a neuroradiologist.[18] Banked nonneoplastic tissue ($n = 24$ slides) from patients who had received surgery on a previous institutional review board–approved study for treatment-refractory seizures were collected as controls.

The RT recurrence study was meant to survey the location of recurrence relative to sMRI abnormalities before the start of RT. All patients ($n = 13$) were part of a separate institutional review board–approved prospective Phase II sMRI-RT trial at Emory University (2014–2015). Inclusion criteria were the same as the FGS trial, and individuals with MRI-incompatible implants, medical conditions that compromise RT tolerance, or previous cranial radiation were excluded. Patients were scanned with the sMRI sequence ≤1 week prior to the beginning of RT and monitored every 1–3 months after completion of RT with standard MRI. Recurrence was determined according to the RANO criteria, and location of recurrence was noted relative to pre-RT metabolic abnormalities.

Image Acquisition and Processing

Whole-brain sMRI combining 3D echo-planar spectroscopic imaging, generalized autocalibrating partially parallel acquisitions, and elliptical k-space encoding was conducted (echo time [TE]/repetition time [TR]/flip angle [FA] = 17.6 ms/1551 ms/71 degrees) on a 3 T MRI scanner with a 32-channel head coil array (Siemens Medical). This single average sMRI sequence has a scan time of ~19 min. Intracellular water signal was collected in an interleaved manner for signal normalization and registration with anatomical images. Raw data were processed using MIDAS (Metabolite Imaging and Data Analysis System)[11,19] to give DICOM (Digital Imaging and Communications in Medicine) images

with nominal voxel size of 4.4 mm × 4.4 mm × 5.6 mm. Metabolite maps generated included choline (Cho), creatine (Cr), and *N*-acetylaspartate (NAA), as well as Cho/NAA, Cho/Cr, and NAA/Cr ratio maps. Also acquired were T1-weighted 3D magnetization-prepared rapid-acquisition gradient echo (1 mm^3, TR/TE/FA = 2300 ms/3.4 ms/9 degrees), T2w fluid-attenuated inversion recovery (FLAIR) images (TR/TE/FA = 10 000 ms/121 ms/90 degrees), and diffusion-weighted images (TR/TE/FA = 5400 ms/105 ms/90 degrees, b = 0/1000). Spectroscopic MRI maps were then imported into VelocityAI (Varian Medical Systems), an FDA 510(k)–cleared image analysis suite for the processing of multimodal medical images, for registration to the surgical planning MRI and resampling into the planning MRI space.

Tissue Sampling and Fluorescence Measurement

5-Aminolevulinic acid (Gliolan, Medac) was administered to patients orally (20 mg/kg body weight) 3–5 h before surgery. Cho/NAA ratio maps were coregistered with surgical planning MRIs and imported into the StealthStation neuronavigation system (Medtronic) to guide tissue sampling. Anatomy from water signal maps was visually compared with high-resolution anatomical imaging to verify coregistration accuracy. Specimens (1–2 per patient) were sampled in a biopsy-like fashion from areas exhibiting elevated Cho/NAA and visible fluorescence using a location-reporting probe before surgical debulking, in order to minimize navigation error due to resection-related brain shift (Fig. 1A–C). Twenty-six tissue specimens (11 from contrast-enhancing tissue, 11 from T2w/FLAIR abnormal tissue, and 4 from tissue devoid of either abnormality) were sampled in this manner. Multiple fluorescence measurements (3–5 per sample) were made immediately *ex vivo* using a hand-held spectrometer with the tip 5–10 mm from the tissue.[20]

Sox2 Immunohistochemistry

Paraffin-embedded 5-μm sections of sampled tissue were stained for tumor infiltration using a marker for SRY (sex determining region Y)-box 2 (Sox2). Immunohistochemistry against Sox2 was performed with rabbit monoclonal antibody (1:500, ab92494; Abcam) according to the manufacturer's instructions (Dako). Visualization was established using Dako EnVision+ Dual (mouse and rabbit) Link System-HRP (K4061) and diaminobenzidine (K3467; Dako), and slides were counterstained with hematoxylin. Samples from control patients were stained similarly.

Automated Histology Slide Analysis

Tumor infiltration in terms of Sox2 density (Sox2-positive area/total tissue area) was quantified using automated, whole-slide

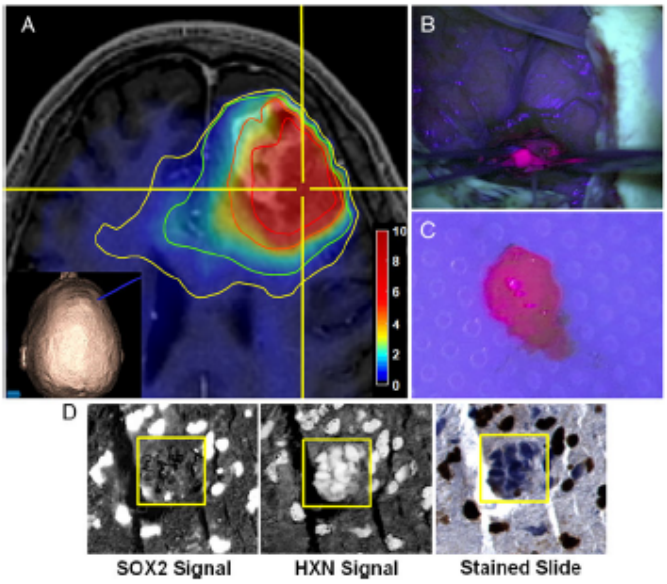


Fig. 1. Procedure for tissue sampling and histological analysis using sMRI and 5-ALA FGS. (A) View of anatomical and metabolic data in neuronavigation station with Cho/NAA ratio contours (yellow, 1.5-fold; green, 2-fold; orange, 5-fold; red, 10-fold increases in Cho/NAA over normal contralateral white matter). The inset image shows the 3D reconstruction of the patient surface anatomy along with the navigation probe (blue). (B) The region of metabolic abnormality was identified using a stereotactic technique with a location-reporting probe, and fluorescence was visualized using intraoperative microscopy. (C) Tissue was sampled in a biopsy-like fashion before debulking, and fluorescence was measured *ex vivo*. (D) Automated nuclear segmentation, digital unmixing (pictured), and nuclear classification using machine-learning techniques allowed the generation of a Sox2 density metric that was correlated with sMRI and *ex vivo* fluorescence signal. HXN, hematoxylin. Color bar depicts fold changes.

image analysis. Sox2-stained sections were digitized at 40× magnification using Hamamatsu's High-Resolution Nanozoomer 2.0HT Whole-Slide Scanner. Automatic segmentation of nuclear boundaries was performed by digitally deconvolving hematoxylin and Sox2 signals into separate image channels (Fig. 1D). An adaptive Gaussian mixture model was trained to classify image pixels into glass, tissue, and nuclear regions using maximum likelihood optimization of the hematoxylin signal. A graph-cutting approach was used to smooth nuclear segmentation, while a marker-based watershed method was used to separate clumped nuclei. Features extracted from each nucleus were used to train a random forest classifier to label each as Sox2-positive or -negative. Total tissue area was computed as the sum of nuclear and tissue areas. Sox2 and total tissue areas were generated by multiplying the number of pixels classified as Sox2-positive or tissue, respectively, by the pixel dimensions (0.5 μm × 0.5 μm).

Spectroscopic MRI–Sox2 Analysis

Each set of coregistered metabolite volumes was imported into MATLAB (v8.4.0; MathWorks) for preprocessing and analysis. Contralateral white matter contours were used to estimate normal brain signal parameters and generate standardized maps of the abnormality index for each metabolite (AI_{metab}) (Fig. 2). To account for potential navigation error, sMRI values to be correlated with Sox2 density were sampled from AI_{metab} maps using an 8 mm isotropic region of interest (ROI) centered on the location of tissue extraction (Fig. 2C, blue box).

Statistical Methods

Statistical analyses were performed with the MATLAB Statistics and Machine Learning Toolbox, were 2-sided, and had significance set at $P \leq .05$. Differences in normal and tumor tissue Sox2 densities were evaluated using a one-way ANOVA with Tukey–Kramer's multiple comparisons correction. Standardized AI_{metab} maps were generated for each sMRI volume using a linear scaling function (Fig. 2). Mean differences and effect size for metabolite abnormalities in necrotic, contrast-enhancing, and T2w-hyperintense regions were evaluated using a MANOVA. All fluorescence measurements for a piece of tissue were averaged to generate a mean fluorescent signal. Correlations among sMRI markers, mean *ex vivo* fluorescence, Sox2 density, and PFS were evaluated using Pearson's correlation coefficient (ρ) with a null hypothesis of no correlation.

Results

Spectroscopic MRI Shows Metabolic Abnormalities beyond Anatomical MRI

Coregistration of sMRI maps with conventional MRI allows the display of sMRI-detectable metabolites throughout the entire brain and illustrates the metabolic heterogeneity within GBM tumors (Fig. 3A and B). The signal-to-noise ratio (SNR) for the sMRI sequence, calculated as the mean area under the NAA peak divided by the peak-to-peak noise, is outstanding (SNR = 39.7 ± 14.7), resulting in highly sensitive, quantitative metabolite maps (see Supplementary Fig. S1 for spectra). In the case pictured, increases in Cho, a marker of membrane synthesis and cellular proliferation, and decreases in NAA, a marker of neuronal integrity, expand well beyond the T1w-CE and T2w signal abnormalities, indicating the potential infiltration of tumor cells across the genu of the corpus callosum. Conversely, Cr, a marker of cellular energetics, remains relatively unchanged, with the exception of the central necrotic portion of the tumor, where it is nearly absent. The intracellular water signal is acquired in an interleaved fashion with the spectral data (Fig. 2C) and serves as a source of anatomical features for affine

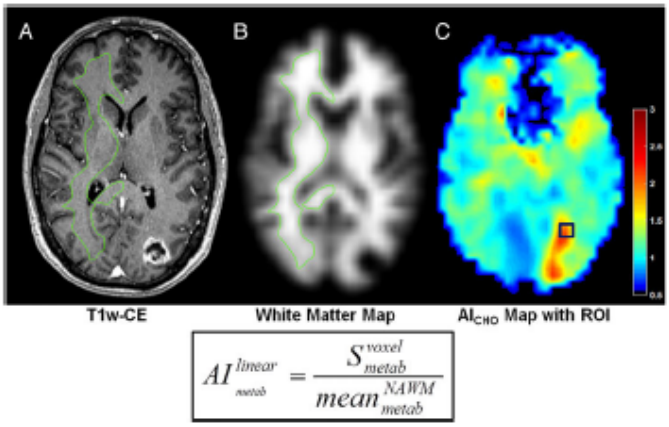


Fig. 2. Metabolite signal normalization scheme used for sMRI analyses. (A and B) Normal appearing white matter (NAWM) in hemisphere contralateral to the tumor was segmented using a white matter probability map to estimate mean normal brain signal. (C) Normalized AI maps by linear scaling using the value in this contour. These are presurgical images of the patient in Fig. 5C. AI_{CHO}, normalized choline abnormality index map; green contour, NAWM segmentation. Color bars depict fold changes.

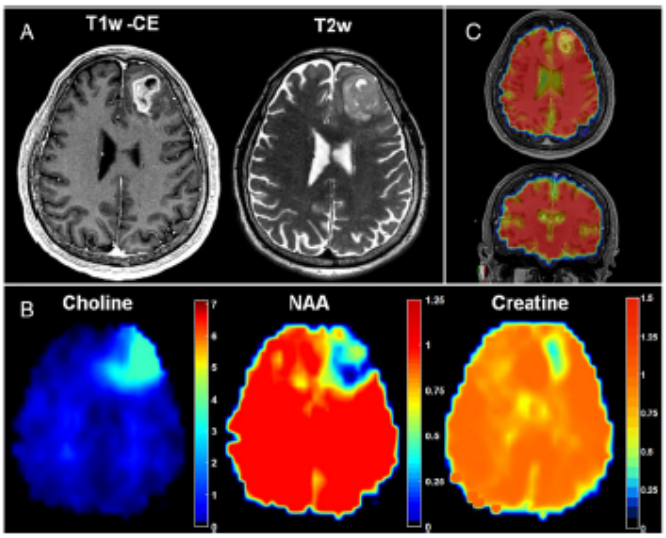


Fig. 3. Spectroscopic MRI quantitatively maps small-molecule metabolism throughout the entire brain and describes metabolic abnormalities outside of conventional anatomical MRI. (A and B) Metabolite maps (Cho, NAA, and Cr) show abnormalities beyond T1w-CE or T2w imaging and give insight into the metabolic heterogeneity of the tumor and surrounding tissue. (C) Internal water signal is used as a denominator of metabolite signal, allowing the generation of absolute metabolite concentrations. Color bars depict fold changes.

image registration, as well as a denominator for the absolute quantification of metabolite concentrations.

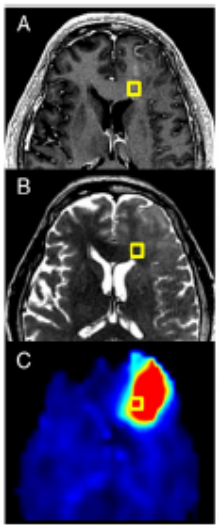
Over 1.3 million voxels from segmented necrotic, contrast-enhancing, and T2w-hyperintense tissue ROIs were evaluated for metabolic abnormalities. The average fold change in each metabolite for all 20 presurgical patients can be found in Supplementary Table S1 along with the percent variance in each region explained by metabolic changes (ie, effect size for group mean difference, η^2). There was a statistically significant difference in the mean metabolite abnormalities observed in each ROI from these patients, indicating that each exhibits a distinct metabolic profile (Wilks' lambda [Λ; first dimension] = 0.64, $P < .001$; Wilks' Λ [second dimension] = 0.94, $P < .001$). Though each imaging value exhibited a statistically significant contribution, only Cho/NAA, Cho/Cr, and NAA exhibited moderate to strong effect in differentiating each ROI ($\eta^2 = 0.11$, 0.21, and 0.10, respectively).

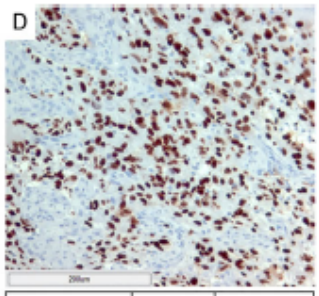
Integration of Spectroscopic MRI into the Neuronavigation System

Maps of sMRIs were integrated into the surgical neuronavigation station by fusion with standard anatomical MRIs to allow real-time guidance of surgeons to metabolically abnormal tissue (Fig. 1A). Each sMRI map was resampled into the anatomical MRI space for transfer to the neuronavigation system. Fusion of these images with the neuronavigation system's fiducial coordinate system allows the real-time guidance of surgical instruments to a selected target with exceptional accuracy. Contours representing various degrees of metabolic abnormality to be targeted are generated to describe abnormality thresholds.

Automated Histology Slide Image Analysis Gives an Objective Marker of Tumor Infiltration

An automated whole-slide image analysis approach was developed to objectively quantify the density of immunostained tumor cells in tissue specimens. Sox2 is a transcription factor known to maintain pluripotency in stem cells; however, immunohistochemistry for Sox2 shows remarkable specificity for infiltrating neoplastic cells in glioma.[21] A hematoxylin counterstain allows the delineation of nuclear boundaries; and the deconvolution of hematoxylin and Sox2 signals into separate image channels with digital unmixing allows the automated classification of Sox2-positive and -negative populations (Fig. 1D).[22] Sox2 density (mm^2 Sox2/mm^2 tissue), a quantitative metric of tumor infiltration in a tissue section, can then be calculated as the area of Sox2-positive nuclei over the area of total tissue on each slide. Using this method, the Sox2 densities in GBM (contrast-enhancing and nonenhancing regions) from Cho/NAA abnormal regions and control tissue samples were found to be 0.037 ± 0.048, 0.035 ± 0.040, and 0.001 ± 0.0009 mm^2 Sox2/mm^2 tissue, respectively. Sox2 densities in nonenhancing and enhancing tissue were found to be significantly elevated relative to tissue acquired from controls ($P < .01$ and $P < .001$, respectively), although no significant difference in Sox2 density was observed between them ($P = .97$). Although too few were acquired for a properly powered comparison ($n = 4$), as sampling was generally targeted within T2w abnormal regions, tissue samples outside of T2w hyperintensities also exhibited elevated Sox2 density, with a mean of 0.065 ± 0.040 mm^2 Sox2/mm^2 tissue (Fig. 4A–D). Objective histological analysis not only confirms that tumor infiltration occurs in regions with Cho/NAA abnormalities regardless of





E

Biomarker	ρ	*P* value
NAA	−0.42	.04*
NAA/Cr	−0.56	<.01*
Cho	0.69	<.001*
Cho/NAA	0.82	<0001*
DWI-ADC	0.18	.39

Fig. 4. A normalized metric of tumor infiltration, Sox2 density, identifies tumor outside of conventional imaging and exhibits striking correlations with sMRI biomarkers. Though no obvious abnormality can be found on preoperative T1w-CE (A) or T2w imaging (B) in this patient, a striking elevation in Cho/NAA (C) on sMRI suggests substantial tumor infiltration. (D) A light micrograph of tissue (including 200 μm scale bar) from the gold box showed elevations in Sox2 density along with the microvascular proliferation and nuclear atypia suggestive of GBM. (E) Statistically significant correlations were seen between various normalized metabolic markers and Sox2 density, with Cho/NAA exhibiting the strongest association. *Significant at $P < .05$. DWI-ADC, diffusion-weighted image–apparent diffusion coefficient.

contrast enhancement, but also suggests that elevations in Cho/NAA may be able to identify infiltration beyond T2w abnormalities.

Spectroscopic MRI Markers Exhibit Significant Correlations with Sox2 Density

AI_{metab} maps depicting standardized metabolic changes between patients were used to evaluate the correlation between sMRI markers and Sox2 density. Significantly elevated Sox2 densities were found in all tissues from Cho/NAA abnormal regions exhibiting T1w-CE and T2w abnormalities, as well as in tissue outside of both abnormalities. Cho/NAA and Cho were the markers most highly correlated with Sox2 density, exhibiting strong, statistically significant associations (Fig. 4E and Supplementary Table S2). NAA and NAA/Cr maps both exhibited moderate negative correlations, while Cho/Cr and Cr did not exhibit significant correlations with Sox2 density ($\rho = 0.35$, $P = .08$ and $\rho = 0.24$, $P = .23$, respectively). In addition, the apparent diffusion coefficient, a diffusion-weighted imaging marker generally associated with the cellularity of tissue, did not exhibit significant correlation with Sox2 density ($\rho = 0.18$, $P = .39$). The coupling of stereotactic tissue sampling with objective histological analysis suggests a striking relationship between sMRI metabolic abnormalities and the infiltration of GBM into normal-appearing brain tissue.

Ex vivo Tissue Fluorescence Correlates with sMRI Markers and Sox2 Density

The average fluorescence signal measured in tumor specimens *ex vivo* was compared with both sMRI markers and Sox2 density. Fluorescence was measurable in all tissues from Cho/NAA abnormal regions with an average fluorescence signal of $2.15 \times 10^6 \pm 1.29 \times 10^6$. These measurements exhibited a strong, statistically significant correlation with Sox2 density ($\rho = 0.64$, $P = 5E\text{-}6$). Furthermore, Cho/NAA and Cho exhibited statistically significant associations with *ex vivo* fluorescence ($\rho = 0.36$, $P < .0001$; $\rho = 0.40$, $P < .001$). Thus, not only is *ex vivo* fluorescence highly associated with a histological marker of tissue infiltration (Sox2 density), but it also exhibits significant associations with metabolic markers generated preoperatively with sMRI.

Cho/NAA Identifies Regions at High Risk for Tumor Recurrence

All patients in this analysis had histopathologically confirmed GBM, completed RT (30 fractions of 2 Gy), and received follow-up care at a single institution. Five of the 13 patients on trial had documented T1w-CE progression (38.5%) as of August 2015, and each recurrence was confirmed by serial imaging or histopathology. While both Cho and Cho/NAA measures showed high correlations with infiltration, Cho/NAA was chosen to evaluate recurrence, as it is a more sensitive marker for identifying regions at risk for recurrence.[23,24] Red contours depicting 2-fold elevations in Cho/NAA (compared with contralateral normal-appearing white matter) are shown on each image to depict regions with a high likelihood of tumor infiltration.[25] This level of elevation equates to a mean Z-score of 6.62 in these patients, suggesting the identification of metabolic abnormalities with >99.999% confidence.

All patients who had tumor recurrence in the follow-up period showed contrast enhancement in regions that exhibited Cho/NAA abnormalities before RT; a few examples of this can be found in Fig. 5. Row A in Fig. 5, Cho/NAA elevation at the posterior aspect of the tumor resection cavity predates the appearance of a T1w-CE lesion in that region by roughly 5 months. This lesion continued to grow after the recurrence date, resulting in increased spatial agreement with the pre-RT 2-fold Cho/NAA abnormality (Z-score = 7.57 [tissue classified as abnormal with >99.999% confidence]). Similarly, in a patient with a large frontal GBM, pre-RT Cho/NAA abnormalities anterior to the resection cavity and even across the midline approximate later tumor recurrence volumes nearly 5 months after completion of RT (Fig. 5B). The morphology of the T1w-CE lesion continued to evolve throughout the follow-up period to further approximate the morphology of the pre-RT 2-fold Cho/NAA abnormality (Z-score = 5.75 [tissue classified as abnormal with >99.999% confidence]).

In some cases where GBM recurrence was observed, the recurrence sites exhibited clear metabolic abnormalities before surgical resection. For example, the 40-year-old patient depicted in Fig. 5C exhibited a striking tail of Cho/NAA elevation that coursed along the occipital horn of the left lateral ventricle even

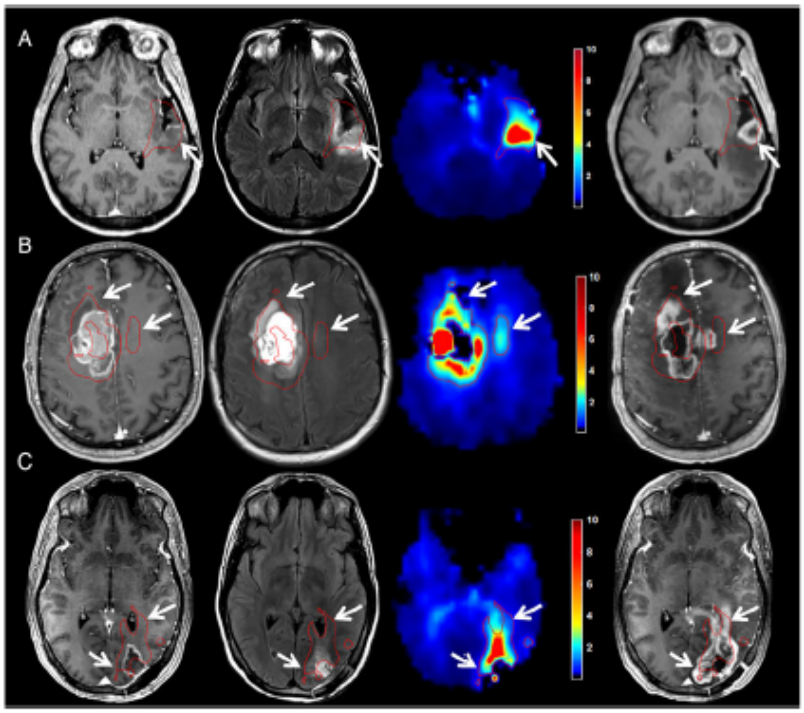


Fig. 5. Abnormalities in Cho/NAA describe regions at high risk for recurrence before RT in GBM. Coregistered T1w-CE (first column), T2w/FLAIR (second column), and Cho/NAA (third column) images taken before RT are shown with first recurrence on T1w-CE imaging after RT (fourth column). (A) In a patient with no residual contrast-enhancing disease and a T2w abnormality that surrounded the entire resection cavity, increased Cho/NAA at the posterior aspect of the resection cavity is spatially coherent with the site of first recurrence 5 months after RT. (B) Cho/NAA abnormalities anterior and contralateral to enhancing tumor before RT predate expansion into these regions 4 months after RT. Though no T2w abnormality was found contralaterally before RT, the metabolic signature of tumor was present. (C) Pre-RT Cho/NAA map clearly shows infiltration of subependymal space that becomes contrast enhancing 4 months later. The red contour illustrates the regions that exhibit a Cho/NAA abnormality ≥2-fold higher than normal contralateral brain. Color bars depict fold changes.

before surgery (Fig. 2C). This abnormality continued to grow through the duration of RT, ultimately resulting in overt tumor invasion along the trajectory of the 2-fold Cho/NAA abnormality (Z-score = 6.53 [tissue classified as abnormal with >99.999% confidence]).

Cho/NAA Ratio in T2w-Hyperintense Regions Correlates with Progression-Free Survival

Segmentation of necrotic, T1w-CE, and T2w abnormalities using a previously described semi-automated, blob-based algorithm allowed the comparison of quantitative sMRI findings with survival outcomes.[18] When Cho/NAA statistics in each segmentation were analyzed (mean, median, maximum, etc), a striking relationship between Cho/NAA values in the T2w abnormality and PFS was identified (Fig. 6). Patients who exhibited high maximum Cho/NAA values within the T2w abnormality (not including necrosis or contrast-enhancing tissue) preoperatively had shorter PFS than those with lower values in this region ($\rho = -0.74$, $P = .012$). No trend between Cho/NAA values within necrotic or enhancing regions and PFS were found, however.

Discussion

GBM is a devastating disease, with the vast majority of patients exhibiting recurrence within 6 months despite aggressive treatment.[2] It has long been suspected that the standard treatment regimen is not optimally effective because conventional imaging does not allow reliable targeting of the entire GBM tumor: T1w-CE imaging does not identify infiltrative margins and T2w imaging is not specific for tumor tissue.[26–28] Conversely, spectroscopic techniques are not limited by contrast diffusion (unlike T1w-CE imaging) and are not obscured by the presence of edema (unlike T2w imaging).[14,15] Although prospective spectroscopy-histology studies have generated compelling results in the past, the low spatial resolution and limited field of view of sequences used in these studies, and even those currently available clinically, hinder the clinical use of the technology severely.[12,13,29–31] As such, spectroscopy has been

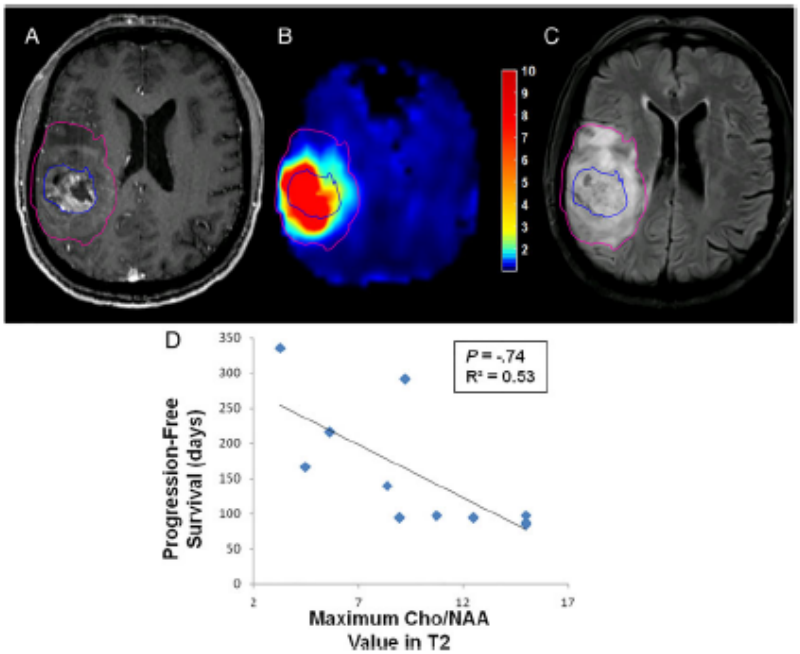


Fig. 6. Peritumoral Cho/NAA abnormalities are associated with PFS. (A–C) Patients with high maximum Cho/NAA values within the T2w-hyperintense region outside of contrast-enhancing and necrotic tissue before RT appear to exhibit poorer survival, in terms of PFS, than those (D) who have lower maximal Cho/NAA values in this region. Pink contour, T2w-hyperintense region; blue contour, T1w-CE region. Color bar depicts fold changes.

relegated to a supportive role in glioma diagnosis only. To overcome such imaging limitations, a spectroscopy pipeline utilizing a state-of-the-art pulse sequence and processing tools was developed for the generation of high-resolution, whole-brain sMRIs that are easily imported into standard intraoperative neuronavigation stations. Moreover, to overcome bias in histological interpretation, quantitative image analysis techniques were used for the objective and automated evaluation of microscopic tumor infiltration.

The combination of standardized, high-resolution metabolic mapping, precise stereotactic tissue extraction, and quantitative tissue section analysis allows the correlation of metabolic abnormality with histology at an unprecedented level of accuracy. Combining these tools, we confirm the presence of tumor-infiltrated tissue beyond T1w-CE and T2w lesions, as well as the similarity of tumor infiltration in metabolically abnormal contrast-enhancing and nonenhancing peritumoral regions. These results imply substantial tumor infiltration beyond regions conventionally targeted for resection or RT. The results also suggest that the absence of contrast enhancement does not signify the absence of bulk tumor, thus supporting previous suspicions concerning the inadequacy of anatomical imaging for therapy planning in GBM. Furthermore, sMRI metrics exhibit significant associations with 2 quantitative measures of tissue infiltration—Sox2 density and *ex vivo* tissue fluorescence—supporting the hypothesis that sMRI identifies tumor-infiltrated tissue in vivo. Lastly, Cho/NAA not only identifies regions at high risk for contrast-enhancing recurrence, but also shows a significant association with PFS in a small cohort of GBM patients. Taken together, the findings in this work represent the first in-human study to (i) combine high-resolution, whole-brain sMRI, and 5-ALA FGS for real-time intraoperative neuronavigation in GBM, (ii) describe sMRI abnormalities using both ex vivo fluorescence and quantitative histological metrics, and (iii) survey the capacity that sMRI may have for assessing clinical outcomes such as the location of recurrence and time to recurrence.

This study has limitations common to other pilot neurosurgical studies: small sample sizes for both tissue sampling and recurrence analyses. However, even given the sample size, the analytical techniques are robust enough to describe striking relationships among histology, tissue fluorescence, and sMRI markers. Moreover, the recurrence and PFS data remain unchanged, further supporting the claim that sMRI can identify tumor-infiltrated tissue. Lastly, we were unable to sample normal tissue outside of Cho/NAA abnormal and fluorescent regions in FGS patients, making it impossible to determine baseline Sox2 densities and diagnostic accuracy. This is a problem common to neurosurgical studies, as it is considered negligent to acquire normally functioning tissue, which often requires sampling of the contralateral hemisphere in GBM, at the risk of causing extensive neurological injury. Though unfortunate, this is a component of neurosurgical studies that is insurmountable and thus is accepted in the field. Even in light of these limitations, the data support the claims that sMRI can

identify brain regions that are tumor infiltrated, regions that are at high risk for recurrence, and regions that could be specifically targeted with surgery and RT in an attempt to decrease the likelihood of local progression in GBM.

High-resolution, whole-brain sMRI could prove to be an excellent method for obtaining the complementary metabolic information necessary to preoperatively identify sites of significant tumor infiltration and to direct 5-ALA FGS to tumor-infiltrated regions that appear normal on conventional MRI. Furthermore, as the intensity of metabolic abnormality in the T2w-hyperintense component of the tumor is shown to be associated with progression, the expansion of high-dose RT boost volumes ($\geq$60 Gy) to encompass these regions may possibly decrease the rate of local recurrence as well. Apart from GBM, a number of other intracranial tumors lie within the exciting potential of sMRI to improve their diagnosis, targeting, and response assessment. This is especially true for lower-grade gliomas, which often do not contrast-enhance on T1w-CE MRI, making surgery and RT target planning difficult. Thus, the addition of sMRI to RT dosage planning in low-grade glioma could result in a brand new, clinically important target ROI. Most importantly, the addition of sMRI to the surgical and RT management of gliomas would represent a paradigm shift in the field of image-guided therapy away from targeting surrogate markers using tracer-based imaging techniques (eg, contrast enhancement, standardized tracer uptake in PET) to targeting abnormal tissue regions by measuring endogenous biological processes.

Encouragingly, many of the technical pitfalls of sMRI implementation that have plagued its clinical implementation in the past have now been surmounted, and further development of more sophisticated sMRI analysis and integration pipelines appears promising. Although further standardization and automation of the clinical workflow is required, tracer-independent metabolic mapping with sMRI would provide accurate brain tumor metabolism information to neurosurgeons and radiation oncologists treating glioma patients. The clinical integration of sMRI into therapy planning and response assessment in glioma would represent a paradigm shift in the management of these patients, potentially giving physicians a new tool to improve survival with this debilitating disease beyond the current standard of care.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

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Conflict of interest statement. C.G.H. has received intellectual fees from Nx Development Corp. C.G.H. is also a consultant for Nx Development Corp.

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