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Mycophenolate Mofetil Combined With Radiation Therapy in Glioblastoma

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PHASE 0/1 DOSE ESCALATION STUDY OF MYCOPHENOLATE MOFETILCOMBINED WITH RADIATION IN GLIOBLASTOMA

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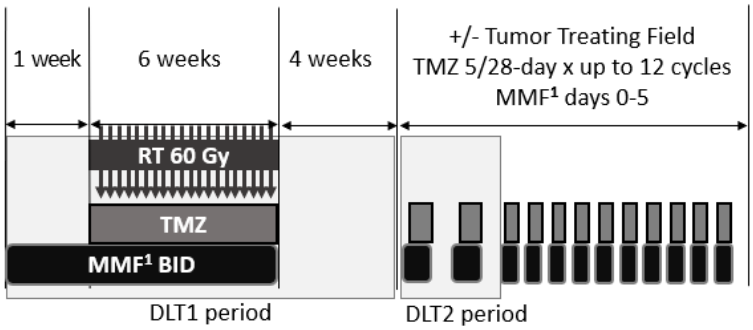
ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AST	Aspartate Aminotransferase
BID	Twice per day
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTSU	Clinical Trials Support Unit
DLT	Dose Limiting Toxicity
DSMC	Data and Safety Monitoring Committee
GBM	Glioblastoma
GTP	Guanosine triphosphate
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IND	Investigational New Drug
IRB	Institutional Review Board
IV (or iv)	Intravenously
KPS	Karnofsky Performance Status
MMF	Mycophenolate Mofetil
MTD	Maximum Tolerated Dose
NANO	Neurologic Assessment in Neuro-Oncology
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
p.o.	per os/by mouth/orally
PR	Partial Response
RANO	Response Assessment for Neuro-Oncology
RT	Radiation Therapy
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase

TMZ	Temozolomide
UaP	Unanticipated Problem
WBC	White Blood Cells

STUDY SCHEMA

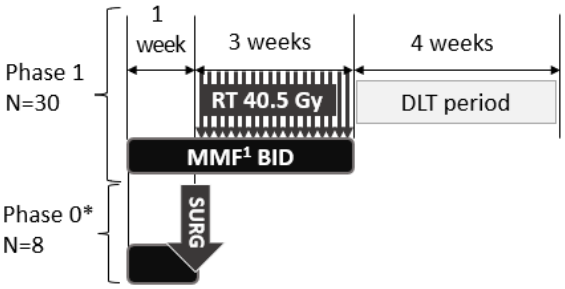
Newly diagnosed GBM Phase 1: N=30



[1]Phase 1 MMF dose levels (TITE-CRM)
1: 250mg BID
2: 500mg BID
(Starting) 3: 1000mg BID
4: 1500mg BID
5: 2000mg BID

*Phase 0 MMF dose (n=2 per arm)
Arm A: 500mg BID
Arm B: 1000mg BID
Arm C: 1500mg BID
Arm D: 2000mg BID

Recurrent GBM Phase 0/1



Abbreviations:

GBM	Glioblastoma
MMF	Mycophenolate mofetil
TMZ	Temozolomide
RT	Radiotherapy
DLT	Dose limiting toxicity
SURG	Surgery

STUDY SYNOPSIS

Title	PHASE 0/1 DOSE ESCALATION STUDY OF MYCOPHENOLATE MOFETIL COMBINED WITH RADIATION IN GLIOBLASTOMA
Phase	Phase 0 & 1
Methodology	Open label, single center, dose escalation study
Study Duration	Projected accrual will begin May 2020 and end May 2024. Follow up will end three years after the accrual of the last patient.
Study Center(s)	University of Michigan
Objectives	Primary objectives: - Phase 0: To measure the concentration of mycophenolic acid (MPA) in tumors from patients who undergo re-resection for Glioblastoma (GBM). - Phase 1 Recurrent GBM: To determine the dose-limitingtoxicity (DLT) and maximally tolerated dose (MTD) of Mycophenolate Mofetil (MMF) when combined with re-irradiation in patients with recurrent GBM. - Phase 1 Newly Diagnosed GBM: To determine the DLT and MTD of MMF when combined with standard upfront chemoradiation in patients with newly diagnosed GBM. Secondary objectives: - Phase 0: To determine GTP (guanosine triphosphate) concentrations in recurrent GBM tissue re-resected after 1 week of MMF administration. - Phase 1: To describe the adverse events associated with MMFwhen administered with re-irradiation in recurrent GBM patients, and with standard upfront chemoradiation in newly diagnosed GBM. - Phase 1: To compare the overall survival of patients with GBMtreated with MMF and radiation to historical controls - Phase 1: To compare the patterns of recurrence after radiation with concurrent MMF to patterns of failure in patients treated with radiation without MMF.
Number of Subjects	Phase 0: 8 Phase 1 (recurrent): 30 Phase 1 (newly diagnosed): 30

Study Product(s), Dose, Route, Regimen	Mycophenolate mofetil, 500-2000mg orally twice daily (phase 0); Mycophenolate mofetil, 250-2000mg orally twice daily (phase 1 Recurrent arm) Mycophenolate mofetil, 250-2000mg orally twice daily (phase 1 Newly Diagnosed arm)
Duration of Administration	1 week prior to re-resection (Phase 0) 1 week prior to, and concurrent to re-irradiation (Phase 1 Recurrentarm) 1 week prior to, and concurrent to upfront chemoradiation and cyclic chemotherapy (Phase 1 newly diagnosed arm)
Statistical Methodology	TITE-CRM (Section 11)

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

Glioblastoma is the most common and aggressive primary brain tumor in adults. Gliosarcoma is a diagnosis closely related to, and treated identically to glioblastoma in standard of care. Upon diagnosis, patients first undergo surgical resection followed by radiation therapy with concurrent and adjuvant temozolomide (TMZ) [1]. Despite these standard treatments, most patients recur within one year of diagnosis and fewer than 10% live beyond five years.

When a GBM recurs following initial treatment there is no consensus standard of care. Treatment options include re-resection, re-irradiation, initiation of anti-angiogenic therapy, next-line cytotoxic chemotherapy or a clinical trial. Treatment decisions at the time of recurrence depend on the clinical context. For example, re-resection is frequently used to pathologically confirm recurrence or when re-resection will leave little residual disease[2, 3]. Re-irradiation is frequently used if the recurrence is localized and a sufficient length of time (e.g., >6 months) has passed since the initial course of radiation[3]. Next line systemic therapies are used when failures are early or multifocal. Anti-angiogenic therapy may be preferred when there is symptomatic vasogenic edema.

Re-irradiation of recurrent GBM has been used clinically for decades [4-6]. A single institution experience from the University of Heidelberg utilized a radiation dosing schema of 36 Gy in 2 Gy fractions in 53 patients with recurrent glioblastoma. Radiation was given without concurrent chemotherapy and resulted in a median survival of 8 months from the time of re-irradiation without significant treatment-related toxicity [7]. Similar results were seen in the largest case series to date from Thomas Jefferson University. In this study, 147 patients with recurrent GBM were treated with hypofractionated reirradiation to a total dose of 35 Gy in 3.5 Gy fractions. Most received conventional initial therapy (60 Gy with concurrent temozolomide) and nearly 1/3 received concurrent chemotherapy with hypofractionated radiation. Treatment was well-tolerated with no hospitalizations for acute or delayed toxicities and one single patient experiencing a late grade 3 CNS toxicity (headaches). Median survival from time of re-irradiation was 10-11 months. The results these studies compare favorably to overall survivals for systemic therapy alone or the anti-angiogenic therapy bevacizumab [8, 9]. Because of this safety and efficacy, re-irradiation (35 Gy in 3.5 Gy fractions) is currently being evaluated in phase 3 clinical trials (RTOG 1205) in comparison with bevacizumab monotherapy and results are pending.

Despite the efficacy of radiation in both newly diagnosed and recurrent GBM, radiation resistance remains an important barrier for improving clinical outcomes. In primary GBM, approximately 80% of patients recur within the high dose radiation field[10, 11]. In recurrent GBM that is re-irradiated, the patterns of failure appear similar. Indeed, a single institution study of re-irradiation in 31 patients with GBM showed that only 16% of cases had recurrence outside of the radiation field [12]. A larger study of 109

patients who underwent re-irradiation for recurrent GBM was presented in 2015 and showed that 86% of patients recurred within the re-irradiated field [13]. These findings suggest that strategies to overcome radiation resistance are likely to improve outcomes in patients with both primary and recurrent GBM.

Efforts to radiosensitize GBM using molecularly targeted therapies designed to inhibit oncogenic alterations in the tumor have been unsuccessful, likely due to the profound heterogeneity in genomic alterations that drive different cells and regions within an individual GBM [14, 15]. Initial studies from our group and others suggest that there may be common *metabolic* phenotypes that mediate RT resistance in GBM across heterogeneous genotypes [16, 17]. To further investigate this hypothesis, we determined the RT resistance of 23 genomically-distinct cell line models of GBM using the clonogenic assay. In parallel, we performed targeted metabolomic analysis on these cell lines during unperturbed exponential growth. We then performed metabolite- and pathway-level correlations to determine how metabolic phenotypes were associat[illegible]e. Numerous metabolites involve[illegible]te synthesis (inosinates and guanyla[illegible]orrelated with RT resistance, while th[illegible]gy production were associated with radiation sensitivity (**Figure[illegible]**M cell lines further increased guanylates such as GTP immedi[illegible]uggesting that RT may also directly regulate guanylate

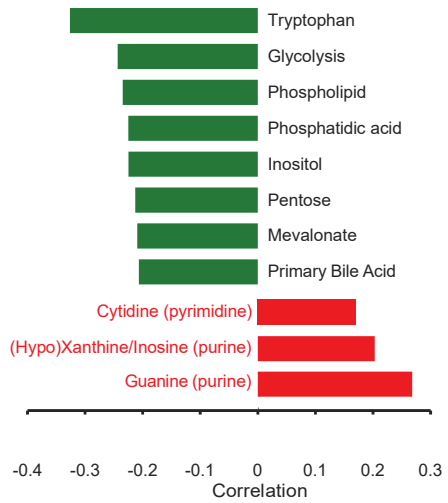


Figure 1. Metabolic pathways significantly associated with radiation resistance (red) or sensitivity (green) in growing unperturbed IDHwtGBM cell lines.

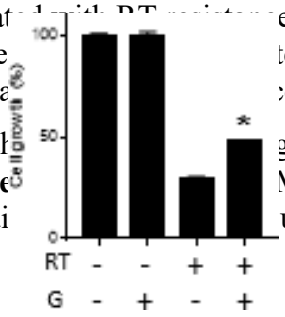


Figure 2. A. IDHwt GBM HF2303 neurospheres were treated with Guanosine (G, 50 μM) or control for 24 hours, irradiated with 6 Gy RT and then replated as single cells., Viability was determined 8 days later using celltiterGlo.* indicates p<0.05 compared to RT alone

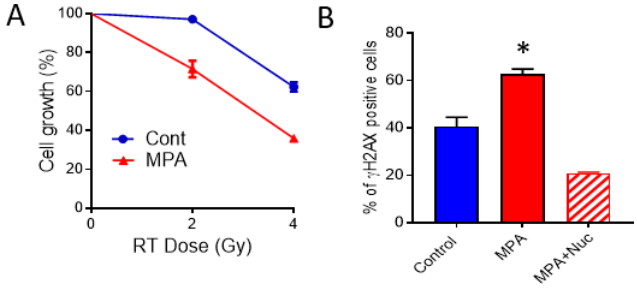


Figure 3. A. IDHwt GBM HF2303 neurospheres were treated with mycophenolic acid (MPA, 10 μM) or control for 24 hours, irradiated with varying doses of RT and then replated as single cells. After 7-14 days, viability was determined using celltiterGlo. **B**. HF2303 spheres were treated with MPA or control for 24 hr and then received a single dose of 4 Gy RT. Six hours after RT, spheres were fixed and stained for γH2AX foci by immunofluorescence. * indicates p<0.05. The ability of MPA to potentiate RT-induced γH2AX foci was reversed by pooled exogenous nucleosides (Nuc)

production**.** Increased guanylate levels are causally related to GBM RT resistance. Treating guanylate-poor RT-sensitive IDHwtGBM cell lines and neurospheres **(Figure 2)** with guanosine protected them from RT and promoted the repair of RT-induced DNA damage. Conversely, inhibiting *de novo* guanylate synthesis with clinically-achievable concentrations of mycophenolic acid (MPA) sensitized multiple GBM cell lines and patient-derived neurospheres **(Figure 3)** to RT by slowing DNA repair in a nucleoside-dependent fashion. Mycophenolate mofetil (the FDA-approved prodrug of MPA) inhibited the ability of flank and orthotopic IDHwtGBM xenografts to increase guanylates after RT and potentiated RT efficacy by increasing DNA damage (**Figure 4).** Depleting pyrimidines with the dihydroorotate dehydrogenase inhibitor teriflunomide did not recapitulate these findings, which suggests that MMF is not acting through non-specific nucleotide depletion. Together, these studies demonstrate a potential reciprocal relationship where RT increases guanylate levels, which in turn promotes DNA repair and RT resistance [18]

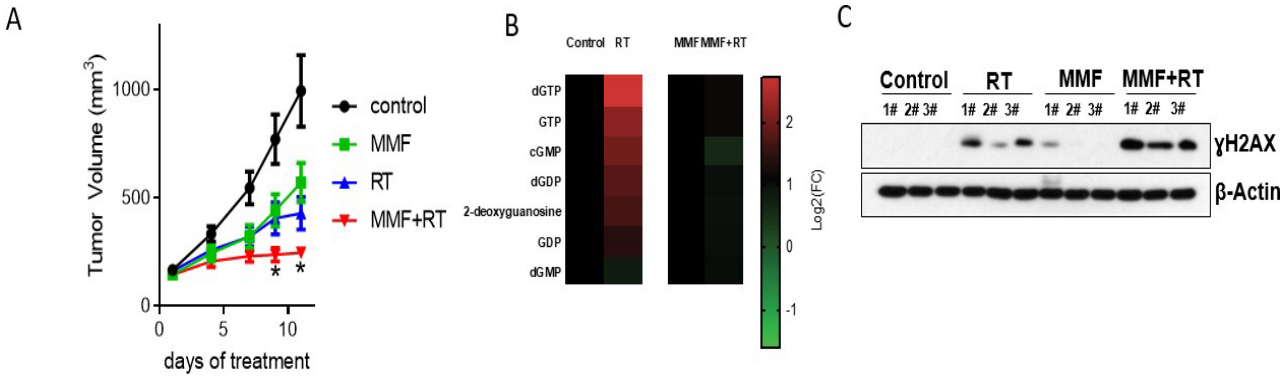


Figure 4. IDHwtGBM xenografts were allowed to grow to ~100 mm^3 in the flanks of immunosuppressed mice and then randomized to receive no treatment, RT (2 Gy x 5), MMF (Mycophenolate Mofetil, the orally bioavailable prodrug of MPA) or RT+MMF. A. Tumor size was measured 2-3x weekly * indicates p<0.05 compared to MMF or RT alone. B and C. Two hours after the second RT dose, three tumors per group were harvested and samples flash frozen for analysis of guanylate levels by mass spectrometry (B) or γH2AX to assess for DNA damage (C).

A recent manuscript suggests that MMF may also improve the efficacy of temozolomide (TMZ), which is the standard chemotherapeutic agent used in GBM [19]. Indeed, TMZ treatment epigenetically upregulates the ciliary GTP-regulated protein ARL13B, which physically associates with the GTP-producing enzyme IMPDH2, one of the molecular targets of MMF. TMZ treatment promotes the activity of IMPDH2 and *de novo* purine synthesis and MMF potentiates the efficacy of TMZ in orthotopic PDX models of GBM [19]. Thus, MMF may increase the efficacy of both primary standard of care treatments for GBM: TMZ and RT.

Therefore, we now propose to investigate the use of MMF in conjunction with RT and temozolomide for patients with GBM to determine if the drug achieves active concentrations in brain tumors and is well tolerated (and potentially efficacious) when combined with radiation and temozolomide.

1.2 Study Agent(s) Background and Associated Known Toxicities

In this study, we will combine re-irradiation, a well-tolerated standard-of-care treatment for recurrent GBM, and mycophenolate mofetil (MMF), an inhibitor of *de novo* GTP synthesis that is FDA approved to prevent organ rejection. As outlined below, the known toxicities of these two treatments are non-overlapping, suggesting that their combination may be safe[20]. In support of this hypothesis, a small pilot study of palliative radiation combined with mycophenolic acid performed in the 1970s reported no severe toxicities [21].

Standard of care for newly diagnosed GBM. Radiation has been a standard of care treatment for GBM since the 1970s with a standard dose of 60 Gy [22]. The Stupp trial established the alkylating agent temozolomide as an additional standard of care treatment. Typically, this is given concurrently with daily radiation followed by monthly cycles for 6-12 months. Patients who have MGMT promoter methylation derive the greatest benefit from TMZ, but patients with unmethylated MGMT promoters also receive temozolomide in most cases[23]. Recently, a randomized trial showed that treatment with an array of electrodes affixed to the scalp ("termed tumor treating fields") also provides a small survival benefit in GBM. Many patients choose not to receive treatment with tumor treating fields due to the burden associated with treatment. Despite these intensive treatments, survival of patients with newly diagnosed GBM remains poor, with medians survivals estimated at 12-15 months in patients with unmethylated MGMT promoter and 22-24 months for those with methylated MGMT promoters[24]. Toxicities associated with radiation include alopecia, fatigue, wound break down and radiation necrosis and cognitive decline. Common side effects with temozolomide include mild cytopenias, and typically manageable nausea and constipation. Common side effects with tumor targeting fields include dermatologic toxicity and headache[24].

Re-irradiation for recurrent GBM

In the largest series to date, 147 patients with recurrent GBM were treated with hypofractionated reirradiation to a total dose of 35 Gy in 3.5 Gy fractions targeted to the tumor volumes with abnormal contrast-enhancement on MRI[25]. Most received conventional initial therapy (60 Gy with concurrent temozolomide) and nearly 1/3 received concurrent chemotherapy with hypofractionated radiation. Treatment was well-tolerated with no hospitalizations for acute or delayed toxicities and a single patient experiencing a late grade 3 CNS toxicity (headaches). Median survival from time of re-irradiation was 10-11 months.

The University of Heidelberg reported on 53 patients with recurrent GBM treated with reirradiation[7]. The median interval between initial treatment and reirradiation was 10 months. Reirradiation was given as 36 Gy in 2 Gy fractions and was targeted to the regions of tumor with abnormal contrast enhancement with an additional 1 cm CTV margin. No patient received concurrent chemotherapy. The median survival following reirradiation was 8 months. No patient developed a grade 3 or greater toxicity.

A smaller study of 44 patients with recurrent GBM from the University of Munich showed similar results[4]. Patients with GBM who recurred 3 months or greater from their initial course of radiation whose tumors were between 0.5-4.5 cm in diameter were prospectively treated with reirradiation and concurrent temozolomide. Radiation was given as 30 Gy in 5 fractions and targeted to both areas of abnormal contrast-enhanced MRI signal and abnormal amino acid tracer signal. The median survival following reirradiation was 8 months. Acute grade 1-2 neurologic toxicity developed in 14% of patients and no patients developed acute grade 3 or higher acute neurologic toxicity.

These studies, and other smaller case reports [5] suggest that reirradiation is both safe and effective for patients with recurrent GBM. Median survival times range between 7 and 11 months (Table 1). Expected toxicities include alopecia, but rates of grade 3 or greater acute neurologic toxicity are very low.

Study	Treatment Regimen	Median OS (from time of reirradiation)	Toxicities
Fogh et. al (n=147)	3.5 Gy x 10 fractions to contrast-enhanced tumor	10 months (grade 3, n=42) 11 months (grade 4, n=105)	N=1 late grade 3 neurotoxicity (headache)
Gilbert et. al (n=88)	6 Gy x 4 weekly fractions with concurrent paclitaxel to contrast-enhanced tumor	7 months (all grade 4)	Poorly described. Seven patients required re-operation for radionecrosis
Combs et. al (n=53)	2 Gy x 18 fractions to contrast-enhanced tumor with 1 cm CTV margin	8 months (all grade 4)	Frequent patchy alopecia, no grade 3 or greater toxicities
Grosu et. al (n=44)	6 Gy x 5 fractions on consecutive days to the contrast-enhanced tumor and regions of altered amino acid tracer uptake. Concurrent temozolomide was also used	8 months (including 8 patient with grade 3 astrocytoma)	Grade 1-2 Neurologic toxicity in 14% patients, managed with steroids. No grade3+ neurologic toxicity

Table 2: Selected results of re-irradiation for patients with high grade glioma

Mycophenolate Mofetil (MMF) Mechanism of Action and Clinical Use

MMF blocks purine synthesis by reversibly inhibiting inosine monophosphate dehydrogenase (IMPDH), the rate limiting step of *de novo* GTP synthesis [26, 27]. MMF is a prodrug of the active metabolite mycophenolic acid (MPA), which inhibits lymphocyte proliferation and antibody production and acts as an immunosuppressant [26]. MMF is indicated for the prophylaxis of organ rejection after allogenic renal,

cardiac, or liver transplants, in combination with other immunosuppressive agents such as cyclosporine, tacrolimus, everolimus, or sirolimus [28-30]. Off label uses of MMF include graft-versus-host disease, autoimmune hepatitis, antirejection therapy after lung transplant, lupus nephritis, myasthenia gravis, psoriasis, and systemic sclerosis. MMF is typically started at 500-1500mg twice daily, with the goal of 1500-3000mg daily dose. [28]

Mycophenolate Mofetil Pharmacodynamics and Pharmacokinetics

MMF exerts its therapeutic effects by depleting GTP, which is a necessary substrate for DNA, RNA and G protein activation [26, 27]. GTP can be synthesized using the *de novo* synthetic pathway, through the action of IMPDH, or via the salvage pathway, through the action of hypoxanthine guanine phosphoribosyl transferase (HGPRT, which is mutated in Lesch-Nyan syndrome). Guanosine nucleotides are necessary substrates for DNA and RNA synthesis, and cells such as B and T lymphocytes are more dependent on the *de novo* guanosine synthesis pathway [26]. Proliferating lymphocytes preferentially rely on *de novo* GTP synthesis. Thus, MMF depletes GTP in these cells leading to apoptosis [31]. Non-dividing neutrophils, which preferentially rely on salvage pathways to generate GTP, are less affected by MMF [31].

MMF is available in both oral and intravenous formulations at 1:1 dose ratio. An enteric-coated, delayed-release formulation of MMF (Myfortic®) is also available, but will not be used in this study. MMF is well absorbed after oral administration and is rapidly hydrolyzed to MPA (the active metabolite) in the liver. The area under the plasma concentration-time curve (AUC) of MMF is generally proportional to dosage, although renal impairment can increase AUC [26]. The peak plasma concentration of MPA is reached between 30 minutes to 2 hours after MMF oral administration [27]. Enterohepatic recirculation of MPA occurs and may contribute to interpatient variability in drug concentrations. MPA is ultimately glucuronidated to MPAG (an inactive metabolite), which is primarily eliminated renally [26].

Mycophenolate Mofetil CNS Penetration

There is evidence to suggest that MPA crosses the blood brain barrier and achieves effective intracranial concentrations.

The pre-clinical characteristics of MPA are promising for CNS penetration. It is likely membrane permable given its low molecular weight (320 g/mol) and lipophilic nature (consensus logP = 2.7). In murine models, accumulation of MPA in the brain was not affected by p-glycoprotein efflux [32].

These favorable chemical properties are supported by empiric evidence. In healthy mice given a single 60 mg/kg oral dose of MMF, the MPA concentration in brain reached 3-5 mg/gm of brain tissue, which is above the active concentration of 10 μM used in our and other preclinical studies [32].

In the setting of GBM, the blood brain barrier is frequently disrupted, as evidenced by the brisk contrast-enhancement seen on MRI imaging. This disruption suggests that the concentrations of MPA that accumulate in GBM tumor tissue may be higher than the concentrations in normal brain. The mass spectrometry studies performed from tissue in the Phase 0 portion of this study will attempt to answer this question.

Known Toxicities of Mycophenolate Mofetil

Adverse events attributed to long-term MMF use in clinical trials that occur at rates over 20% include diarrhea, leukopenia, infection, and vomiting [30]. Because MMF use in this trial will be short-, rather than long-term, we expect significantly less toxicity. MMF is generally better tolerated at 2000 mg/day compared to 3000 mg/day, and the rate of adverse events are felt to be dose related [26]. Gastrointestinal symptoms and bone marrow suppression are the most common side effects, but these typically resolve with dose adjustment. MMF is used widely in patients receiving organ transplantation and it has been shown to have favorable safety profile [33].

Immunosuppressants such as MMF can increase the risk of developing lymphomas and other malignancies (0.7-2.1%) and non-melanoma skin carcinomas (1.6-4.2%), related to the intensity and duration of drug use in patients [30, 34]. The risk of post-transplant lymphoproliferative disorder (0.4-1%) in association with MMF appears to be related to Epstein Barr Virus infection [30]. Patients receiving MMF or other immunosuppressants are at increased risk of developing bacterial, fungal, protozoal, new or reactivated viral infections, and opportunistic infections; the risk increases with the total immunosuppressive load [30]. At daily dose of 2000-3000mg in clinical trials for antirejection therapy after organ transplant, fatal infection / sepsis occurred in 2-5% of patients [34]. Though rare, cases of progressive multifocal leukoencephalopathy (PML) have been reported in patients treated with MMF [30, 34].

In post-transplant patients receiving MMF 3000mg/day, 2.0-3.6% developed neutropenia (ANC < 0.5K/uL), though it may be related to MMF, concomitant medications, viral infections, or combinations of these causes [34]. Neutropenia has been observed most frequently from days 31-180 after transplant in patients treated with MMF for rejection prophylaxis [30, 34]. Pure red cell aplasia cases have been reported in patients who received MMF in combination with other immunosuppressive therapy, though some cases were reversible with dose reduction or discontinuation of MMF [30].

In a small proportion of patients on MMF, serum hepatic enzyme elevations occur but these are usually mild, asymptomatic and resolve spontaneously or with dose reduction. A small number of cases of clinically apparent liver injury have been reported in patients on MMF. The onset of hepatic injury is usually during the first month of therapy, and is usually mild and self-limiting [35]. Particularly in patients with gastrointestinal (GI) disease, GI bleeding requiring hospitalization (1.7-5.4%), ulceration and perforations were observed in clinical trials [30].

MMF is teratogenic, therefore it is contraindicated in pregnancy or those who are breastfeeding, and women of childbearing potential must use highly effective contraceptive methods and be tested for pregnancy before using MMF [27, 28, 34]. Sexually active male patients and their female partners are recommended to use effective contraception during MMF treatment and for at least 90 days after stopping treatment [30]. Females of reproductive potential must use acceptable birth control during the entire MMF therapy and for 6 weeks after stopping MMR [25]. Concomitant use of MMF decrease the systemic exposure to levonorgestrel which may results in reduced combination oral contraceptive effectiveness, Patients should use additional barrier contraceptive methods with combination oral contraceptive [25].

Mycophenolate Mofetil in Anti-Cancer Therapy

MMF has been investigated as an anti-cancer therapeutic since the 1960s[36]. Its target, IMPDH, is upregulated across numerous cancer types including brain, ovarian, skin, bladder, renal cell, and uterine cancers [33]. Since then, many pre-clinical studies have been conducted in malignancies including leukemia, lymphoma, pancreatic cancer, non-small-cell lung adenocarcinoma, and colon cancer that showed suppression of tumor cell growth in cell line and xenograft models of cancer [33]. A single preclinical study using immortalized GBM cell lines has shown that MPA can slow the growth of U87 xenografts grown in the flanks of immunodeficient mice[37].

MMF has also been investigated in patients with cancer in pilot clinical trials. A small trial performed in Madrid, Spain, utilized MMF in patients with resectable pancreatic cancer. The MMF regimen used was 1-2 gm given daily for 5-15 days prior to resection. No grade 3 toxicities were observed. The authors found that MMF did not affect tumor angiogenesis, but they did not investigate other parameters such as GTP concentration, mitotic index or patient outcome[38]. A second trial involved 12 patients with multiple myeloma treated at the University of Maryland with MMF (1-5 gm daily). There were no grade 3 toxicities in the 1-3 gm/day dosing groups, but one of two patients receiving 5 gm daily developed grade 3 thrombocytopenia that ultimately was thought due to progression of his myeloma. Five patients had a response to therapy (stabilization of disease or partial response). Therapy responses were associated with decreased levels of dGTP[39].

The combination of MPA and radiation therapy has been reported to be safe. A case report from 1977 described the use of MPA (800-2400 mg) in 15 patients who were receiving palliative radiation for metastatic cancer of varied histologies. MPA-induced nausea was noted in most patients, but no potentiation of radiation-induced toxicities was noted. Tumor responses were not systematically quantified[21]. To our knowledge, there have been no studies examining the use of MMF or MPA in patients with GBM.

1.3 Rationale

Radiation resistance is one of the major obstacles in treatment of both newly diagnosed and recurrent GBM. The majority of patients with primary and recurrent GBM

experience recurrence within the area of previous radiation, and overcoming RT resistance will likely improve outcomes in both populations[10-13]. Efforts to radiosensitize GBM using molecularly targeted therapies designed to inhibit oncogenic alterations in the tumor have been unsuccessful, likely due to the profound heterogeneity in genomic alterations that drive different cells and regions within an individual GBM [14, 15]. Initial studies from our group and others suggest that there may be common *metabolic* phenotypes that mediate RT resistance in GBM across heterogeneous genotypes [16, 17]. As noted above (Figures 1-4), our group has discovered a causal relationship between guanylate levels and radiation resistance in GBM and shown that depleting GTP with the FDA-approved drugs MPA and MMF radiosensitizes GBM cell lines, neurospheres and xenografts. Similarly, MMF potentiates the efficacy of temozolomide, which is used along with radiation in newly diagnosed GBM. **We now propose to investigate the use of MMF in conjunction with RT with or without temozolomide for patients with GBM to determine (1) if the drug achieves active concentrations inbrain tumors and (2) if it is well tolerated (and potentially efficacious) when combined with radiation and temozolomide.** We will address these questions in this study for patients with both newly diagnosed and recurrent GBM.

The phase 0 component will investigate the MMF drug delivery in patients who are undergoing clinically indicated biopsy or re-resection of GBM. MMF appears to penetrate the CNS, at least in a pharmacokinetic study performed in mice [32], but similar data in human patients are lacking. It is also unknown whether MMF can deplete intratumoral GTP levels. By performing the phase 0 portion of this study on patients with recurrent GBM who are already undergoing planned re-resection of their tumors, we will be able to test to what extent MMF accumulates in GBMs and depletes intratumoral GTP without performing any additional surgical procedures. Patients will take MMF at varying doses for 1 week prior to surgery, and MMF and GTP in tumor will be measured in tissue that was resected at surgery as part of the standard of care.

There are two phase 1 parts of this study. The first is a dose escalation safety and tolerability study of re-irradiation (re-RT) combined with MMF in recurrent GBM patients who have clinical indication for re-RT.. The second is a similarly structured arm where MMF is combined with RT+TMZ and later with TMZ alone (i.e., the standard of care “Stupp regimen).The most common adverse events of RT in GBM are typically fatigue, alopecia, and symptoms related to temporary worsening of tumor burden during and up to approximately 2 months after radiation. The most common adverse effects of temozolomide include cytopenias (most commonly mild thrombocytopenia), nausea and constipation. The most common adverse effects associated with MMF are usually dose limiting and include diarrhea and bone marrow suppression. MMF is typically well tolerated and usually administered as a long-term medication.MMF is well tolerated even in patients receiving additional immunosuppressants immediately following organ transplant, who have more comorbidities than typical patients with GBM. The anticipated overlap of adverse effects or serious adverse events directly related to combination of RT, TMZ and MMF are minimal as the MMF dose widely used is around 2000mg/day-3000mg/day.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

1. Phase 0: To measure the concentration of MPA in tumors from patients who undergo re-resection for GBM.

2. Phase 1 Recurrent GBM: To determine the DLT and MTD of MMF whencombined with re-irradiation in patients with recurrent GBM.

3. Phase 1 Newly Diagnosed GBM: To determine the DLT and MTD of MMF when combined with standard upfront chemoradiation in patients with newly diagnosed GBM.

2.2 Secondary Objectives

1. Phase 0: To determine GTP concentrations in recurrent GBM tissue re-resected after 1 week of MMF administration.

2. Phase 1: To describe the adverse events associated with MMF when administered with re-irradiation in recurrent GBM patients, and with standard up front chemoradiation in newly diagnosed GBM.

3. Phase 1: To compare the overall survival of patients with recurrent GBM treated with MMF and re-irradiation to historical control of patients who were treated with re-irradiation alone (PMID: 20479391).

4. Phase 1: To compare the patterns of recurrence after re-irradiation withconcurrent MMF to patterns of failure in patients treated with re-irradiation alone [13].

2.3 Exploratory Objectives

1. To measure GTP level in re-resected GBM tumor tissue in patients who undergo standard of care re-resection without MMF administration. (HUM00024610)

2. To measure circulating MPA levels in plasma after 1 week of MMF administration and determine if these levels correlate with intratumoral MPA concentrations

3. To measure circulating MPA levels in plasma after 1 week of MMF administration and determine if these levels correlate with survival after, and toxicity with combined treatment with MMF and radiation.

4. Describe overall survival of patients with newly diagnosed GBM treated with MMF combined with standard of care.

2.4 Endpoints

1. The concentration of MPA will be measured by mass spectrometry in recurrent GBM tumor tissue after 1 week (+/- 3 days) of MMF administration prior to standard of care re-resection.

2. DLT will be defined based on the rate of drug related grade 3-5 adverse events experienced for the duration and within 4 weeks of treatment completion with MMF and re-irradiation in recurrent GBM patients. In newly diagnosed patients, DLT1 duration will be within 4 weeks of completion of MMF with radiation and concurrent temozolomide. DLT2 period will be during the first 2 cycles of adjuvant MMF and temozolomide. These will be assessed via NCI's CTCAE version 5.0.

3. GTP will be measured by mass spectrometry in recurrent GBM tissue after 1 week of MMF administration prior to standard of care re-resection.

4. Adverse events associated with MMF and/or re-irradiation will be recorded and assessed via NCI's CTCAE version 5.0.

5. The overall survival of patients with recurrent GBM treated with MMF and re-irradiation will be determined by Kaplan Meier method, to be compared to the historical control of patients who were treated with re-irradiation alone (PMID: 20479391).

6. The overall survival of patients with newly diagnosed GBM treated with MMF and standard of care will be determined by Kaplan Meier method.

7. Patterns of failure will be determined based on standard-of-care MRI in collaboration with treating physicians and multidisciplinary team as previously described [40].

3.0 PATIENT ELIGIBILITY

Subjects must meet all of the inclusion and exclusion criteria to be enrolled to the study. Study treatment may not begin until a subject is enrolled.

3.1 Inclusion Criteria

Phase 0 Inclusion Criteria

1. Age 18 or older.

2. KPS 60 or greater.

3. Recurrent glioblastoma or recurrent gliosarcoma or recurrent WHO Grade 4 astrocytoma.

4. Multidisciplinary brain tumor board consensus of tumor progression based on imaging and-or clinical factors.

5. Candidate for clinically indicated re-resection or biopsy of glioblastoma or gliosarcoma per treating physician(s).

6. ANC ≥ 1,500 cells/mm^3 within 14 days prior to enrollment.

7. Patient (men and childbearing age women) agrees to the use of highly effective contraception during study participation and for at least 6 weeks for female patients and 90 days for male patients after final MMF administration.

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

Recurrent GBM Phase 1 Inclusion Criteria

1. Age 18 or older.

2. KPS 60 or greater.

3. Recurrent glioblastoma or recurrent gliosarcoma or WHO Grade 4 astrocytoma.

4. Multidisciplinary brain tumor board consensus of tumor progression based on imaging and-or clinical factors.

5. Candidate for clinically indicated re-irradiation of glioblastoma or gliosarcoma per treating physician(s).

6. ANC ≥ 1,500 cells/mm^3 within 14 days prior to enrollment.

7. Patient (men and childbearing age women) agrees to the use of highly effective contraception during study participation and for at least 6 weeks for female patients and 90 days for male patients after final MMF administration.

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

9. No more than one prior course of radiation for GBM.

Newly diagnosed GBM Phase 1 Inclusion Criteria

1. Age 18 or older.

2. KPS 60 or greater.

3. Newly diagnosed glioblastoma or gliosarcom or WHO Grade 4 astrocytoma.

4. No prior chemotherapy or radiation treatment for glioblastoma or gliosarcoma.

5. Candidate for upfront standard of care chemoradiation for glioblastoma or gliosarcoma per treating physician(s), to start no earlier than 14 days post-operatively from last definitive surgery for glioblastoma or gliosarcoma (if more than one surgery done. Ex. biopsy prior to resection).

6. ANC ≥ 1,500 cells/mm^3 within 14 days prior to enrollment.

7. Patient (men and childbearing age women) agrees to the use of highly effective contraception during study participation and for at least 6 weeks for female patients and 90 days for male patients after final MMF administration.

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

3.2 Exclusion Criteria

Phase 0 Exclusion Criteria

1. Lack of histopathological diagnosis of the tumor.

2. Gliomatosis cerebri pattern ofdisease.

3. Leptomeningeal disease.

4. Use of bevacizumab within 8 weeks of study enrollment.

5. Known history of HIV.

6. Active hepatitis B or C infection

7. Active systemic or CNS infection.

8. Grade 4 lymphopenia (if ALC <0.5, patient must be on *Pneumocystis jirovecii* prophylaxis).

9. Estimated CrCl < 25 ml/min

10. History of organ transplantation

11. Patients with known hypoxanthine-guanine phosphoribosyl-transferase deficiency.

12. Serious intercurrent illness

13. History of allergic reaction or hypersensitivity to mycophenolate mofetil or mycophenolic acid or any component of the drug product.

14. Known immunosuppressive condition from autoimmune disease, immune deficiency syndrome, or chronic immunosuppressive therapy.

15. Inability to undergo MRI brain with and without contrast.

16. Medical contraindication for MMF per treating physician(s).

17. Pregnant or lactating women.

18. Patients with known phenylketonuria

19. Patients undergoing biopsy who are deemed unlikely to have sufficient tissue to spare for research purposes (e.g., those whose tumors are in an eloquent brain location where all tissue taken must be used for diagnostic purposes)

Recurrent GBM Phase 1 Exclusion Criteria

1. Lack of histopathological diagnosis of the tumor.

2. Gliomatosis cerebri pattern ofdisease.

3. Leptomeningeal disease.

4. Use of bevacizumab within 8 weeks of study enrollment.

5. Radiation within 6 months prior to study enrollment.

6. Prior surgery within 4 weeks of re-irradiation.

7. Known history of HIV.

8. Active hepatitis B or C infection

9. Active systemic or CNS infection.

10. Grade 4 lymphopenia (if ALC <0.5, patient must be on *Pneumocystis jirovecii* prophylaxis).

11. Estimated CrCl < 25 ml/min

12. History of organ transplantation

13. Patients with known hypoxanthine-guanine phosphoribosyl-transferase deficiency.

14. Serious intercurrent illness

15. Increase in steroid requirement within 7 days of study enrollment (stable or decreasing dose allowed).

16. History of allergic reaction or hypersensitivity to mycophenolate mofetil or mycophenolic acid or any component of the drug product.

17. Known immunosuppressive condition from autoimmune disease, immune deficiency syndrome, or chronic immunosuppressive therapy.

18. Inability to undergo MRI brain with and without contrast.

19. Medical contraindication for MMF per treating physician(s).

20. Pregnant or lactating women.

21. Patients with known phenylketonuria

Newly Diagnosed GBM Phase 1 Exclusion Criteria

1. Lack of histopathological diagnosis of the tumor.

2. Gliomatosis cerebri pattern ofdisease.

3. Leptomeningeal disease.

4. Prior chemotherapy or radiation therapy for glioblastoma or gliosarcoma.

5. Known history of HIV.

6. Active hepatitis B or C infection

7. Active systemic or CNS infection.

8. Grade 4 lymphopenia (if ALC <0.5, patient must be on *Pneumocystis jirovecii* prophylaxis).

9. Estimated CrCl < 25 ml/min

10. History of organ transplantation

11. Patients with known hypoxanthine-guanine phosphoribosyl-transferase deficiency.

12. Serious intercurrent illness

13. Increase in steroid requirement within 7 days of study enrollment (stable or decreasing dose allowed).

14. History of allergic reaction or hypersensitivity to mycophenolate mofetil or mycophenolic acid or any component of the drug product.

15. History of hypersensitivity reactions to temozolomide or any other ingredients in temozolomide and dacarbazine.

16. Known immunosuppressive condition from autoimmune disease, immune deficiency syndrome, or chronic immunosuppressive therapy.

17. Inability to undergo MRI brain with and without contrast.

18. Medical contraindication for MMF per treating physician(s).

19. Pregnant or lactating women.

20. Patients with known phenylketonuria

4.0 SUBJECT SCREENING AND REGISTRATION PROCEDURES

Screening / baseline tests are to be completed within 28 days of enrollment.

After informed consent is obtained and PRIOR to the initiation of protocol therapy all patients satisfying the inclusion/exclusion criteria must have eligibility confirmed by the

CTSU. The patient will not be considered registered and enrolled in the study until all information is confirmed by the CTSU Data Manager.

5.0 TREATMENT PLAN

5.1 Treatment Dosage and Administration

5.1.1 Overview

In the phase 0 component of the study, patients with recurrent / progressive GBM will receive MMF for 1 week prior to re-resection deemed clinically indicated by treating physician(s). In the phase 1 portion of the study, patients with recurrent / progressive GBM will receive MMF (dose escalation study) for 1 week prior to re-irradiation deemed clinically indicated by treating physician(s), and for the duration of the re-irradiation. Phase 0 patients will be allowed to enroll on the phase 1 study after 4 weeks following the surgery if meeting Phase 1 eligibility criteria. Dosing details are described below. For both the phase 0 and phase 1 portions of this trial, protocol treatment will begin within 28 business days of enrollment to the study.

5.1.2 Phase 0:

Patients with recurrent / progressive GBM with clinical indication for re-resection will be identified. Patients will be assigned to a dose level and receive outpatient MMF orally twice daily for 1 week prior to re-resection. We will enroll 2 patients per dose level beginning at 500 mg PO BID and increasing from there. MMF will be taken on an empty stomach (1 hour before or two hours after eating). The final dose will be taken the morning of surgery. At the time of re-resection, the following samples will be obtained:

1. N=3 flash frozen samples from the contrast-enhancing tumor (one sample to assess tumor content, one to assess GTP levels, one to assess MPA levels)
2. N=3 flash frozen samples from the non-enhancing tumor (one sample to assess tumor content, one to assess GTP levels, one to assess MPA levels)
3. N=1 serum sample at the time of tumor resection (to assess plasma MPA concentrations)
    a. Serum (at least 1 mL) will be collected in a red-top tube and mycophenolic acid concentration will be determined by clinical laboratory at the University of Michigan.

Phase 0 patients who meet the eligibility criteria for phase 1 component of the study will be allowed to enroll on the phase 1 portion of the study.

Five patients who undergo standard of care re-resection for recurrent GBM without MMF administration who have consented to tumor banking will be identified (HUM00024610). In these patients, equivalent samples to above #1 and #2 will be obtained to assess GTP levels in untreated recurrent GBM tissue. We expect that recurrent GBM tissue obtained from

MMF-treated patients on the phase 0 portion of this trial will have decreased GTP levels compared to these control patients who did not receive MMF prior to surgery. We expect that these control patients will have undetectable MPA (i.e., serve as negative controls) and have normal levels of GTP (i.e., will serve as positive controls).

Phase 0 Schema								
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
AM MMF		X	X	X	X	X	X	X
PM MMF	X	X	X	X	X	X	X	
Craniotomy								X

Phase 0 MMF dose arms	
Dose Level	**Dose of the Study Agent**
Arm A (n=2)	500 mg PO BID
Arm B (n=2)	1000 mg PO BID
Arm C (n=2)	1500 mg PO BID
Arm D (n=2)	2000 mg PO BID

The analysis of phase 0 samples will be conducted at the end of both phase 0 and 1 studies to optimize interpretation of phase 1 result.

5.1.3 Phase 1:

Recurrent GBM Phase 1

This phase is a dose-escalation to determine the maximum tolerated dose of MMF when administered concurrently with radiation for patients with recurrent GBM and to provide an efficacy estimate at that dose. Patients with recurrent / progressive GBM with clinical indication for re-irradiation will be identified. Patients will receive outpatient MMF orally twice daily for 1 week prior to and concurrent to the re-irradiation. The final dose of MMF will be given the evening of the final dose of radiation. MMF dose will be escalated per TITE-CRM algorithm described in section 11.2, with the starting dose of 1000mg twice daily. We expect to enroll a total of 30 patients on this arm of phase 1 component of the study. RT total dose will be 40.5 Gy in 15 fractions (typically administered over three consecutive weeks).

Phase 1 Schema								
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8 through RT end date**
AM MMF		X	X	X	X	X	X	X
PM MMF	X	X	X	X	X	X	X	X
RT								X

Phase 1 MMF Dose-Escalation Schedule		
Dose Level	**Dose of the Study Agent**	**Minimum Number of Patients**
Level 1	250 mg PO BID	n/a
Level 2	500 mg PO BID	n/a
Level 3	1000 mg PO BID	2
Level 4	1500 mg PO BID	2
Level 5	2000 mg PO BID	2

Newly Diagnosed GBM Phase 1

This phase is a dose-escalation to determine the maximum tolerated dose of MMF when administered concurrently with standard of care upfront treatment (radiation with concurrent temozolomide, followed by adjuvant temozolomide with or without tumor treating field) in patients with newly diagnosed GBM and to provide an efficacy estimate at that dose. Patients with newly diagnosed GBM meeting eligibility criteria will be identified. Patients will receive outpatient MMF orally twice daily for 1 week prior to and concurrent to the radiation. The final dose of MMF during radiation period will be given the evening of the final dose of radiation. The final dose of MMF during radiation period will be given the evening of the final dose of temozolomide each cycle. During adjuvant phase, patients will receive outpatient MMF orally twice daily one day prior to and concurrent to cyclic temozolomide.

MMF dose will be escalated per TITE-CRM algorithm described in section 11.2, with the starting dose of 1000mg twice daily. We expect to enroll a total of 30 patients on this arm of phase 1 component of the study. RT total dose will be 60 Gy in 30 fractions (typically administered over six consecutive weeks). Temozolomide given concurrent to radiation, and during adjuvant setting will be per standard of care. Typical dose of temozolomide concurrent to radiation is 75mg/m2/d, and 150-200mg/m2/d x 5 out of 28 day cycles during adjuvant setting.

Phase 1 Schema with radiation								
Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8 through RT end date**
AM MMF		X	X	X	X	X	X	X
PM MMF	X	X	X	X	X	X	X	X
RT								X

Phase 1 Schema with adjuvant cyclic temozolomide (TMZ)						
Day	**0**	**1**	**2**	**3**	**4**	**5**
AM MMF		X	X	X	X	X
PM MMF	X	X	X	X	X	X
PM TMZ		X	X	X	X	X

Phase 1 MMF Dose-Escalation Schedule		
Dose Level	**Dose of the Study Agent**	**Minimum Number of Patients**
Level 1	250 mg PO BID	n/a
Level 2	500 mg PO BID	n/a
Level 3	1000 mg PO BID	2
Level 4	1500 mg PO BID	2
Level 5	2000 mg PO BID	2

5.1.4. Mycophenolate Mofetil (MMF)

5.1.4.1 MMF will be administered as an oral capsule or as tablet(s) each morning and evening as detailed above (at least 1 hour before or 2 hours after eating). Tablets will not be crushed and capsules will not be crushed or opened. Proton pump inhibitors (PPIs) should be avoided if possible. If a patient is taking aluminum or magnesium supplements, they must be taken at least 2 hours before or 2 hours after MMF. Patients should be instructed to take a missed dose as soon as they remember unless it is less than 2 hours to the next scheduled dose. In this case they should skip the dose and not double up. If patient experiences emesis after the MMF dose, patient should not re-take the vomited dose. Patients should record taking the drug, missed doses, and any adverse effect in the provided drug diary.

5.1.4.2 For the phase 0 portion of this study, MMF will be given as monotherapy prior to surgical resection. Patients will start at a 500 mg BID dose. After two patients have been accrued to this dose level, two will be accrued to the 1,000 mg BID dose level, followed by two patients at 1500 mg BID and two patients at 2000 mg BID, which will complete the phase 0 portion of the study. MMF doses up to 2000 mg BID are well-tolerated doses in severely immunocompromised patients undergoing bone marrow transplantation and we expect no complications in patients receiving this as monotherapy prior for one week prior to surgery. However, if issues such as poor wound-healing develop at a dose level N, we will accrue patients at a dose level N-1. If these issues occur at an MMF dose of 500 mg BID, we will drop the phase 0 dose to 250 mg BID.

5.1.4.3 For the phase 1 portion of the study, MMF will be given prior to and concurrently with radiotherapy. MMF will also be given with adjuvant temozolomide in the newly diagnosed arm. The dose of MMF will be escalated according to the TITE-CRM design (see section 11)

5.1.5. Radiation Therapy (RT) in phase 1

All radiation treatment planning and administration will be performed at the University of Michigan.

Immobilization/Simulation: Within three weeks prior to treatment initiation, patients will undergo CT simulation in a thermoplastic immobilization device and MRI scan with and without contrast containing at minimum T1 pre-contrast, T1 post-contrast and T2 FLAIR imaging sequences. These imaging sequences will be registered together in the radiation oncology treatment planning system.

Target Delineation: Radiation targets will be defined on the CT dataset using information from relevant MRI sequences. A gross tumor volume (GTV) will be defined that encompasses only gross disease and the operative cavity (typically enhancing disease on T1 post-contrast MRI). For patients with newly diagnosed GBM, a clinical target volume will be defined per standard of care. Typically, this will involve an expansion of 1.7 cm that is limited by anatomic barriers to spread such as bone, the tentorium or the falx cerebri. After expansion, this may be adjusted further to ensure coverage of any suspicious regions seen on FLAIR MRI sequences. For patients with recurrent GBM who are receiving a second course of radiation, the elective CTV will be much smaller, typically between 0 and 5 mm depending on the treating physician's discretion. A planning target volume (PTV) will be defined based on daily imaging and reproducibility (typically a 3 mM uniform expansion on the CTV).

Organs at Risk: The following critical normal tissues will be defined as per the standard of care: Brain, brainstem, cochlea, optic nerves, optic chiasm. Treatment planning will aim to meet the following dose constraints: Brainstem (D10cc $\leq$ 54 Gy, D0.03cc $\leq$ 45 Gy), cochlea (D0.03cc $\leq$ 45 Gy), optic nerves (D0.03cc $\leq$ 54 Gy), optic chiasm (D0.03cc $\leq$ 54 Gy). Brain dose will be limited to as low as reasonably achievable (ALARA). In rare cases these dose constraints may be exceeded due to close proximity to tumor.

Treatment Planning and Radiation Dose: For patients with recurrent GBM receiving a second course of RT patients, the PTV will receive 40.5 Gy in 2.7Gy fractions. For patients with newly diagnosed GBM, the PTV will receive 60 Gy in 2 Gy fractions. Minimum dose to the PTV will be 95% of the prescription dose, except in cases where there is an adjacent organ at risk that must be avoided. The maximum dose to the PTV will be 110% of prescription.

5.1.6. Temozolomide

Temozolomide capsules are an approved oral chemotherapeutic drug for the treatment of adult patients with newly diagnosed glioblastoma concomitantly with radiotherapy and then as adjuvant treatment. Treatment with temozolomide will be determined as per standard-of-care for the individual patient by the treating oncologist. It is anticipated that, for most patients, concomitant temozolomide 75mg/m2/day will be taken with upfront RT and the duration of concomitant temozolomide will coincide with the duration of RT, and 150-200mg/m2/day on 5 out of 28 days cycles for up to 12 cycles. During adjuvant cyclic temozolomide period, MMF will be combined for up to 12 cycles of adjuvant temozolomide. The dosing and timing of temozolomide therapy will be per standard of care, deemed appropriate by the treating oncologist.

Dose limiting toxicity (DLT) will be evaluated for the duration of study treatment, and for 4 weeks after the completion. Patients will be allowed to initiate treating physician's choice next line treatment after the DLT evaluation period. Patients will be followed for overall survival.

5.2 Dosage Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Subjects who are non-evaluable for toxicity will be replaced to reach 30 evaluable subjects in each phase 1 cohorts. Each patient will be assessed for the development of toxicity according to the Time and Events Table (Section 8.1). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.
Dose adjustments should be made according to the system showing the greatest degree of toxicity.

5.3 MMF Dose Modification. Recommended dose modifications for MMF are as follows:

A CBC with differential will be drawn prior to the initiation of MMF treatment and then weekly during concurrent radiation and MMF. Dose reduction, delay or discontinuation of MMF will be decided weekly by the treatment team according to hematologic and non-hematologic adverse events (AEs) as recommended below*. If the administration of MMF has to be interrupted, the radiotherapy will proceed as normal. Missed doses of MMF will not be "made up" at the end of radiotherapy.

Delay and Dose Reduction: If one or more of the following are observed

- ANC < 1,500 cells/mm^3 to $\geq$ 500 cells/mm^3
- Platelet count < 100K cells/mm^3 to $\geq$ 25K cell/mm^3
- Grade 3 non-hematologic AE (except alopecia, fatigue and nausea and vomiting not yet on maximum anti-emetic therapy)

Then treatment with concomitant MMF will be held until all of the following conditions are met:

- ANC $\geqslant$1,500 cells/mm^3
- Platelet count $\geqslant$100 cells/mm^3
- Grade $\leqslant$2 non-hematologic AE (except alopecia, fatigue and nausea and vomiting not yet on maximum anti-emetic therapy)

As soon as all of the above conditions are met, MMF will be resumed with the dose reduced by one level (see dose level table in section 5.1.3).

Discontinuation: If one or more of the following are observed

- ANC < 500 cells/mm^3
- Platelet count < 25K cells/mm^3
- Grade 4 non-hematologic AE (except alopecia, nausea and vomiting not yet on maximum anti-emetic, and fatigue)

Then treatment with MMF will be permanently discontinued and radiation will be held. Radiation will be restarted once non-hematologic toxicity returns to grade $\leqslant$2 (except alopecia, fatigue and nausea and vomiting not yet on maximum anti-emetic therapy)

5.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment will occur for approximately 1 week for Phase 0, 4 weeks for Phase 1 (recurrent), and 5 weeks if a patient with recurrent GBM participated in both phases. Patients with newly diagnosed GBM will receive 7 weeks of MMF during upfront chemoradiation and then MMF with cyclic temozolomide starting the day before starting temozolomide x 5 days each cycle for total of 6 days per cycle (up to 12 cycles). Alternatively, therapy will be discontinued when one of the following criteria apply:

- Disease progression as defined in Section 8.5.
- Inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient voluntarily withdraws from treatment **OR**
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

5.5 Off Treatment Criteria

Patients will be removed from protocol therapy when any of the criteria listed in Section 6.2 apply. Document in the source the reason for ending protocol therapy and the date the patient was removed from treatment. All patients who discontinue treatment should comply with protocol specific follow-up procedures as outlined in Section 5.6. The only exception to this requirement is when a

subject withdraws consent for all study procedures or loses the ability to consent freely.

5.6 Duration of Follow-Up

All patients will be followed for survival, and for progression of neurological symptoms and for evidence of treatment failure by MRI. Patients who have tumor progression, as defined below, will not be removed from the protocol and will continue to be followed endpoints including survival, salvage therapies, etc. Date and cause of death will be documented, if known. Total follow up period will be for three years after the last trial patient enrollment. Outside of standard of care visits, survival will be determined by phone call every 6 months.

5.7 Off Study Criteria

Patients can be taken off study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation from study will be documented and may include:

1. Patient withdraws consent (termination of treatment and follow-up);

2. Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment;

3. Patient is unable to comply with protocol requirements;

4. Treating physician judge's continuation on the study would not be in the patients' best interest;

5. Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);

6. Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;

7. Lost to Follow-up. If a research subject cannot be located to document survival after a period of 2 years, the subject may be considered "lost to follow-up." All attempts to contact the subject during the two years must be documented;

8. Termination of the study by The University of Michigan, Sponsor, or the FDA;

9. Patient completes protocol treatment and follow-up criteria.

5.8 Contraception

Females of reproductive potential must use acceptable birth control during the entire MMF therapy and for 6 weeks after stopping MMR [25]. Concomitant use of MMF decrease the systemic exposure to levonorgestrel which may results in reduced combination oral contraceptive effectiveness, Patients should use additional barrier contraceptive methods with combination oral contraceptive [25]. Sexually active male patients and/or their female partners must use effective contraception during treatment of the male patient and for at least 90 days after cessation of treatment [25].

5.9 Patient Replacement

Patients who do not undergo surgery or who are found to have treatment effect rather than recurrent GBM in the phase 0 portion may be replaced as allowed by trial finances. For the phase 1 portion, patients who enroll but do not begin radiation therapy will be replaced as finances allow.

6.0 Drug therapy (Mycophenololate Mofetil)

6.1 Description: Mycophenolate mofetil is a 2-morpholinoethyl ester of mycophenolic acid (MPA), an immunosuppressive agent; inosine monophosphate dehydrogenase (IMPDH) inhibitor. The chemical name for mycophenolate mofetil (MMF) is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.

6.2 Warnings and Precautions: For patients with increased risk for skin cancer, exposure to sunlight and UV light should be limited by wearing protective clothing and using a sunscreen with a high protection factor. Administration of live attenuated vaccines should be avoided while receiving MMF. Patients will not be allowed to donate sperm while receiving MMF and from 90 days following discontinuation of MMF treatment. Patients should not donate blood during therapy and for at least 6 weeks following discontinuation of MMF treatment. MMF may impact the ability to drive and use machines. Patients should avoid driving or using machines if they experience somnolence, confusion, dizziness, tremor or hypotension during treatment with MMF.

6.3 Drug interactions:

Drugs and drug groups that may reduce MMF and or MPA systemic exposure are listed in the table below:

Antacids with Magnesium or Aluminum Hydroxide	
Clinical Impact	Concomitant use with an antacid containing magnesium or aluminum hydroxide decreases MPA systemic exposure, which may reduce MMF efficacy.

Prevention or Management	If needed, administer magnesium or aluminum hydroxide containing antacids at least 2 hours before and after MMF administration.
Proton Pump Inhibitors (PPIs)	
Clinical Impact	Concomitant use with PPIs decreases MPA systemic exposure, which may reduce MMF efficacy.
Prevention or Management	Monitor patients for alterations in efficacy when PPIs are co-administered with MMF.
Examples	Lansoprazole, pantoprazole
Drugs that Interfere with Enterohepatic Recirculation	
Clinical Impact	Concomitant use with drugs that directly interfere with enterohepatic recirculation, or indirectly interfere with enterohepatic recirculation by altering the gastrointestinal flora, can decrease MPA systemic exposure, which may reduce MMF efficacy.
Prevention or Management	Monitor patients for alterations in efficacy or MMF related adverse reactions when these drugs are co-administered with MMF.
Examples	Trimethoprim/sulfamethoxazole, bile acid sequestrants (cholestyramine), rifampin as well as aminoglycoside, cephalosporin, fluoroquinolone and penicillin classes of antimicrobials
Drugs Modulating Glucuronidation	
Clinical Impact	Concomitant use with drugs inducing glucuronidation decreases MPA systemic exposure, potentially reducing MMF efficacy, while use with drugs inhibiting glucuronidation increases MPA systemic exposure, which may increase the risk of MMF related adverse reactions.
Prevention or Management	Monitor patients for alterations in efficacy or MMF related adverse reactions when these drugs are co-administered with MMF.
Examples	Telmisartan (induces glucuronidation); isavuconazole (inhibits glucuronidation).
Calcium Free Phosphate Binders	
Clinical Impact	Concomitant use with calcium free phosphate binders decrease MPA systemic exposure, which may reduce MMF efficacy.
Prevention or Management	Administer calcium free phosphate binders at least 2 hours after MMF.
Examples	Sevelamer

MMF may affect the systemic exposure of drugs or drug groups that are listed in the table below:

Drugs that Undergo Renal Tubular Secretion

Clinical Impact	When concomitantly used with MMF, its metabolite MPAG, may compete with drugs eliminated by renal tubular secretion which may increase plasma concentrations and/or adverse reactions associated with these drugs.
Prevention or Management	Monitor for drug-related adverse reactions in patients with renal impairment.
Examples	Acyclovir, ganciclovir, probenecid, valacyclovir, valganciclovir
Combination Oral Contraceptives	
Clinical Impact	Concomitant use with MMF decreased the systemic exposure to levonorgestrel, but did not affect the systemic exposure to ethinylestradiol, which may result in reduced combination oral contraceptive effectiveness.
Prevention or Management	Use additional barrier contraceptive methods.

Additional information these drug interactions can be found in the latest MMF drug label.

6.4 **Human Toxicity:** Long-term MMF use in clinical trials that occur at rates over 20% include diarrhea, cytopenias, infection, and vomiting, but these symptoms may be primarily due to the other immunosuppressive therapies given along with MMF and the long term nature of immunosuppressive treatment when given in the transplant setting [30]. Indeed, in the renal transplant randomized study that compared MMF to azathioprine, the incidence of peripheral edema (27-29%), constipation (18-22%), nausea (20-25%), and infection (20-24%) were similar between treatment arms[30]. However, diarrhea was significantly higher in the MMF arms (31% in the 2 g/day and 36% in the 3 g/day arm) than in the azathioprine arm (21%). Similar results were seen in the randomized comparison of MMF and azathioprine in cardiac transplant patients (diarrhea in 45% of patients on MMF and 34% of patients on azathioprine). In this study, infection again occurred at similar rates in both arms (35-37%)[30]. In patients who have not undergone organ transplantation, MMF-induced toxicity is less common. Serious infections occurred in fewer than 10% of lupus patients treated with MMF (1000 mg BID), which was similar to the rate of serious infection in patients receiving azathioprine [41]. Over the three-year treatment course of MMF in this trial, approximately 25% of patients had to discontinue treatment due to toxicity. Despite its immunosuppressive mechanism of action, initiation of MMF is rarely associated with cytopenias when used to treat immunocompetent patients [42].

A comprehensive listing of adverse events associated with **long-term** MMF dosing from LexiComp® (https://online.lexi.com) is as follows: (Incidences include concomitant use with cyclosporine and corticosteroids. In general, lower doses used in renal rejection patients had less adverse effects than higher doses.

Rates of adverse effects were similar for each indication, except for those unique to the specific organ involved).

Incidence >10%:

Cardiovascular: Hypertension (18% to 79%), edema (17% to 68%), hypotension (34%), tachycardia (22% to 23%), lower extremity edema (16%)

Central nervous system: Pain (25% to 79%), headache (11% to 59%), insomnia (24% to 52%), dizziness (34%), depression (20%), chills (3% to <20%), confusion (3% to <20%), drowsiness (3% to <20%), hypertonia (3% to <20%), malaise (3% to <20%), myasthenia (3% to <20%), paresthesia (3% to <20%)

Dermatologic: Skin rash (26%), ecchymoses (20%), cellulitis (3% to <20%)

Endocrine & metabolic: Hyperglycemia (44% to 48%), hypercholesterolemia (46%), hypomagnesemia (20% to 39%), hypokalemia (9% to 37%), hypocalcemia (11% to 30%), increased lactate dehydrogenase (24%), hyperkalemia (22%), acidosis (3% to <20%), weight loss (3% to <20%), hyperuricemia (13%), hyperlipidemia (10% to 12%), hypophosphatemia (9% to 11%)

Gastrointestinal: Abdominal pain (22% to 63%), nausea (27% to 56%), diarrhea (24% to 53%), constipation (38% to 44%), vomiting (20% to 39%), decreased appetite (25%), dyspepsia (19% to 23%), esophagitis (3% to <20%), gastric ulcer (3% to <20%), gastritis (3% to <20%), gastrointestinal hemorrhage (3% to <20%), hernia of abdominal cavity (3% to <20%), intestinal obstruction (3% to <20%), stomatitis (3% to <20%), upper abdominal pain (14%), flatulence (10% to 13%)

Genitourinary: Urinary tract infection (29% to 33%), hematuria (3% to <20%)

Hematologic & oncologic: Leukopenia (19% to 46%), anemia (20% to 45%), leukocytosis (22% to 43%), thrombocytopenia (24% to 38%), benign skin neoplasm (3% to <20%), disorder of hemostatic components of blood (3% to <20%), neoplasm (3% to <20%), pancytopenia (3% to <20%), skin carcinoma (3% to <20%; non-melanoma: 1% to 12%)

Hepatic: Increased liver enzymes (25%), hepatitis (3% to <20%), increased serum alkaline phosphatase (3% to <20%)

Infection: Bacterial infection (27% to 40%), viral infection (31%), cytomegalovirus disease (4% to 22%), fungal infection (11% to 12%)

Neuromuscular & skeletal: Asthenia (35% to 49%), tremor (12% to 34%), back pain (6% to 12%), arthralgia (7% to 11%)

Renal: Increased serum creatinine (10% to 42%), increased blood urea nitrogen (37%)

Respiratory: Dyspnea (31% to 44%), cough (41%), pleural effusion (34%)

Miscellaneous: Fever (13% to 56%)

Incidence 1% to 10%:

Cardiovascular: Exacerbation of hypertension (<10%), peripheral edema (<10%), phlebitis (4%), thrombosis (4%)

Central nervous system: Anxiety (<10%), fatigue (<10%)

Dermatologic: Acne vulgaris (<10%), pruritus (<10%)

Endocrine & metabolic: Diabetes mellitus (<10%)

Gastrointestinal: Abdominal distension (<10%), gastroesophageal reflux disease (<10%), gingival hyperplasia (<10%), oral candidiasis (<10%)

Genitourinary: Urinary retention (<10%)

Hematologic & oncologic: Lymphocele (<10%), severe neutropenia (2% to 4%), malignant neoplasm (≤2%), malignant lymphoma (1%), lymphoproliferative disorder (≤1%)

Hepatic: Abnormal hepatic function tests (<10%)

Infection: Influenza (<10%), wound infection (<10%), herpes simplex infection (6% to 8%), herpes zoster infection (4% to 5%), sepsis (2% to 5%)

Neuromuscular & skeletal: Muscle cramps (<10%), myalgia (<10%), peripheral pain (<10%)

Ophthalmic: Blurred vision (<10%)

Renal: Renal insufficiency (<10%), renal tubular necrosis (<10%)

Respiratory: Dyspnea on exertion (<10%), nasopharyngitis (<10%), pneumonia (<10%), sinusitis (<10%), upper respiratory tract infection (<10%)

Incidence not defined:

Gastrointestinal: Mucocutaneous candidiasis

Infection: Protozoal infection

Respiratory: Pharyngitis, respiratory tract infection

Incidence <1%, postmarketing and/or case reports: Agranulocytosis, alopecia, anorexia, atypical mycobacterial infection, BK virus, bone marrow failure, bronchiectasis (Boddana 2011; Rook 2006), colitis, duodenal ulcer, endocarditis, esophageal ulcer, gastrointestinal perforation, hematemesis, hemorrhagic colitis, hemorrhagic gastritis, hypersensitivity reaction, hypogammaglobulinemia (Boddana 2011; Keven 2003; Robertson 2009), interstitial pulmonary disease, Kaposi sarcoma, lymphadenopathy, lymphopenia, melena, meningitis, osteomyelitis, pancreatitis, peritonitis, polyomavirus infection, progressive multifocal leukoencephalopathy, pulmonary edema, pulmonary fibrosis, pure red cell aplasia, reactivation of disease (HCV), reactivation of HBV, tuberculosis, venous thrombosis, wheezing, xerostomia

6.5 **Pharmaceutical Data:** MMF is a white to off-white crystalline powder. It is slightly soluble in water (43 µg/mL at pH 7.4); the solubility increases in acidic medium (4.27 mg/mL at pH 3.6). It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol. The apparent partition coefficient in 1-octanol/water (pH 7.4) buffer solution is 238. Mycophenolate mofetil is available commercially from both Genentech/Roche and generic suppliers as 250 mg oral capsules or 500 mg oral tablets.

6.6 **Administration: See section 5.1.4.1.**

6.7 **Storage:** Mycophenolate mofetil will be stored at controlled room temperature with excursions permitted to 15° to 30°C (59° to 86°F).

6.8 **Supplier:** MMF is available both in generic formulations and as CellCept, manufactured by Roche/Genentech. These formulations have similar efficacy and toxicity profiles for patients undergoing organ transplantation[43]. MMF is commercially available for purchase from Roche/Genentech and numerous generic pharmaceutical manufacturers. It will be provided to study subjects free of charge.

6.9 **Drug accountability:** The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of MMF. The drug accountability records will capture drug receipt, drug dispensing, drug return and final disposition. Remaining, expired, or unused drug that is returned by patients will be destroyed on site according to the institution standard operating procedure for drug destruction and documented on the drug accountability records.

7.0 Temozolomide

Temozolomide is a standard-of-care treatment for adult patients with newly diagnosed glioblastoma concomitantly with radiotherapy and then as adjuvant treatment. Treatment with temozolomide will be determined as per standard-of-care for the individual patient by the treating oncologist. It is anticipated that, for most patients, concomitant temozolomide 75mg/m2/day will be taken with upfront RT and the duration of concomitant temozolomide will coincide with the duration of RT, and 150-200mg/m2/day on 5 out of 28 day cycles for 6-12 cycles. During adjuvant cyclic temozolomide period, MMF will be combined for up to 12 cycles of adjuvant temozolomide. The dosing and timing of temozolomide therapy will be per standard of care, deemed appropriate by the treating oncologist.

In patients receiving temozolomide, toxicities known compatible with temozolomide as the cause will not be considered as DLTs from the treatments administered as part of this study.

Patients treated with temozolomide have reportedly experienced myelosuppression including prolonged pancytopenia, which may result in aplastic anemia, which in some cases has resulted in a fatal outcome. In some cases, exposure to concomitant medications associated with aplastic anemia including carbamazepine, phenytoin, and sulfamethoxazole/trimethoprim complicates assessment. Geriatric patients and women have been shown in clinical trials to have a higher risk of developing myelosuppression.

Cases of myelodysplastic syndrome and secondary malignancies, including myeloid leukemia, have also been observed.

Prophylaxis against Pneumocystis carinii pneumonia will be left to the discretion of the treating physician. There may be a higher occurrence of PCP when temozolomide is administered during a longer dosing regimen. However, all patients receiving temozolomide, particularly patients receiving steroids, should be observed closely for the development of PCP regardless of the regimen.

Grade 3/4 neutropenia occurred in 8% and Grade 3/4 thrombocytopenia in 14% of patients treated with temozolomide. The most common side effects associated with temozolomide therapy are nausea, vomiting, anorexia, alopecia, headache, fatigue, constipation, convulsions, weakness, and thrombocytopenia.

8.0 Patient Assessments and Data Collection

8.1 Study Calendar

Phase 0

	Pretreatment evaluation: Days -28 days	MMF alone: Day 1-7	Surgery: Day 8 (+/-3 days[f])	2 weeks post-op (+/- 10 days)
Informed Consent	X			
History & Physical exam(including NANO scale [a])	X			X
Karnofsky performanceStatus	X			X
Tumor MPA concentration measurement			X	
Toxicity Evaluations	X	X	X	X
Patient pill Diaries		X	X	X
Pathologyreport	X			
Prior resectionrecord	X			
CBC with diff	X	Weekly	X	X
CMP[b]	X			X
MRI[c]	X			
Pregnancy Test(child bearing age women)	X[e]			
MMF Administration[d]		X	X	
Blood MPA concentration measurement[g]			X	

[a]See Appendix I

[b]CMP should include sodium, potassium, chloride, CO2, BUN, creatinine, glucose, calcium, albumin, protein, total bilirubin, alkaline phosphatase, ALT, and AST.

[c]MRI during follow up period will be at the discretion of treating physician.

[d]See dosing schema (section 5.1.2)

[e]Within 14 days prior to MMF start date

[f]Allow up to 3 days delay in surgery date. In such cases, BID MMF dosing will continue until the morning of surgery. If surgery happens earlier than expected, the final MMF dose will still be the morning of surgery.

[g] Serum sample at the time of tumor resection (to assess plasma MPA concentrations)

Phase 1 Recurrent GBM

	Pretreatment evaluation: Days -28-0	MMF alone: Day 1-7	RT+MMF: Beginning day 8 (+/- 3 days)[g]	4 weeks after RT completion (+/- 7 days)	Survival Follow Up
Informed Consent	X				
History & Physical exam (including NANO scale[a])	X			X	
Karnofsky Performance Status	X			X	
Toxicity (include DLT) Evaluations	X	X	Weekly	X	
MRI[b]	X			X	X[h]
Patient Pill Diaries	X	X	X	X	
Pathology report	X				
Prior radiation record	X				
CBC with diff	X	Weekly	Weekly	X	
CMP[c]	X		X	X	
QOL (MDASI[d])	X			X	
Pregnancy Test (child bearing age women) [e]	X[f]				
MMF Administration [f]		X	X		
Phone Calls (q6 month)					X[h]
Blood MPA concentration measurement[i]			X		

[a]See Appendix I

[b]MRI during follow up period will be at the discretion of treating physician. Modified RANO willbe used to assess MRI during follow up.

[c]CMP should include sodium, potassium, chloride, CO2, BUN, creatinine, glucose, calcium, albumin, protein, total bilirubin, alkaline phosphatase, ALT, and AST.
[d]See Appendix II
[e]Within 14 days prior to MMF start date
[f]See dosing schema (Section 5.1.3)
[g]Allow up to 3 days delay in start of radiation (continue MMF monotherapy in such case), and up to 5 days for RT completion date delay.
[h]If patient is not followed at the University of Michigan, survival will be assessed by phone call every 6 months until 3 years after trial enrollment. We will attempt to obtain MRI reports from these patients as well to assess for progression.
[i]Serum (at least 1 mL) will be collected in a red-top tube on the first day of radiation therapy.

Phase 1 Newly Diagnosed GBM

	Pretreatment evaluation: Days -28-0	MMF: Day 1-7	RT+MMF: Beginning day 8 (+/- 3 days)[h]	4 weeks after RT completion (+/- 7 days)	SOC adjuvant TMZ (each cycle)	Survival Follow Up
Informed Consent	X					
History & Physical exam (including NANO scale[a])	X			X	X[i]	
Karnofsky Performance Status	X			X	X[i]	
Toxicity (include DLT) Evaluations	X	X	Weekly	X	X[i]	
MRI[b]	X			X		X[j]
Patient Pill Diaries	X	X	X	X	X[i]	
Pathology report	X					
Prior radiation record	X					
CBC with diff	X	Weekly	Weekly	X	X[i]	
CMP[c]	X		X	X		
QOL (MDASI[d])	X			X		
Pregnancy Test (child bearing age women) [e]	X[f]					
MMF Administration [f]		X	X		Start 1 day before TMZ	
Temozolomide [g]			X		X	
Phone Calls (q6 month)						X[j]
Blood MPA concentration measurement[k]			X			

[a]See Appendix I

[b]MRI during follow up period will be at the discretion of treating physician. Modified RANO willbe used to assess MRI during follow up.

[c]CMP should include sodium, potassium, chloride, CO2, BUN, creatinine, glucose, calcium, albumin, protein, total bilirubin, alkaline phosphatase, ALT, and AST.

[d]See Appendix II
[e]Within 14 days prior to MMF start date
[f] See dosing schema (Section 5.1.3)
[g] Dosing and schedule per standard of care.
[h]Allow up to 3 day delay in start of radiation (continue MMF monotherapy in such case), and up to 5 days for RT completion date delay.
[i] Clinical evaluation and CBC with differential to be done up to 10 days prior to, to the date of temozolomide day 1 each cycle. Cycle 1 items may be fulfilled by the studies completed at the 4 weeks post-RT follow up.
[j]If patient is not followed at the University of Michigan, survival will be assessed by phone call every 6 months until 3 years after trial enrollment. We will attempt to obtain MRI reports from these patients as well to assess for progression.
[k]Serum (at least 1 mL) will be collected in a red-top tube on the first day of radiation therapy.

8.2 Toxicity Assessment

Toxicity will be assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Toxicity will be assessed on each patient prior to treatment, weekly during radiation therapy and at each follow-up visit. Dose-limiting hematologic and non-hematologic toxicities will be defined differently.

Dose limiting toxicity (DLT) will be assessed in phase 1 groups. In the recurrent GBM phase 1 (DLT) and the newly diagnosed GBM phase 1 (DLT1), dose limiting toxicity will be based on events occurring up to 4 weeks after the completion of radiation and MMF. In order to be declared a dose-limiting toxicity, an adverse experience must be at least possibly related (i.e. definitely, probably, or possibly) to study therapy.

In newly diagnosed phase 1 cohort, DLT (DLT2) will be determined based on events occurring during cycle 1 and 2 of adjuvant cyclic temozolomide (day 0 of cycle 1 through day 28 of cycle 2).

8.3 Dose Limiting Toxicity

- Grade 4-5 hematological toxicity with the exception of grade 4 neutropenia lasting for <7 days because of its transient nature. Asymptomatic grade 4 lymphopenia will not be considered DLT because ALC <200/mm3 is frequently observed in patients with GBM, and the clinical management in this situation usually involves *Pneumocystis jirovecii* prophylaxis.
- Grade 4-5 non-hematologic events that are not clearly due to the underlying disease or extraneous causes.
- Grade 3 or grade 4 neutropenia with fever >38.5°C and/or infection requiring

antibiotic or anti-fungal treatment

- Treatment related grade ⩾3 non-hematological toxicities, except
    - Nausea, vomiting, or diarrhea that in the opinion of the investigator occurs in the setting of inadequate supportive care measures and lasts for less than 48 hours
    - Any grade alopecia
    - Inadequately treated hypersensitivity reactions
    - Clinically non-significant, treatable or reversible lab abnormalities including: glucose, uric acid, etc.
- Missed doses: if a patient misses more than 50% of the scheduled doses of MMF it will be considered a DLT (unless it is due to poor patient compliance in the absence of treatment-related toxicity)
- A delay of >2 weeks in the scheduled administration of radiation due to drug-related toxicities, which do not qualify as a DLT per the guidelines above will be considered a DLT (except if the delay is due to a cause unrelated to the disease or treatment under study, e.g., family emergency or auto accident)

9.0 Evaluation Criteria and Endpoints

9.1 Response Criteria

Modified RANO (mRANO, PMID 28108885) criteria will be used primarily to evaluate objective tumor response. Measurable disease is defined as bi-dimensionally contrast enhancing lesions with clearly defined margins by MRI scan. Two-dimensional, perpendicular measurements of contrast enhancing tumor size, excluding the resection cavity along with any cysts or areas of central macroscopic necrosis, will be used for response assessment.

Up to a total of five target measurable lesions are included as target lesions. Measurable disease is defined as contrast enhancing lesions with a minimum size of both perpendicular measurements ≥ 10mm. Non-measurable disease is defined as lesions that are < 10mm in either perpendicular dimensions, lesions that lack contrast enhancement (non-enhancing disease), or lesions that contain a poorly defined margin that cannot be measured or segmented with confidence.

The post-radiotherapy MRI brain obtained around 4 weeks post-RT will be used as the baseline study.

Formal response at the following time points will be assessed after baseline assessment using MRI and clinical information obtained approximately every 2-3 months after chemoradiation at an interval seen as appropriate per standard of care by treating physician(s).

9.2 Complete Response (CR)

Complete response requires all following:

1. Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. The first scan exhibiting disappearance of all enhancing measurable and non-measurable disease is considered "preliminary CR". If the second scan exhibits measurable enhancing disease with respect to the "preliminary CR" scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered "preliminary PD" (note confirmed PD requires at least two sequential increases in tumor volume). If the second scan continues to exhibit disappearance of enhancing disease or emergence of non-measurable disease (< 10mm bidimensional product), it is considered a *durable CR*.
2. Patients must be off corticosteroids (or on physiologic replacement doses only).
3. Stable or improved clinical assessments (i.e. neurological examinations).

Note: Patients with non-measurable disease only at baseline cannot have CR; the best response possible is stable disease (SD).

9.3 Partial Response (PR)

Partial response requires all following:

1. ≥50% decrease in sum of products of perpendicular of all measurable enhancing lesions compared with baseline, sustained for at least 4 weeks. The first scan exhibiting ≥50% decrease in sum of products of perpendicular diameters of all measurable enhancing lesions compared with baseline is considered "preliminary PR". If the second scan exhibits PD with respect to the "preliminary PR" scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered "preliminary PD" (note confirmed PD requires at least two sequential increases in tumor volume). If the second scan exhibits SD, PR, or CR, it is considered a *durable PR*.
2. Steroid dose should be the same or lower compared with baseline scan.
3. Stable or improved clinical assessments.

Note: Patients with non-measurable disease only at baseline cannot have PR; the best response possible is stable disease (SD).

9.4 Stable Disease (SD)

Stable disease requires all following:

1. Does not qualify for CR, PR, or PD as defined in this section. Note this also applies to patients that demonstrate PsR when the confirmation scan does not show PD or PsP when the confirmation scan does not show PR/CR.
2. In the event that corticosteroid dose was increased (for new symptoms/signs) without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that the steroid increase was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

9.5 Progression (PD)

1. Progressive disease (PD) requires all following:At least two sequential scans separated by at ≥4 weeks both exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions. The first scan exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions should be compared to the smallest tumor measurement obtained either at baseline (if no decrease) or best response (on stable or increasing steroid dose) and is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the "preliminary PD" scan, it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD or PR/CR, this scan showing "preliminary PD" is noted as "pseudoprogression", PsP, and the patient should continue on therapy until a second increase in tumor size relative to the PsP scan is observed. Note that any new *measurable* (>10mm x 10mm) enhancing lesions should *not* be immediately considered PD, but instead should be added to the sum of bidimensional products or total volume representing the entire enhancing tumor burden.
2. In the case where the baseline or best response demonstrates no measurable enhancing disease (visible or not visible), then any new *measurable* (>10mm x 10mm) enhancing lesions are considered PD *after* confirmed by a subsequent scan ≥4 weeks exhibiting ≥25% increase in sum of products of perpendicular relative to the scan first illustrating new measurable disease. The first scan exhibiting new measurable disease is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the "preliminary PD" scan it is considered "confirmed PD". If the second scan at least 4 weeks later exhibits SD, CR, PR, or becomes non-measurable, this scan showing "preliminary PD" is noted as "pseudoprogression", PsP. Note that any new *measurable* (>10mm x 10mm) enhancing lesions on the subsequent scan following the preliminary PD scan should *not* be immediately considered confirmed PD, but instead should be added to the sum of bidimensional products or total volume representing the entire enhancing tumor burden.

3. Clear clinical deterioration not attributable to other causes apart from tumor (e.g. seizures, medication adverse effects, therapy complications, stroke, infection) or attributable to changes in steroid dose.
4. Failure to return for evaluation as a result of death or deteriorating condition.

9.5.1 Progression free survival. Time from date of registration to the date of documented progressive disease, other disease related therapy or death.

Subjects who withdraw from the study due to reasons other than study-related toxicity prior to initiation of radiotherapy will not be included in the response / survival analysis.

Images obtained after initiation of anti-VEGF therapy such as bevacizumab initiation will be considered non-evaluable for response, and subjects will be censored for progression free survival analysis.

9.5.2 Freedom from local progression. Time from date of registration to the date of documented local progressive disease.

9.5.3 Overall Survival. Time from date of registration to date of death or last follow up.

Subjects who withdraw from the study due to reasons other than study-related toxicity prior to initiation of radiotherapy will not be included in the response / survival analysis.

10.0 Adverse Events

10.1 Adverse Event Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial and is done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Data on adverse events will be collected from the time of the initial study enrollment through 30 days after the last dose of MMF. Any serious adverse event that occurs more than 30 days after the last dose of MMF and is considered related to the study treatment must also be reported. Serious Adverse Events (SAEs) will continue to be followed until:

- Resolution or the symptoms or signs that constitute the serious adverse event return to baseline;
- There is satisfactory explanation other than the study treatment for the changes observed; or
- Death.

The investigator is responsible for the detection, documentation, grading and

assignment of attribution of events meeting the criteria and definition of an AE or SAE. The definitions of AEs and SAEs are given below. It is the responsibility of the principal investigator to ensure that all staff involved in the trial is familiar with the content of this section.

Any medical condition or laboratory abnormality with an onset date before initial study treatment is considered to be pre-existing in nature. Any known pre-existing conditions that are ongoing at time of study entry should be considered medical history.

All events meeting the criteria and definition of an AE or SAE, as defined in Section 9.2, occurring from the initial study enrollment through 30 days following the last dose of MMF must be recorded as an adverse event in the patient's source documents and on the CRF regardless of frequency, severity (grade) or assessed relationship to the study treatment.

In addition to new events, any increase in the frequency or severity (i.e., toxicity grade) of a pre-existing condition that occurs after the patient begins study treatment is also considered an adverse event.

10.2 Definitions

10.2.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

- Diagnostic and therapeutic non-invasive and invasive (i.e., surgical) procedures will not be reported as adverse events. However, the medical condition for which the procedure was performed must be reported if it meets the definition of an adverse event unless it is a pre-existing (prior to protocol treatment) condition.
- Symptoms of the original or targeted disease are not to be considered adverse events for this study. The following symptoms are indicative of underlying disease GBM or gliosarcoma and will not be reported as adverse events (unless the event is considered serious):
    - Tumor progression causing neurologic signs or symptoms such as headache, nausea, vomiting, cognitive changes or focal neurologic deficits
- Abnormal laboratory values or test results constitute adverse events if they induce clinical signs or symptoms or require therapy. They are to be captured under the signs, symptoms or diagnoses associated with them.
- Unrelated events will be reported according to the IRB guidelines.
- During the course of an adverse event, severity and/or causality may

change. For CRF documentation this adverse event represents one entity from onset to resolution and the worst of the observed categories shall be attributed.

- When an AE reoccurs after it disappeared, it should be handled as a new AE. However, AEs that occur intermittently can be recorded as a single AE.

10.2.2 Serious Adverse Event

An adverse event is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event

  An adverse even is considered 'life-threatening' if, in the view of either the investigator, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization for $\geq$ 24 hours.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical event
  - Any event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition of "Serious Adverse Event". Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that do not result in inpatient hospitalization or the development of drug dependency or drug abuse

Previously planned (prior to signing the informed consent form) surgeries should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study. Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient's medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (for example, for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. However, if the preexisting condition worsened during the course of the study, it should be reported as an SAE.

10.2.3 Expected Adverse Events

An adverse event (AE) is considered "expected" if:

- For approved and marketed drugs or devices, those adverse events are described in the approved Package Insert (Label).
- For investigational new drugs or devices, those adverse events are described in the FDA Investigator's Brochure.
- In clinical research studies, information on expected adverse events is also summarized in the protocol and in the consent document. See section 6.4 for the list of expected adverse events related to the drug under study.

10.2.4 Unexpected Adverse Event

An adverse event (AE) is considered "unexpected" if it is not described in the Package Insert, Investigator's Brochure, in published medical literature, in the protocol, or in the informed consent document.

10.3 Adverse Event Characteristics and Attributions

10.3.1 CTCAE Term

AE description and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0. can be downloaded from the CTEP web site. (http://ctep.cancer.gov)

10.3.2 Attribution of the AE

The investigator or co-investigator is responsible for assignment of attribution.

Definite – The AE *is clearly related* to the study intervention.
Probable – The AE *is likely related* to the study intervention.
Possible – The AE *may be related* to the study intervention.
Unlikely – The AE *is doubtfully related* to the study intervention.
Unrelated – The AE *is clearly NOT related* to the study intervention.

10.4 Serious Adverse Event Reporting Guidelines

10.4.1 The Principal Investigator must be notified within 2 business day of study team's knowledge of any event meeting the criteria and definition of a serious adverse event, regardless of attribution, occurring during the study or within 30 days of the last administration of the study related intervention.

10.4.2 The investigator must report all events meeting the criteria and definition of a serious adverse event as per the current institutional IRB reporting requirements.

10.4.3 The Study Team will coordinate with the Michigan Institute for Clinical

and Health Research (MICHR) IND/IDE Investigator Assistance Program (MIAP) office for the reporting of any and all IND safety reports to the FDA as per the requirements outlined in 21 CFR 312.32. This includes reporting of all Serious Adverse Events (SAEs) that are both unexpected and related to the drug as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting. If the unexpected and related SAE is either fatal or life-threatening, then the SAE must be reported as soon as possible but in no case later than 7 calendars days after the sponsor's initial receipt of the information.

A summary of all non-expedited safety reports will be submitted in the annual report.

10.5 Routine Reporting

All other adverse events will be reported annually as part of regular data submission if they meet the institutional IRB guidelines. Adverse events will no longer be reported if the patient has another GBM-directed therapy.

10.6 Reporting of Unanticipated Problems

Upon becoming aware of any incident, experience, or outcome (not related to an adverse event) that may represent an unanticipated problem, the investigator should assess whether the incident, experience, or outcome represents an unanticipated problem. The incident, experience or outcomes is considered unanticipated if it meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency);
2. Related or possibly related to participation in the research; and
3. Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the investigator determines that the incident, experience, or outcome represents an unanticipated problem, the investigator must report it to the IRB according to the local IRB policies.

10.7 Stopping Rules

When a new subject is enrolled, the probability of toxicities at each dose level will be estimated. The trial will stop early if the estimated probability of dose-limiting toxicity at the lowest dose level exceeds 35.

11.0 CORRELATIVES/SPECIAL STUDIES

11.1 Sample collection and methodology

For patients undergoing re-resection or biopsy in the phase 0 portion of this study, the following samples will be obtained at the time of craniotomy:

1. N=3 flash frozen samples from the contrast-enhancing tumor (one sample to assess tumor content, one to assess GTP levels, one to assess MPA levels)

2. N=3 flash frozen samples from the non-enhancing tumor (one sample to assess tumor content, one to assess GTP levels, one to assess MPA levels)
3. N=1 serum sample at the time of tumor resection (to assess plasma MPA concentrations)
    a. Serum (at least 1 mL) will be collected in a red-top tube and mycophenolic acid concentration will be determined by clinical laboratory at the University of Michigan

For patients on the phase 1 portion of this study, serum (at least 1 mL) will be collected in a red-top tube on the first day of radiation therapy. Mycophenolic acid concentrations in this sample will be determined by the clinical laboratory at the University of Michigan. GTP and other nucleobase species will be measured by an in-house metabolomics platform operated by Dr. Wahl and co-investigator Dr. Lyssiotis[44]. Mycophenolic acid concentrations in tissue samples will be analyzed by the pharmacokinetics core at the University of Michigan using a mass-spectrometry-based assay.

11.2 Specimen Banking

Patient samples collected for this study will be retained in a locked -80 °C Freezer in the Wahl Laboratory (MedSci1 room 4433). Specimens will be stored indefinitely or until they are used up. If future use is denied or withdrawn by the patient, best efforts will be made to stop any additional studies and to destroy the specimens.

Specimens being stored long-term for potential use not outlined in the protocol are subject to University policy governing tissue sample collection, ownership, usage, and disposition within all UMMS research repositories. http://msa.med.umich.edu/policies/governing-tissue-sample-collection-ownership-usage-disposition-within-all-umms-research

11.3 Optional analysis of prior and subsequent surgical tissues.

Patients will have the option of signing an optional consent form that would allow the research team to obtain pathologic tissue from prior or subsequent resections of brain tumor in order to compare GTP or other metabolite levels at the time of initial resection or future recurrence.

12.0 STATISTICAL CONSIDERATIONS

This is a phase 0/I dose-escalation trial to determine the maximum tolerated dose of MYCOPHENOLATE MOFETIL when administered with radiation, in patients with recurrent glioblastoma, and in patients with newly diagnosed glioblastoma with standard upfront radiation and temozolomide. Dose-escalation will be managed by the TITE-CRM algorithm, with the goal of establishing the target dose. Section 11.2 and 11.4 describes the TITE-CRM design in detail and provides operating characteristics under several simulation scenarios.

12.1 Phase 0 Component

The phase 0 component aims to assess the delivery of MMF to tumors and its effect on depletion of GTP, in patients with recurrent glioblastoma. The samples collected from both accrued patients and control patients will be measured for primary and secondary endpoints.

Number of Patients: 8 subjects will be accrued to the phase 0 component of this trial. The control group consists of 5 patients who undergo standard of care re-resection without MMF administration who have consented to tumor banking as part of another study. We expect that these control patients will have undetectable MPA (i.e., serve as negative controls) and have normal levels of GTP (i.e., will serve as positive controls). Based on recent recruitment, it is expected that these 13 patients will be accrued in approximately two years.

Sample Collection: For each patient, samples will be collected from both contrast-enhancing and non-enhancing tumors. There are no repeated measurements.

Primary Endpoint: The concentration of MPA (the active metabolite of MMF) in tumors at the time of craniotomy will be measured by mass spectrometry on a continuous scale.

Secondary Endpoint: The concentrations of GTP in both recurrent GBM tissue and plasma after one week of MMF administration will be measured by mass spectrometry on a continuous scale.

Statistical Analysis Plan: The primary objective is to assess the delivery of MMF to tumors. We propose to fit two regression models for comparison of tumor tissue MMF concentrations between two patient groups while adjusting for the MRI contrast-enhancement status: one model with binary MMF exposure, MMF vs. control; and the other use dose as continuous exposure (0, 500, 1000, 1500 mg).

The Secondary objective is to evaluate the intratumoral GTP deletion. We plan to analyze the data using the same regression framework as in the primary objective.

The correlative study aims to model the relationship between tumor MPA levels to plasma MPA levels. We propose to use regression for modeling this relationship. The outcome is MPA concentration in tumor tissue, and the independent variables are plasma MPA concentration and MRI contrast-enhancement status.

Sample Size and Power: We conduct power analysis using a 1-sided 0.05 level t-test. Because of varying dosages, we assume the variance of MPA concentration in the MMF group is twice as large as that in the control group.

A sample size of 13 (8 patients with MMF and five control patients) will be able to provide >80% power to detect a large effect size of 1 standard deviation of the MMF group.

12.2 Phase 1 Study Design in Recurrent Glioblastoma/Study Endpoints

Number of Patients: 30 subjects evaluable for toxicity will be accrued to the phase 1 portion of this trial. This includes any patient who receives phase 1 medication/treatment. Subjects who are non-evaluable for toxicity (ie withdraw from trial prior to initiating treatment) will be replaced. Based on recent recruitment, it is expected that 10-15 subjects can be accrued to this trial per year. The trial will be run until either 30 patients evaluable for toxicity have been enrolled OR the estimated probability oftoxicity at the lowest dose level exceeds 35%, in which case the trial would be stopped early.

Dose Assignment: The first patient will be treated at dose level 3. Each subsequent patient's dose level will be assigned using the TITE-CRM algorithm. When a new subject is enrolled, the probability of toxicity at each dose is estimated, based on the toxicity data accrued up to that time, using a one-parameter logistic dose-toxicity model described below. The subject is assigned to the highest dose with estimated probability of toxicity closest to but less than the target rate of 30%, subject to two dose-escalation restrictions: (1) the dose of subject i cannot be more than one level greater than the dose of subject i-1; (2) at least one patient must have completed the entire DLT observation period at Dose j before a subject can be treated at Dose j+1.

Target Dose: The target dose will be that which produces DLT at a frequency of 0.30. That is, the goal of the trial is to treat patients at the highest dose associated with DLT in not more than 30% of patients.

Initial Estimates of Probability of Toxicity: Before any subjects are treated, the estimated probability of toxicity at each dose level is given in the following table. The sensitivity of operating characteristics of the trial (number of toxicities, quality of estimation of target dose) to these estimates is assessed by means of Monte Carlo simulations.

Dose Level	Prior P(DLT)	Probability of DLT		
		Scenario 1	Scenario 2	Scenario 3
1	.05	.05	.10	.10
2	.10	.10	.15	.20
3	.15	.15	.20	.30
4	.20	.20	.30	.45
5	.25	.25	.40	.60

Dose (d), initial estimate of probability of toxicity for dose d (drs), rescaled dose values, and true probabilities of toxicities used in Monte Carlo simulations (scenarios 1-3) of TITE-CRM trials.

Dose-toxicity function: A continuous, monotonically increasing function relating treatment dose to the probability of toxicity is employed to allow subjects‟ experience at any dose to contribute to the estimation of the probability of toxicity at all doses. The function is an approximation to the true state of nature; the trial estimates are robust against misspecification of this function. This trial will use a logistic dose-toxicity model:

$$p(DLT|d)=\exp(3+\alpha \bullet drs)/(1+\exp(3+\alpha \bullet drs))$$

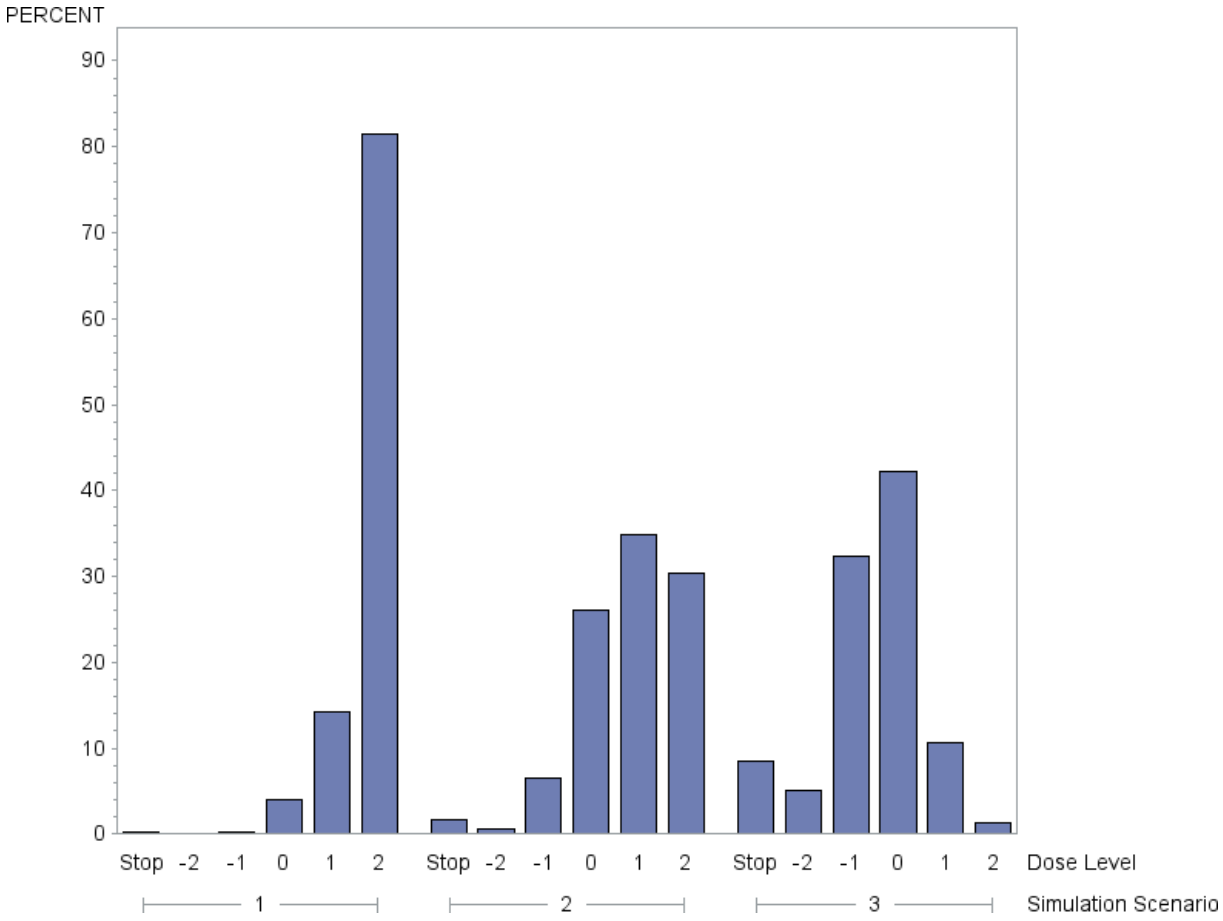
where α, the dose-toxicity parameter, is to be estimated, and *drs* is the rescaled dose, calculated from the initial estimate of probability of toxicity (pd), assuming α=1.

Estimation of p(DLT|d): The prior distribution of the dose-toxicity parameter, α, is Gaussian, with mean 1 and standard deviation 0.3. At any time, the probability of toxicity at each dose is estimated by calculating the expected value of alpha, given the prior and the trial data up to that time, and entering the expected value of alpha into the logistic dose-toxicity function. At a given point of time, the datum of subject i is represented by the triple (di, yi, wi), where di denotes the dose the subject received, yi the response (0=no DLT, 1=DLT) and wi the weight (see table X below) derived from the portion of the approximately 210 day observation period the subject has completed at the given time, or, if the subject has experienced DLT, 1. The expectation of α can be calculated using a straightforward univariate numerical integration, and is implemented in a SAS code that has been used in a number of trials.

Observation Period: Observation for DLT (as defined in Section 8.3/8.4) will begin on the first day of treatment, and continue for 28 days following completion of radiation and MMF. Subjects who are rendered unevaluable for DLT during this period for reasons unrelated to toxicity of treatment (e.g., disease progression) will not be counted towards the accrual goal, but will be fractionally counted in the estimation of the probability of toxicity. The fractional weight will be linear over the 28-day period so for example a patient who has completed 14 days without toxicity would receive a weight of .50.

Operating Characteristics: Operating characteristics, estimated by means of Monte Carlo simulation, are presented below. The primary goal of the simulations is to check the design robustness against the misspecification of the dose-conditional probability of toxicity. For this presentation, 1000 trials were simulated for each of three sets of characteristics (Section 12.1.5), where the true state of nature was as specified in the protocol (Scenario 1), more toxic than assumed (Scenario 2), and much more toxic than assumed, with a rapid increase in toxicity near the target rate (Scenario 3). Metrics presented include the distribution of selected target doses (a measure of estimation efficacy), the number of observed toxicities (a measure of risk) and the numbers of subjects treated at each dose (indicating the number of subjects treated at potentially

therapeutic doses). In each of the 3 simulations, the correct dose is the most frequently selected dose. When the priors are correct the highest dose level is selected in greater than 80% of trials. When the priors are far off, as in the case of scenarios 2 and 3, the correct dose level is still selected in approximately 40% of trials. In all scenarios, the selected MTD is within 1 dose level of the true MTD in greater than 80% of trials. Simulation 3 represents the potentially riskiest state of nature, but even in this case the median number of DLT‟s is 9 (30% of the total 30 patients). In all three simulations, the average number of subjects treated at the target dose or within 1 level, is greater than 20, allowing for Phase 2a estimates of efficacy. It has been previously demonstrated [30] that, in general, the TITE-CRM design is not significantly riskier (in terms of number of toxicities/subjects treated) than the „traditional‟ design, while better estimating the target dose, and treating a greater number of subjects at potentially therapeutic doses.



Cumulative Number of DLTs Per Trial			
Simulation	10th Percentile	Median	90th Percentile
1	3	6	8
2	6	8	10
3	7	9	11

Median Number of Patients Treated at Each Dose Level					
	Dose Level				
Simulation	1	2	3	4	5
1	0	1	7	7	14
2	1	3	9	9	8
3	3	6	10	6	2

Operating characteristics of example TITE-CRM trial: Upper plot shows the percent of simulated trials selecting each dose level as the target dose. In the table showing the average number of patients treated at each dose level, the numbers may not sum to 30 due to rounding or to the fact that some trials stop early and thus enroll fewer than 30 patients.

12.3 Phase 1 Data Analysis Plan in Recurrent Glioblastoma Patients

Primary Objective: To determine the target dose and toxicity profile of MMF when administered concurrently with radiation in patients with recurrent glioblastoma.

At the end of the trial, once all evaluable subjects have completed observation, p(DLT|d) will be calculated for each dose using the tite-CRM method (one parameter logistic regression model with prior distribution on the parameter alpha). The final estimated target dose will be the dose d with estimated p(DLT|d) closest to the target rate but not exceeding 30%. To further characterize toxicity as a function of dose, we will use maximum likelihood to fit a two parameter logistic regression model to the data from this trial. This more flexible model typically provides estimates of toxicity very close to the tite-CRM estimates at and near the target dose, but better estimates toxicity at doses further from the selected target dose. The numbers of subjects treated at each dose and the numbers experiencing DLT will be tabulated. Toxicities at each dose level will be tabulated, categorized by grade and by whether they are not, possibly, probably or definitely related to treatment. Toxicities not considered dose limiting may also be summarized in this manner and modeled as a function of dose level.

Secondary Objective 1: To estimate the efficacy of this regimen (combined with standard systemic therapy) at the target dose, as determined by progression-free survival, overall survival, freedom from local progression and response rates. Overall survival (OS), progression-free survival (PFS) and freedom from local progression (FFLP) will be summarized by Kaplan-Meier curves and characterized by descriptive statistics such as median OS and PFS times. For OS, subjects alive at the time of last FU will be censored at that time. Similarly, for

PFS and FFLP, subjects alive and without progression will be censored at the last date on which they were assessed for progression. A likelihood ratio test will be used to compare the estimated median OS with historical controls. Cox proportional hazards regression models will be used to estimate OS, PFS and FFLP as a function of dose and possibly other covariates such as age. Response will be assessed per mRANO. The number and proportion of patients with progressive disease, stable disease, partial and complete response will be calculated for each dose level and overall. Logistic regression models will be used to model probability of any response as a function of dose level. Exploratory analyses will be conducted to assess for any relation between MPA levels and efficacy outcomes.

12.4 Phase 1 Study Design in Newly Diagnosed Glioblastoma/Study Endpoints

Number of Patients: 30 subjects evaluable for toxicity will be accrued. This includes any patient who receives phase 1 medication/treatment. Subjects who are non-evaluable for toxicity (ie withdraw from trial prior to initiating treatment) will be replaced. Based on recent recruitment, it is expected that 10-15 subjects can be accrued to this trial per year. The trial will run until either 30 patients evaluable for toxicity have been enrolled, OR the estimated probability of toxicity at the lowest dose level exceeds 35%, in which case the trial would be stopped early. Note at least 6 patients should be enrolled before early stopping is considered.

The primary study endpoints are the DLTs defined in Section 2.4. A DLT will be any of the events specified in Section 8.3 during the radiation therapy and the corresponding observation window (DLT1), and then re-assessed during the course of TMZ and the corresponding observation window (DLT2).

Dose Assignment: In the radiation component, the first patient will be enrolled to dose level 3 of MMF in combination with radiation. Each subsequent patient's dose level will be assigned using the TITE-CRM algorithm. When a new subject is enrolled, the probability of toxicity at each dose is estimated, based on the toxicity data accrued up to that time, using a one-parameter logistic dose-toxicity model described below. The subject is assigned to the highest dose with an estimated probability of toxicity closest to, but less than the target rate of 30%, subject to two dose-escalation restrictions: (1) the dose of subject i cannot be more than one level greater than the dose of subject i-1; (2) at least one patient must have completed the entire DLT observation period at Dose j before a subject can be treated at Dose j+1.

In the TMZ component, the first patient will be treated at dose level 3 unless they experienced DLT1 in which case they will be treated at dose level 2. Patients who have DLT1 in the radiation phase will be treated with MMF at one dose level lower than they received in the radiation component, or at the lowest dose level if they received the level 1 with radiation and had DLT1. Otherwise, the subsequent dose assignment will be managed by the TITE-CRM algorithm, the same as described above.

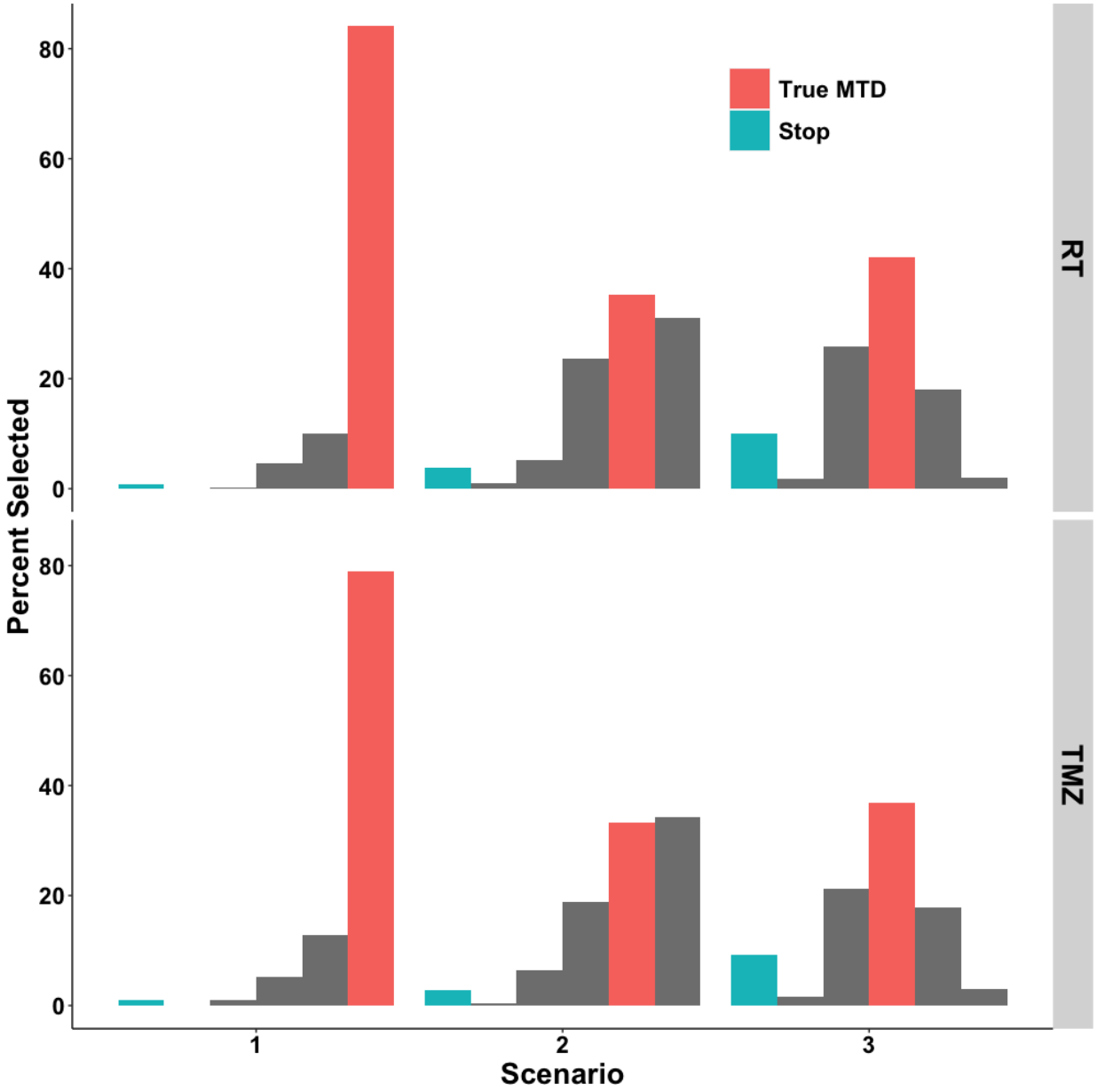
Target Dose: In both components, the target dose will produce DLTs at a frequency of 0.30. The goal of the trial is to treat patients at the highest dose associated with DLT in not more than 30% of patients.

Initial Estimates of Probability of Toxicity: The following table summarized the estimated probability of toxicity at each dose level. The prior probabilities are assumed to be the same in both treatment components.

Dose Level	Prior P(DLT)	Probability of DLT		
		Scenario 1	Scenario 2	Scenario 3
1	.05	.05	.10	.10
2	.10	.10	.15	.20
3	.15	.15	.20	.30
4	.20	.20	.30	.45
5	.25	.25	.40	.60

Observation Period: Observation for DLT1 will begin on the first day of treatment, and continue for 28 days following completion of radiation and MMF. Observation for DLT2 will begin on the first day of adjuvant MMF in cycle 1 and continue for 28 days after the first dose of MMF in cycle 2 (total duration of approximately 8 weeks). Subjects who are rendered unevaluable for DLT during this period for reasons unrelated to the toxicity of treatment (e.g., disease progression) will not be counted towards the accrual goal but will be fractionally counted in the estimation of the probability of toxicity. The fractional weight will be linear over the 8 weeks, so for example, a patient who has completed 4 weeks without toxicity would receive a weight of .50.

Operating Characteristics: Operating characteristics in both radiation and TMZ components were assessed using Monte Carlo simulation. One thousand trials were simulated under three scenarios, where the true state of nature was as specified in the protocol (Scenario 1), more toxic than assumed (Scenario 2), and much more harmful than assumed, with a rapid increase in toxicity near the target rate (Scenario 3). Metrics presented include the distribution of selected target doses (a measure of estimation efficacy), the number of observed toxicities (a measure of risk), and the numbers of subjects treated at each dose (indicating the number of subjects treated at potentially therapeutic doses).



Cumulative Number of DLTs Per Trial (Median (IQR))		
Scenario	RT	TMZ
1	7 (5, 8)	6 (5, 8)
2	9 (8, 10)	8 (7, 9)
3	9 (8, 10)	9 (7, 10)

Median Number of IQR of Patients Treated at Each Dose Level						
	Dose					
	Scenario	1	2	3	4	5
RT	1	0 (0, 0)	0 (0, 1)	4 (3, 6)	2 (1, 6)	**23 (14, 26)**
	2	0 (0, 0)	0 (0, 3)	7 (4, 12)	**7 (3, 11)**	7 (2, 17)
	3	0 (0, 2)	4 (0, 10)	**11 (5, 15)**	3 (1, 8)	1 (0, 3)
TMZ	1	0 (0, 1)	1 (0, 2)	2 (1, 4)	8 (6, 10)	**18 (11, 21)**
	2	0 (0, 2)	3 (1, 5)	6 (3, 9)	**9 (6, 12)**	7 (2, 14)
	3	2 (0, 4)	6 (3, 11)	**8 (4, 12)**	5 (2, 8)	1 (0, 4)

In the RT component, the correct dose is the most frequently selected in all three scenarios. And in the TMZ component, the correct dose is the most frequently selected in Scenarios 1 and 3; while in Scenario 2, there is still a decent probability of selecting the right MTD. Note Scenario 2 has the most difficult setup for establishing DLT, in terms that the true DLT rate at dose levels 4 and 5 are close. In both components, when the priors are correct the highest dose level is selected in greater than 80% of trials. In all scenarios, the selected MTD is within 1 dose level of the true MTD in greater than 80% of trials. All scenarios have reasonable number of DLTs, the median number of DLTs are less than or equal to 9, i.e., 30% of patients. In all three scenarios, the median number of subjects treated at the target dose or within 1 level, is greater than 20, allowing for Phase 2a estimates of efficacy.

Data Analysis Plan

At the end of the trial, once all evaluable subjects have completed observation, the DLT rate at each dose level in both components will be estimated using one-parameter logistic regression models. The estimated MTDs will be the dose d1 (and d2) with estimated rate of DLT1 (and DLT2) closest to the target rate but not exceeding 30%. The numbers of subjects treated at each dose level and DLT incidence in both components will be summarized.

Overall survival (OS), progression-free survival (PFS) and freedom from local progression (FFLP) will be summarized by Kaplan-Meier curves and characterized by descriptive statistics such as median OS and PFS times. Cox proportional hazards regression models will be used to estimate OS, PFS and FFLP as a function of MMF dose in both components and possibly other covariates such as age. Patient response will be assessed per mRANO and analyzedwith regression models with dose in both components.

13.0 DATA AND SAFETY MONITORING

This study will be monitored in accordance with the NCI approved University of Michigan Rogel Cancer Center Data and Safety Monitoring Plan.

The study team will provide continuous review of the data and patient safety and meet monthly or more frequently depending on the activity of the protocol. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and

integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness. At these regular meetings, the protocol specific Data and Safety Monitoring Report form will be completed and signed by the Principal Investigator or by one of the co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Rogel Cancer Center Data and Safety Monitoring Committee (DSMC) on a monthly basis for independent review.

14.0 REFERENCES

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Appendix I: Neurologic Assessment in Neuro-Oncology (NANO) Scale

Neurologic Assessment in Neuro-Oncology (NANO) Scale

Scoring assessment is based on direct observation and testing performed during clinical evaluation and is not based on historical information or reported symptoms. Please check one answer per domain. Please check "Not assessed" if testing for that domain is not done. Please check "Not evaluable" if a given domain cannot be scored accurately because of preexisting conditions, comorbid events, or concurrent medications.

Patient identifier: _____

Date assessment performed (day/month/year): _____

Study time point (ie, baseline, cycle 1, day 1, etc): _____

Assessment performed by (please print name): _____

Domains	**Key Considerations**
Gait 0 ☐ Normal 1 ☐ Abnormal but walks without assistance 2 ☐ Abnormal and requires assistance (companion, cane, walker, etc.) 3 ☐ Unable to walk ☐ Not assessed ☐ Not evaluable	• Walking is ideally assessed by at least 10 steps
Strength 0 ☐ Normal 1 ☐ Movement present but decreased against resistance 2 ☐ Movement present but none against resistance 3 ☐ No movement ☐ Not assessed ☐ Not evaluable	• Test each limb separately • Recommend assess proximal (above knee or elbow) and distal (below knee or elbow) major muscle groups • Score should reflect worst performing area • Patients with baseline level 3 function in one major muscle group/limb can be scored based on assessment of other major muscle groups/limb

1

Ataxia (Upper Extremity)

0 ☐ Able to finger-to-nose touch without difficulty
1 ☐ Able to finger-to-nose touch but difficult
2 ☐ Unable to finger-to-nose touch
☐ Not assessed
☐ Not evaluable

- Nonevaluable if strength is compromised
- Trunk/lower extremities assessed by gait domain
- Particularly important for patients with brainstem and cerebellar tumors
- Score based on best response of at least 3 attempts

Sensation

0 ☐ Normal
1 ☐ Decreased but aware of sensory modality
2 ☐ Unaware of sensory modality
☐ Not assessed
☐ Not evaluable

- Recommend evaluating major body areas separately (face, limbs, and trunk)
- Score should reflect worst performing area
- Sensory modality includes but not limited to light touch, pinprick, temperature, and proprioception
- Patients with baseline level 2 function in one major body area can be scored based on assessment of other major body areas

Visual Fields

0 ☐ Normal
1 ☐ Inconsistent or equivocal partial hemianopsia (≥quadrantanopsia)
2 ☐ Consistent or unequivocal partial hemianopsia (≥quadrantanopsia)
3 ☐ Complete hemianopsia
☐ Not assessed
☐ Not evaluable

- Patients who require corrective lenses should be evaluated while wearing corrective lenses
- Each eye should be evaluated, and score should reflect the worst performing eye

Facial Strength

0 ☐ Normal
1 ☐ Mild/moderate weakness
2 ☐ Severe facial weakness
☐ Not assessed
☐ Not evaluable

- Particularly important for brainstem tumors
- Weakness includes nasolabial fold flattening, asymmetric smile, and difficulty elevating eyebrows

Language

0 ☐ Normal

1 ☐ Abnormal but easily conveys meaning to examiner

2 ☐ Abnormal and difficulty conveying meaning to examiner

3 ☐ Abnormal; if verbal, unable to convey meaning to examiner; OR nonverbal (mute/global aphasia)

☐ Not assessed

☐ Not evaluable

- Assess based on spoken speech; nonverbal cues or writing should not be included
- **Level 1**: Includes word-finding difficulty; few paraphasic errors/neologisms/word substitutions; but able to form sentences (full/broken)
- **Level 2**: Includes inability to form sentences (<4 words per phrase/sentence); limited word output; fluent but "empty" speech

Level of Consciousness

0 ☐ Normal

1 ☐ Drowsy (easily arousable)

2 ☐ Somnolent (difficult to arouse)

3 ☐ Unarousable/coma

☐ Not assessed

☐ Not evaluable

- None

Behavior

0 ☐ Normal

1 ☐ Mild/moderate alteration

2 ☐ Severe alteration

☐ Not assessed

☐ Not evaluable

- Particularly important for frontal lobe tumors
- Alteration includes but is not limited to apathy, disinhibition, and confusion
- Consider subclinical seizures for significant alteration

Appendix II: M. D. Anderson Symptom Inventory – Brain Tumor (MDASI-BT)

Date: _____ **Institution:**_____

Participant Initials: _____ **Hospital Chart #:**_____

Participant Number: _____

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?	○	○	○	○	○	○	○	○	○	○	○

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MDASI-BT - 2006

Date: _____ **Institution:**_____

Participant Initials: _____ **Hospital Chart #:**_____

Participant Number: _____

	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 life?

Symptoms frequently interfere with how we feel and 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?	○	○	○	○	○	○	○	○	○	○	○

MDASI-BT - 2006