Full Study Protocol and Statistical Analysis Plan

Study Title: A Phase 1b/2 Study of Repeat rAdiation, Minocycline, and Bevacizumab in patients with recurrent gliOma (RAMBO)

Protocol Version 10: 12/03/2015

NCT #: NCT01580969
Unique Protocol ID: HCI55264
U of U IRB#: IRB_00055264

Principal Investigator: Adam Cohen, MD, MS
Huntsman Cancer Institute

A Phase 1b/2 Study of Repeat rAdiation, Minocycline, and Bevacizumab in patients with recurrent gliOma (RAMBO)

Trial ID: HCI55264/ IRB# 55264

Principal Investigator

Adam Cohen, MD
Huntsman Cancer Institute
2000 Circle of Hope
Salt Lake City, UT 84112
801-587-4024
adam.cohen@hci.utah.edu

Sub-investigator(s)

Howard Colman, MD, PhD
Huntsman Cancer Institute
2000 Circle of Hope
Salt Lake City, UT 84112
801-585-0617
howard.colman@hci.utah.edu

Dennis Shrieve, MD
Huntsman Cancer Institute
Radiation Oncology
2000 Circle of Hope
Salt Lake City, UT 84112
801-581-2396
dennis.shrieve@hci.utah.edu

Sean Strope, PA
Huntsman Cancer Institute
2000 Circle of Hope
Salt Lake City, UT 84112
801-587-4024
Sean.Strope@hci.utah.edu

Karen Salzman, MD
Huntsman Cancer Institute
University of Utah School of Medicine
Department of Radiology
2000 Circle of Hope
Salt Lake City, UT 84112
801-581-4624
karen.salzman@hsc.utah.edu

John Hoffman, MD
Professor of Radiology and Neurology
Director of Nuclear Medicine
University of Utah School of Medicine
Director: Molecular Imaging Program
Huntsman Cancer Institute
University of Utah School of Medicine
1950 Circle of Hope Suite 6810
Salt Lake City, UT 84112-5560
Telephone: (801) 587-4064
Fax: (801) 587-4008
Email: john.hoffman@hci.utah.edu

Randy Jensen, MD, PhD
Huntsman Cancer Institute
University of Utah School of Medicine
Department of Neurosurgery
2000 Circle of Hope
Salt Lake City, UT 84112
801-585-0617
randy.jensen@hsc.utah.edu

Matthew Poppe, MD
1950 Circle of Hope
Salt Lake City, UT 84112
Matthew.poppe@hci.utah.edu

David Gaffney, MD, PhD
2000 Circle of Hope
Salt Lake City, UT 84112
David.gaffney@hci.utah.edu

Gita Suneja, MD
2000 Circle of Hope
Salt Lake City, UT 84112
Gita.suneja@hci.utah.edu

Angela Gerrard, NP
2000 Circle of Hope
Salt Lake City, UT 84112
Angela.gerrard@hci.utah.edu

Investigational agents	Bevacizumab and Minocycline
IND Number	Exempt

Historical Protocol Versions

Version 1: 6-20-12
Version 2: 7-23-2012
Version 3: 8-24-2012
Version 4: 9-12-2012
Version 5:12-17-2012
Version 6: 2-26-2013
Version 7: 04-04-2013
Version 8: 08-08-2013
Version 9: 09-04-2014
Version 10: 12-03-2015

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Abbreviation or Term[1]	Definition/Explanation
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
β-HCG	Beta-human chorionic gonadotropin
BID	Twice daily
BP	Blood pressure
BUN	Blood urea nitrogen
CBC	Complete blood count
CNS	Central nervous system
CR	Complete response
CRF	Case report form
CSF-1	Colony stimulating factor-1 [also known as macrophage stimulating factor (M-CSF)]
CSF-1R	Colony stimulating factor receptor (also known as Fms)
CT	Computed tomography
CTCAE	Common Toxicity Criteria for Adverse Events
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
DLT	Dose Limiting Toxicity
ECG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
HIV	Human immunodeficiency virus
HR	Heart rate
hr	Hour or hours
IC_{50}	Half maximal inhibitory concentration
i.e.	Id est (that is)
IEC	Independent ethics committee
INR	International normalized ratio

Abbreviation or Term[1]	Definition/Explanation
IRB	Institutional review board
IU	International unit
IV	Intravenous, intravenously
LDH	Lactate dehydrogenase
MedRA	Medical Dictionary for Drug Regulatory Activities
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
PD	Pharmacodynamic(s)
PFS	Progression Free Survival
PK	Pharmacokinetic(s)
PO	Per os (administered by mouth)
PR	Partial response
PT	Prothrombin time
PTT	Partial thromboplastin time
SAE	Serious adverse event
$T_{1/2}$	Terminal elimination half-life
T_3	Triiodothyronine
T_4	Thyroxine
T_{max}	Time of maximum observed concentration
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
WBC	White blood cell

[1] All of these abbreviations may or may not be used in protocol.

PROTOCOL SIGNATURE

I confirm that I have read this protocol, and I will conduct the study as outlined herein and according to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practice, and the applicable laws and regulations of the federal government. I will promptly submit the protocol to the IRB for review and approval. I understand that any modification made during the course of the study must first be approved by the IRB prior to implementation except when such modification is made to remove an immediate hazard to the subject.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study treatment, the conduct of the study, and the obligations of confidentiality.

Note: This document is signed electronically through submission and approval by the Principal Investigator in the University of Utah IRB Electronic Research Integrity and Compliance Administration (ERICA) system.

STUDY SUMMARY

Title	**A Phase 1b/2 Study of Repeat rAdiation, Minocycline, and Bevacizumab in patients with recurrent gliOma (RAMBO)**
Protocol Number	IRB # 55264
IND	Exempt
Phase	Phase 1b/2
Study Objective(s)	The primary objective is the safety of minocycline and bevacizumab during and 4 weeks after reirradiation. The secondary objectives are, the response rate, PFS3, PFS6, and effects on quality of life and cognition from repeat radiation, minocycline and bevacizumab.
Study Design	This will be an open label dose finding trial to assess the safety and efficacy of repeat radiation with bevacizumab and minocycline in patients with recurrent glioma. The maximum tolerated dose will be established using 3 patients per cohort and there will be an expansion cohort (step 2) at the MTD. A total of 26 patients will be treated over 3 years. Dose limiting toxicities will be monitored during radiation and for 1 month afterward. Response rate and PFS will be based on MRI using RANO criteria. Quality of life and cognition will be assessed before treatment and 1, 3, and 6 months after radiation.
Study Duration	Patient recruitment period is approximately 30 months.
Number of Patients/Sites	Enrollment will be 26 patients at HCI, with approximately 6-9 patients in step 1 and 12 patients in step 2.
Study Procedures	After providing informed consent, patients will undergo screening for eligibility to participate in the study. Screening will start within 21 days prior to dosing. Subjects will have an MRI within 21 days of starting radiation. QOL and cognition measures will be performed within 21 days of starting radiation. Radiation will be given with parameters determined on an individual basis by the radiation oncologist. Bevacizumab will be continued at 10mg/kg IV every 2 weeks. Minocycline will be given twice a day starting at 100mg PO BID. MRI, QOL, and cognitive tests will be obtained 1, 3 and 6 months after the end of radiation.
Key Patient Selection	<u>Inclusion</u> 1. Male or female patients ≥18 years old with a life expectancy of at

Criteria	least 8 weeks 2. Radiographically proven recurrent (≥ first relapse), intracranial glioma 3. Previous treatment with external beam radiation 4. Radiographic progression on current or prior bevacizumab treatment by RANO criteria 5. Women of child-bearing potential must have a negative pregnancy test within 7 days of initiation of dosing and must agree to use an acceptable method of birth control while on study drug and for 3 months after the last dose. Women of non-childbearing potential may be included if they are either surgically sterile or have been postmenopausal for ≥1 year. Men who are able to father a child must also agree to use an acceptable method of birth control while on study drug, and for 3 months after the last dose. 6. Karnofsky performance status of ≥50 7. Adequate hematologic, hepatic, and renal function (absolute neutrophil count ≥1.0 x 10^9/L, Hgb >9 g/dL, platelet count ≥50 x 10^9/L, AST/ALT ≤2.5x ULN, creatinine ≤1.5x ULN) 8. Prothrombin time/international normalized ratio (PT INR) < 1.4 for patients not on warfarin confirmed by testing within 14 days prior to study registration 9. Patients on full-dose anticoagulants (e.g., warfarin or LMW heparin) must have no active bleeding or pathological condition that carries a high risk of bleeding, and must be on a stable dose of oral anticoagulant for 14 days or on a stable dose of low molecular weight heparin for 14 days 10. Willing and able to provide written informed consent prior to any study related procedures and to comply with all study requirements <u>Exclusion</u> 1. Use of an investigational drug within 14 days or within 5 half-lives of the investigational drug, whichever is shorter 2. Less than 6 months since the end of previous radiation 3. History of Grade 2 (CTCAE v4) or greater acute intracranial hemorrhage 4. A concurrent active cancer that requires non-surgical therapy (e.g. chemotherapy, radiation, adjuvant therapy). 5. Patients with serious illnesses, uncontrolled infection, medical conditions, or other medical history including abnormal laboratory results, which in the investigator's opinion would be likely to interfere with a patient's participation in the study, or with the interpretation of the results

	6. Women of child-bearing potential who are pregnant or breast feeding 7. Unstable angina and/or congestive heart failure in the last 6 months, transmural myocardial infarction within the last 6 months, New York Heart Association grade II or higher congestive heart failure requiring hospitalization within 12 months prior to registration, evidence of recent (within 14 days of registration) myocardial infarction by EKG (only required if clinically indicated), serious or inadequately controlled cardiac arrhythmia, significant vascular and peripheral vascular disease, evidence of bleeding diathesis or coagulopathy 8. History of stroke, cerebral vascular accident (CVA) or transient ischemic attack within 6 months 9. Serious or non-healing wound, ulcer, or bone fracture or history of abdominal fistula, gastrointestinal perforation, intra-abdominal abscess major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to registration, with the exception of the craniotomy for tumor resection 10. Chronic obstructive pulmonary disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration 11. Active connective tissue disorders, such as lupus or scleroderma, that in the opinion of the treating physician may put the patient at high risk for radiation toxicity 12. Uncontrolled symptomatic hypertension that cannot be controlled with anti-hypertensive agents.
Dosage and Regimen	Radiation will be administered per the judgment of the radiation oncologist. Bevacizumab will be given 10mg/kg every 2 weeks. Minocycline will be given twice a day starting at 100mg PO BID with dose modification in each cohort based on adverse event rate in previous cohorts.
Safety and Tolerability Assessments	Physical examinations, vital signs, and adverse events, will be used to assess safety and tolerability.
Primary Endpoint	Adverse event rate
Important Secondary Endpoints	PFS3, PFS6, response rate, change in MDASI-BT at 1, 3, and 6 months, and change in COGSTATE at 1, 3, and 6 months
Statistical Considerations	Descriptive statistics will be used to estimate endpoint rates.

.0 OBJECTIVES

1.1 Primary Objectives

1.1.1 To assess the safety of the combination of bevacizumab, minocycline and repeat radiation

1.2 Secondary Objectives

1.2.1 To assess thePFS3, PFS6, and response rate of bevacizumab, minocycline and repeat radiation as well as the course over time of QOL and cognition.

2.0 BACKGROUND

Gliomas are the most frequent brain tumor, accounting for approximately 12% to 15% of all brain tumors (CBTRUS). The peak incidence occurs between the ages of 45 and 70 years. Patients with recurrent glioma are incurable. Up front treatment options include surgery, radiation, and temozolomide chemotherapy (Stupp et al.). According to the National Cancer Institute, patients with brain tumors that are either infrequently curable or unresectable should be considered candidates for clinical trials that evaluate new drugs and biological response modifiers following radiation therapy.

Bevacizumab is FDA approved for glioblastoma that progresses after temozolomide therapy and is NCCN recommended for all gliomas after progression on temozolomide (Brem et al.). There is no FDA approved therapy for gliomas following progression on bevacizumab. The median progression free and overall survival after progression on bevacizumab is 1 and 3 months, respectively (Kreisl et al.). Because of the risk of rebound edema and retrospective data suggesting a survival advantage, many neuro-oncologists continue bevacizumab after progression. (D. A. Reardon)

Although historically repeat radiation for gliomas was considered prohibitively toxic, modern radiation techniques allow for repeat radiation in many cases. (Kim et al. ; Combs et al. ; Vordermark et al. ; Fogh et al.) Most studies of re-irradiation have not reported response rates or progression free survival. Moreover, most if not all patients in these series did not receive bevacizumab prior to re-irradiation, making them more favorable than current patients. Coombs et al reported on re-irradiation of 172 patients with recurrent gliomas, 54 of whom had glioblastoma. (Combs et al.) The median PFS of the glioblastoma patients was 5 months, with a PFS6 of 28%. Fugh reported on 105 patients with high grade gliomas who received repeat radiation with hypofractionation, of whom 53 were glioblastoma. Although 60% of patients had stable disease at 3 months, only 10% had a partial response by Macdonald criteria.

Two studies have directly addressed the question of reirradiation with concomitant bevacizumab. Niyazi treated thirty patients with repeat radiation, twenty of whom also received bevacizumab (Niyazi et al.). There was one grade 3 deep venous thrombosis and one grade 4 wound dehiscence. The response rate was not recorded. The PFS6 was 72% for radiation with bevacizumab and 24% for radiation alone. Gutin treated 25 bevacizumab-naïve patients with

epeat radiation and bevacizumab (Gutin et al.). There were three grade 3 toxicities, an intratumoral bleed, wound dehiscence, and bowel perforation. The response rate was 50% and PFS6 was about 64%. Neither of the previous studies included patients who had progressed on bevacizumab, who might have a different adverse event rate or response rate. Moreover, no studies have documented quality of life or cognition following repeat radiation.

Resistance to bevacizumab and to radiation is incompletely understood. Induction of alternate promoters of angiogenesis, increased invasion via induction of matrix metalloproteinases and c-MET, hypoxia induced recruitment of bone marrow-derived cells, and cooption of normal vessels have all been hypothesized to play a role in bevacizumab resistance. (Bergers and Hanahan ; Lucio-Eterovic et al.) Radioresistance has been hypothesized to be due to a combination of GBM stem cells, which may be protected by mTOR, survivin, gamma secreatase, Hypoxia-induced NF-KB signaling, or COX-2, EGFR-mediated double strand DNA repair, SOCS-gene loss, and microglia induction. (Chakravarti et al. ; Lal et al. ; Jiang et al. ; Rich ; Zhou et al. ; Murat et al. ; Hatanpaa et al. ; Lin et al. ; Anandharaj et al. ; Hellstrom et al. ; Zhuang et al.) Antiangiogenic agents, including bevacizumab, can increase the effectiveness of radiation in vivo.(Lee et al. ; Geng et al.)

Gene expression studies have identified multiple subtypes of glioblastoma including a mesenchymal (MES) subtype associated with increased angiogenesis and poor survival (Phillips et al., Colman et al.). Promoter analysis of the MES subtype and in glioblastoma stem cells (GSCs) has identified several transcription factors including STAT3, CEBPB, and TAZ associated with the MES subtype (Carro et al; Bhat et al). Recent studies have identified activation of NFkB as a key signaling factor promoting the MES subtype through upregulation of the MES transcription factors (Vaillant et al; Bhat et al) in GSCs and human tumors. Furthermore, activation of NFkB in GSCs by TNF treatment resulted in a MES shift, increased invasion, and radiation resistance. Blockade of NFkB activation by IkB-a was sufficient to prevent these TNF induced effects. Investigation of potential source of NFκB activation in GBM suggested a role of microglia. Indeed, addition of supernatant from activated microglia was sufficient to activate NFκB and MES transition in GSCs that could be blocked by IκB-□ (Vaillant et al.). To test the role of microglia and NFκB activation on treatment resistance in vivo, treatment with minocycline, an inhibitor of microglia activation, led to a reduction of tumor grade and down-regulation of MES markers in intracranial GSC xenograft models (Vaillant et al.). Taken together, these data indicate an important role for microglia activation of NFκB in the induction and maintenance of the MES phenotype and radiation resistance in GBM and GSCs. These data also suggest that blockade of NFκB and/or inhibition of microglia activation may be attractive therapeutic approaches for downregulating MES transition and overcoming treatment resistance in GBM.

Minocycline is a tetracycline-derivative that is FDA approved as an antibiotic. It also has anti-inflammatory properties that are not shared by all members of the tetracycline family. Minocycline has potential as an anti-glioma agent and as a radiation sensitizer for glioma. Minocycline inhibits matrix metalloproteinase expression by microglia, thus reducing glioma invasion and expansion. (Markovic et al.) Minocycline also induces glioma cell death via

utophagy and apoptosis.(Liu et al.) Animals treated with local administration of minocycline to tumor xenografts have improved survival. (Weingart et al.)

Minocycline also decreases inflammation following hypoxic or cytotoxic injury to the brain.(Nutile-McMenemy et al.) As such, it is currently being studied for neurodegenerative disorders. Microglia has been implicated in resistance to radiation. As described above, intracranial GSC xenograft studies in Dr. Colman's lab and labs of his collaborators have shown that minocycline reverses resistance to radiation in these molecularly faithful models of GBM tumors.

Because of its lipophilicity, minocycline is 100% bioavailable and does penetrate the brain. Minocycline is established to penetrate the CNS in humans and is the recommended antimicrobial for various meningoencephalitides (Tsai et al. ; Agustin et al. ; Fang et al.) In one study, after a single 200mg dose of minocycline, the CSF to serum ratio in one subject is 0.54. (Carney et al.) In another study of a single subject, after several days of treatment at 100mg twice a day, the CSF to serum ratio was 0.10-0.2. (Macdonald et al.) In dogs, the concentration of minocycline in the brain is between on-half and three times that in the serum. (Kelly and Kanegis ; Barza et al.) In rats, after 5-7 days of oral treatment, the brain concentration of minocycline is approximately equal to plasma concentrations. (Smith et al.) In horses, after 2 days of oral treatment, the CSF concentration of minocycline is approximately 50% of plasma concentration. (Schnabel et al.)

Minocycline can inhibit TNF secretion and NF-κB activation at concentrations less than 5μM, which is the serum concentratiom achieved after a single dose of 200mg. (Bernardino et al. ; Huang et al.) Microglia proliferation and activation may be suppressed at minocycline concentrations as low as 20 nM. (Tikka et al. ; Tikka et al.) At the usual dose of 100mg twice a day, the serum concentration averages about 4μM after several days. (Macdonald et al.) Two people treated at 400mg per day had serum levels of 6-12 μM. Therefore, although 100mg twice a day may be achieve high enough doses in the brain to induce a mesenchymal to proneural shift, higher doses may be necessary.

The long term tolerability of minocycline has been studied in patients with ALS, in whom the mean long-term tolerated dose of minocycline was 387mg per day. (Gordon et al.) Adverse effects were mainly gastrointestinal upset and nonsignificant increases in transaminases. It is likely that glioblastoma patients would have higher tolerance for these effects than ALS patients, and antiemetics and bowel motility agents used as supportive care for temozolomide may ameliorate the gastrointestinal effects. Minocycline doses of 5mg/kg/day for 5 days in combination with radiation have been well tolerated in animals without significant skin toxicity. (Sotomayor et al. ; Teicher et al.) This is not surprising because minocycline is considered much less phototoxic than other tetracyclines and does not share their release of ionized oxygen in response to UV light. (Hasan and Khan) Daily intravenous doses of 10mg/kg/day for 3 days were tolerated by stroke patients in the MINOS dose finding study (Fagan et al.) In a long-term study in people with Parkinson's disease, 77% of people were able to take 200mg twice a day for 18 months without quitting. Therefore, we propose to start at the usual antimicrobial dose of 100mg PO BID and increase up to a maximum dose of 800mg PO BID. Because the long term

afety of minocycline is well established, we will limit the phase 1 component of this study to acute toxicity that occurs during or immediately after chemoradiation.

Therefore, we hypothesize that addition of minocycline and bevacizumab to reirradiation for GBM after progression on bevacizumab will increase efficacy without adding significant toxicity. Because minocycline has not been tested with radiation in humans and other drugs in the same class (such as tetracycline) can have photosensitizing effects, we will first establish the safety of this combination and then extend the study to assess for evidence of efficacy. As a single agent, the mean long-term tolerated dose of minocycline is 387mg per day. (Gordon et al.) Adverse effects were mainly gastrointestinal upset and nonsignificant increases in transaminases. Minocycline doses of 5mg/kg in combination with radiation have been well tolerated in animals. (Sotomayor et al. ; Teicher et al.)

2.1 Bevacizumab

2.1.1 Description

Bevacizumab is a recombinant humanized anti-VEGF monoclonal antibody, consisting of 93% human and 7% murine amino acid sequences. The agent is composed of human IgG framework and murine antigen-binding complementarity-determining regions. Bevacizumab blocks the binding of vascular endothelial growth factor (VEGF) to its receptors resulting in inhibition of angiogenesis.

2.1.1 Bevacizumab Safety

Adverse Events >10%:
Cardiovascular: Hypertension (23% to 67%; grades 3/4: 5% to 18%), thromboembolic event (≤21%; grades 3/4: 15%; venous thrombus/embolus: 8%; grades 3/4: 5% to 7%; arterial thrombosis 6%; grades 3/4: 3%), hypotension (7% to 15%)
Central nervous system: Pain (31% to 62%), headache (24% to 37%; grades 3/4: 2% to 4%), dizziness (19% to 26%), fatigue (≤45%; grades 3/4: 4% to 19%), sensory neuropathy (grades 3/4: 1% to 17%; in combination with paclitaxel: 24%)
Dermatologic: Alopecia (6% to 32%), dry skin (7% to 20%), exfoliative dermatitis (3% to 19%), skin discoloration (2% to 16%)
Endocrine & metabolic: Hypokalemia (12% to 16%)
Gastrointestinal: Abdominal pain (50% to 61%; grades 3/4: 8%), vomiting (47% to 52%; grades 3/4: 6% to 11%), anorexia (35% to 43%), constipation (29% to 40%), diarrhea (grades 3/4: 1% to 34%), stomatitis (25% to 32%), gastrointestinal hemorrhage (19% to 24%), dyspepsia (17% to 24%), taste disorder (14% to 21%), flatulence (11% to 19%), weight loss (9% to 20%), nausea (grades 3/4: 4% to 12%)
Hematologic: Hemorrhage (≤40%; grades 3/4: 1% to 5%), leukopenia (grades 3/4: 37%), neutropenia (grade 4: 6% to 27%)
Neuromuscular & skeletal: Weakness (57% to 74%), myalgia (8% to 19%), back pain (≤12%)
Ocular: Tearing increased (6% to 18%)

Renal: Proteinuria (4% to 36%; grades 3/4: ≤7%; median onset: 5.6 months; median time to resolution: 6.1 months)
Respiratory: Upper respiratory infection (40% to 47%), epistaxis (16% to 35%), dyspnea (25% to 26%), rhinitis
Miscellaneous: Infection (≤55%; serious: 9% to 14%; pneumonia, catheter, or wound infections)

1% to 10%:
Cardiovascular: DVT (6% to 9%; grades 3/4: 9%), HF (grades 3/4: 1% to 4%), syncope (grades 3/4: 3%), intra-abdominal venous thrombosis (grades 3/4: 3%), cardio-/cerebrovascular arterial thrombotic event (2% to 4%), left ventricular dysfunction (grades 3/4: 1%)
Central nervous system: Confusion (1% to 6%), abnormal gait (1% to 5%); CNS hemorrhage (1% to 5%; grades 3/4: 1%), reversible posterior leukoencephalopathy syndrome ([RPLS] ≤1%)
Dermatologic: Nail disorder (2% to 8%), skin ulcer (≤6%), rash desquamation (grades 3/4: 3%), wound dehiscence (1% to 6%), acne (≤1%)
Endocrine & metabolic: Dehydration (grades 3/4: 3% to 10%), hyponatremia (grades 3/4: 4%)
Gastrointestinal: Xerostomia (4% to 7%), colitis (1% to 6%), ileus (grades 3/4: 4% to 5%), gingival bleeding (2% to 4%), fistula (1%), gastrointestinal perforation (≤4%), gastroesophageal reflux (≤2%), gingivitis (≤2%), mouth ulceration (≤2%), tooth abscess (≤2%), intra-abdominal abscess (1%), gastritis (≤1%), gingival pain (≤1%)
Genitourinary: Polyuria/urgency (3% to 6%), vaginal hemorrhage (4%)
Hematologic: Neutropenic fever/infection (5%; grades 3 and/or 4: 4% to 5%), thrombocytopenia (5%)
Hepatic: Bilirubinemia (1% to 6%)
Neuromuscular & skeletal: Bone pain (grades 3/4: 4%), neuropathy (other than sensory: grades 3/4: 1% to 5%)
Ocular: Blurred vision (≤2%)
Otic: Tinnitus (≤2%), deafness (≤1%)
Respiratory: Voice alteration (5% to 9%), pneumonitis/pulmonary infiltrates (grades 3/4: 5%), hemoptysis (nonsquamous histology 2%), pulmonary embolism (≤1%)
Miscellaneous: Infusion reactions (<3%)

<1% (Limited to important or life-threatening): Anaphylaxis, anastomotic ulceration, angina, bladder perforation, cerebral infarction; fistula (biliary, bladder, bronchopleural, duodenal, enterocutaneous, esophageal, gastrointestinal, rectal, renal, tracheoesophageal [TE] and vaginal); gastrointestinal ulcer, hemorrhagic stroke, hypersensitivity, hypertensive crises, hypertensive encephalopathy, intestinal necrosis, intestinal obstruction, mesenteric venous occlusion, microangiopathic hemolytic anemia (when used in combination with sunitinib), MI, nasal septum perforation, nephrotic syndrome, pancytopenia, polyserositis, pulmonary hemorrhage, pulmonary hypertension, renal failure, renal thrombotic microangiopathy, sepsis, subarachnoid hemorrhage, toxic anterior segment syndrome (TASS), transient ischemic attack, ureteral stricture wound healing complications

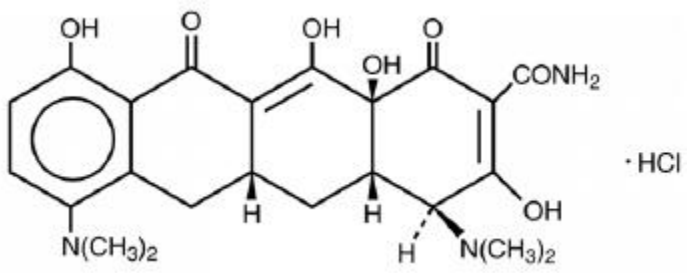
Reported from unlabeled use: Eye disorders: Endophthalmitis, hemorrhage (conjunctival, retinal or vitreous), intraocular inflammation (iritis, vitritis), intraocular pressure increased, ocular hyperemia, ocular pain/discomfort, retinal detachment, visual disturbance, vitreous floaters

2.2 Minocycline

2.2.1 Description

Minocycline hydrochloride, is a semisynthetic derivative of tetracycline, 4,7-Bis(dimethylamino)1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride.

Its structural formula is:



C23H27N3O7•HCl M.W. 493.95

2.2.2 Clinical Pharmacology

Following a single dose of two minocycline hydrochloride 100 mg capsules administered to 18 normal fasting adult volunteers, maximum serum concentrations were attained in 1 to 4 hours (average 2.1 hours) and ranged from 2.1 to 5.1 mcg/mL (average 3.5 mcg/mL).The serum half-life in the normal volunteers ranged from 11.1 to 22.1 hours (average 15.5 hours).

When minocycline hydrochloride capsules were given concomitantly with a high-fat meal, which included dairy products, the extent of absorption of minocycline hydrochloride capsules was unchanged compared to dosing under fasting conditions. The mean Tmax was delayed by one hour when administered with food, compared to dosing under fasting conditions. Minocycline hydrochloride capsules may be administered with or without food.

In previous studies with other minocycline dosage forms, the minocycline serum half-life ranged from 11 to 16 hours in 7 patients with hepatic dysfunction, and from 18 to 69 hours in 5 patients with renal dysfunction. The urinary and fecal recovery of minocycline when administered to 12 normal volunteers was one-half to one-third that of other tetracyclines.

2.2.3 Adverse Reactions

Due to oral minocycline's virtually complete absorption, side effects to the lower bowel, particularly diarrhea, have been infrequent. The following adverse reactions have been observed in patients receiving tetracyclines.

Body as a whole: Fever, and discoloration of secretions.

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, dyspepsia, stomatitis, glossitis, dysphagia, enamel hypoplasia, enterocolitis, pseudomembranous colitis, pancreatitis, inflammatory lesions (with monilial overgrowth) in the oral and anogenital regions. Instances of esophagitis and esophageal ulcerations have been reported in patients taking the tetracycline-class antibiotics in capsule and tablet form. Most of these patients took the medication immediately before going to bed (See DOSAGE AND ADMINISTRATION).

Genitourinary: Vulvovaginitis.

Hepatic toxicity: Hyperbilirubinemia, hepatic cholestasis, increases in liver enzymes, fatal hepatic failure, and jaundice. Hepatitis, including autoimmune hepatitis, and liver failure have been reported (See PRECAUTIONS).

Skin: Alopecia, erythema nodosum, hyperpigmentation of nails, pruritus, toxic epidermal necrolysis, and vasculitis. Maculopapular and erythematous rashes. Exfoliative dermatitis has been reported. Fixed drug eruptions have been reported. Lesions occurring on the glans penis have caused balanitis. Erythema multiforme and Stevens-Johnson syndrome have been reported. Photosensitivity is discussed above (See WARNINGS). Pigmentation of the skin and mucous membranes has been reported.

Respiratory: Cough, dyspnea, bronchospasm, exacerbation of asthma, and pneumonitis.

Renal toxicity: Interstitial nephritis. Elevations in BUN have been reported and are apparently dose related (See WARNINGS). Reversible acute renal failure has been reported.

Musculoskeletal: Arthralgia, arthritis, bone discoloration, myalgia, joint stiffness, and joint swelling.

Hypersensitivity reactions: Urticaria, angioneurotic edema, polyarthralgia, anaphylaxis/ anaphylactoid reaction (including shock and fatalities), anaphylactoid purpura, myocarditis, pericarditis, exacerbation of systemic lupus erythematosus and pulmonary infiltrates with eosinophilia have been reported. A transient lupus-like syndrome and serum sickness-like reactions also have been reported.

Blood: Agranulocytosis, hemolytic anemia, thrombocytopenia, leukopenia, neutropenia, pancytopenia, and eosinophilia have been reported.

Central Nervous System: Convulsions, dizziness, hypesthesia, paresthesia, sedation, and vertigo. Bulging fontanels in infants and benign intracranial hypertension (pseudotumor cerebri) in adults have been reported (see PRECAUTIONS - General). Headache has also been reported.

Other: Thyroid cancer has been reported in the postmarketing setting in association with minocycline products. When minocycline therapy is given over prolonged periods, monitoring for signs of thyroid cancer should be considered. When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discoloration of the thyroid gland. Cases of abnormal thyroid function have been reported.

Tooth discoloration in children less than 8 years of age (see WARNINGS) and also, in adults has been reported.

Oral cavity discoloration (including tongue, lip, and gum) has been reported.

Tinnitus and decreased hearing have been reported in patients on minocycline hydrochloride.

The following syndromes have been reported. In some cases involving these syndromes, death has been reported. As with other serious adverse reactions, if any of these syndromes are recognized, the drug should be discontinued immediately:

Hypersensitivity syndrome consisting of cutaneous reaction (such as rash or exfoliative dermatitis), eosinophilia, and one or more of the following: hepatitis, pneumonitis, nephritis, myocarditis, and pericarditis. Fever and lymphadenopathy may be present.

Lupus-like syndrome consisting of positive antinuclear antibody; arthralgia, arthritis, joint stiffness, or joint swelling; and one or more of the following: fever, myalgia, hepatitis, rash, and vasculitis.

Serum sickness-like syndrome consisting of fever; urticaria or rash; and arthralgia, arthritis, joint stiffness, or joint swelling. Eosinophilia may be present.

3.0 RATIONALE

As described above, there is a strong rationale for the use of bevacizumab and minocycline with repeat irradiation. However, no study has adequately documented the safety and efficacy of repeat radiation with bevacizumab and minocycline in people with recurrent glioma who progress on bevacizumab.

4.0 STUDY DESIGN

4.1 Description

This is an open-label, multi-agent design. Bevacizumab will be administered in accordance with the FDA-approved dose for gliomas, 10mg/kg IV every 2 weeks. Bevacizumab will be continued every two weeks as long as tolerated. One cycle of bevacizumab will be 28 days, with treatments on day1 and day 15. Blood pressure, CBC, CMP, and urine protein level, either by UA or urine protein/creatinine ratio will be checked at the beginning of each cycle.

Radiation planning will be individualized by the radiation oncologist based on the location of the current radiation field relative to prior radiation doses. The length and fractionation will be determined individually by the radiation oncologist.

Minocycline will be given by mouth twice a day starting at about half of the monotherapy maximal tolerated dose, 100 mg PO BID. Minocycline will be started on the day prior to radiation and continued until progression or intolerance. During the combined radiation, minocycline, and bevacizumab treatment, patients will be seen weekly with a CBC, CMP, and adverse event monitoring.

4.2 Dose Limiting Toxicity

Dose level	Dose
-1	75mg PO BID
0	100mg PO BID
1	200mg PO BID
1A (in case of DLT at dose level 1)	150mg PO BID
2	400mg PO BID

The toxicities described below will be considered DLT's if they are felt to be possibly, probably or definitely related to the minocycline. Patients experiencing a DLT will have minocycline stopped until the toxicity resolves to grade 1 or less, at which point the patient may resume minocycline at the next lowest dose level if agreed by the patient, the treating physician, and the PI.

Dose limiting toxicity will be determined in step 1 only during and 4 weeks after radiation. The initial cohort of 3 patients will receive dose level 0 (100mg PO BID). If 2 or 3 patients experience a related grade 3 or higher toxicity or related intolerable grade 2 toxicity (particularly, bullous dermatosis, photosensitivity, scalp pain not able to be controlled by oral pain medication), other than anemia, cholesterol, lymphopenia, or weight gain, which will only be considered dose limiting if grade 5, within 1 month of ending radiation, then the next cohort will receive dose level -1. If 0 or 1 patients experience a related grade 3 or higher toxicity or related intolerable grade 2, other than anemia, cholesterol, lymphopenia, or weight gain, within 1 month of ending radiation, then the dose will be escalated to the next cohort. If 2 or 3 patients experience related grade 3 or higher toxicity or related intolerable grade 2, other than anemia, cholesterol, lymphopenia, or weight gain, within 1 month of ending radiation at dose level -1, then the trial will be stopped. The highest dose at which 1 or 0 patients experience related grade 3 or higher toxicity will be declared the maximum tolerated dose (if dose level -1 or 0) or maximum achieved dose (if dose level 1, 1A or 2) and will be used for step 2.

Enrollment in the dose expansion portion (step 2) will not begin until adverse events have been reviewed by the DSMC and the MTD/maximum achieved dose has been approved by the DSMC.

4.3 Number of Patients

Twenty-six patients will be enrolled in this study.

4.4 Number of Study Centers

The study will be performed at one investigational site.

4.5 Duration of Patient Participation

Bevacizumab will be offered to each patient as long as both the patient and investigator agree that it continues to be well tolerated and to offer potential clinical benefit. Minocycline will be given from the day before the first day of radiation until progression or intolerance. Assessment of dose limiting toxicities for stage 1 will end 4 weeks after the end of radiation. Assessment of adverse events will continue until the patient has been removed from study treatment.

4.6 Duration of Study

Approximately 1 patient is expected to enroll every 2 months, for a total of 6 per year. Radiation treatment usually lasts 3-4 weeks for each patient, so patients will be followed for dose limiting toxicities for a total of 7-8 weeks and for clinical and QOL measures for 6 months. Total recruitment time for the study will be 3 years.

.0 ELIGIBILITY CRITERIA

All patients must participate in the consent process. During the consent process, the person obtaining consent must inform the patient of all elements of informed consent. No protocol-specific procedures, including screening procedures, are to be performed until the patient has signed and dated an institutional review board (IRB)/independent ethics committee (IEC)-approved informed consent form. The study begins with the signing and dating of the informed consent form. Patients must also meet the inclusion and exclusion criteria to be enrolled in the study.

This eligibility checklist is used to determine patient eligibility and filed with signature in the patient research chart.

Patient No. _____

Patient's Initials: (L,F,M) _____

5.1 Inclusion Criteria

Yes/No (Response of "no" = patient ineligible)

_____ 5.1.1 Male or female patients ≥18 years old with a life expectancy of at least 8 weeks

_____ 5.1.2 Radiographically proven recurrent (≥ first relapse), intracranial glioma

_____ 5.1.3 Previous treatment with external beam radiation

_____ 5.1.4 Radiographic progression on bevacizumab by RANO criteria

_____ 5.1.5 Women of child-bearing potential must have a negative pregnancy test within 7 days of initiation of dosing and must agree to use an acceptable method of birth control while on study drug and for 3 months after the last dose. Women of non-childbearing potential may be included if they are either surgically sterile or have been postmenopausal for ≥1 year. Men who are able to father a child must also agree to use an acceptable method of birth control while on study drug, and for 3 months after the last dose.

_____ 5.1.6 Karnofsky performance status of ≥50

_____ 5.1.7 Adequate hematologic, hepatic, and renal function (absolute neutrophil count ≥1.0 x 10^9/L, Hgb >9 g/dL, platelet count ≥50 x 10^9/L, AST/ALT ≤2.5x ULN, creatinine ≤1.5x ULN)

_____ 5.1.8 Prothrombin time/international normalized ratio (PT INR) < 1.4 for patients not on warfarin confirmed by testing within 14 days prior to study registration.

_____ 5.1.9 Patients on full-dose anticoagulants (e.g., warfarin or LMW heparin) must have no active bleeding or pathological condition that carries a high risk of bleeding, and must be on a stable dose of oral anticoagulant for 14 days or on a stable dose of low molecular weight heparin for 14 days

_____ 5.1.10 Willing and able to provide written informed consent prior to any study related procedures and to comply with all study requirements

5.2 Exclusion Criteria

Yes/No (Response of "yes" = patient ineligible)

_____ 5.2.1 Use of an investigational drug within 14 days or within 5 half-lives of the investigational drug, whichever is shorter

_____ 5.2.2 Less than 6 months since the end of previous radiation

_____ 5.2.3 History of Grade 2 (CTCAE v4) or greater acute intracranial hemorrhage

_____ 5.2.4 A concurrent active cancer that requires non-surgical therapy (e.g. chemotherapy, radiation, adjuvant therapy).

_____ 5.2.5 Patients with serious illnesses, uncontrolled infection, medical conditions, or other medical history including abnormal laboratory results, which in the investigator's opinion would be likely to interfere with a patient's participation in the study, or with the interpretation of the results

_____ 5.2.6 Women of child-bearing potential who are pregnant or breast feeding

_____ 5.2.7 Unstable angina and/or congestive heart failure in the last 6 months, transmural myocardial infarction within the last 6 months, New York Heart Association grade II or higher congestive heart failure requiring hospitalization within 12 months prior to registration, evidence of recent (within 14 days of registration) myocardial infarction by EKG (only required if clinically indicated) serious or inadequately controlled cardiac arrhythmia, significant vascular and peripheral vascular disease, evidence of bleeding diathesis or coagulopathy.

_____ 5.2.8 History of stroke, cerebral vascular accident (CVA) or transient ischemic attack within 6 months

_____ 5.2.9 Serious or non-healing wound, ulcer, or bone fracture or history of abdominal fistula, gastrointestinal perforation, intra-abdominal abscess major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to registration, with the exception of the craniotomy for tumor resection.

_____ 5.2.10 Chronic obstructive pulmonary disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration

_____ 5.2.11 Active connective tissue disorders, such as lupus or scleroderma, that in the opinion of the treating physician may put the patient at high risk for radiation toxicity

_____5.2.12 Uncontrolled symptomatic hypertension that cannot be controlled with anti-hypertensive agents

I certify that this patient meets all inclusion and exclusion criteria for enrollment onto this study.

_____ _____ _____
PI Signature Date Time

5.3 Screen Failures

Patients who sign an informed consent form, are not assigned to a treatment, and do not receive test article are defined as screen failures. For all screen failures, the investigator is to maintain a screening log that documents the screening number, patient initials, and reason(s) for screen failure.

5.4 Prior Treatment

Reasonable efforts will be made to determine all relevant treatment received by the patient within 28 days before administration of the test article. All previous treatments for glioma should be recorded.

.0 CONCOMITANT TREATMENT

Concomitant treatment is permitted if the medication is not expected to interfere with the evaluation of safety or efficacy of the study drug. During the study, if the use of any concomitant treatment becomes necessary (e.g., for treatment of an adverse event), the treatment must be recorded on the CRF, including the reason for treatment, generic name of the drug, dosage, route, and date of administration.

7.0 TREATMENT PLAN

Bevacizumab will be administered in accordance with the FDA-approved dose for gliomas, 10mg/kg IV every 2 weeks. Use screening or day 1 weight throughout the study unless there is a 10% weight change. Actual body weight will be used for all dose calculations of bevacizumab. Radiation will be given based on the judgment of the prescribing radiation oncologist. Minocycline will be given by mouth twice a day starting at about half of the monotherapy maximal tolerated dose, 100 mg.

7.1 Bevacizumab

7.1.1 How Supplied, Stored, Packaged and Labeled

Bevacizumab is supplied as a clear to slightly opalescent, sterile liquid ready for parenteral administration. For Bevacizumab, each 100mg (25mg/mL – 4mL fill) and 400 mg (25 mg/mL – 16 mL) glass vial contains bevacizumab with phosphate, trehalose, polysorbate 20, and Sterile Water for Injection, USP.

On receipt, bevacizumab should be stored in the refrigerator (2° to 8°C) and should remain refrigerated until just prior to use. Do not freeze. Do not shake. Shelf-life studies of bevacizumab are continuing. Investigators will be notified when lots have expired. The sterile single use vials contain no antibacterial preservatives; therefore, vials should be discarded eight hours after initial entry.

Bevacizumab will be supplied by the Huntsman Cancer Institute Pharmacy.

7.1.2 Preparation and Administration

Vials contain no preservative and are intended for single use only. The calculated dose should be placed in a 100 mL of 0.9% Sodium Chloride for Injection. Once diluted in 0.9% Sodium Chloride for Injection, the bevacizumab solution must be administered within 8 hours.

Bevacizumab is administered intravenously as a continuous infusion. The initial dose should be administered over a minimum of 90 minutes. If no adverse reactions occur after the initial dose, the second dose should be administered over a minimum of 60 minutes. If no adverse reactions occur after the second dose, all subsequent doses should be administered over a minimum of 30 minutes. If infusion-related adverse

reactions occur, all subsequent infusions should be administered over the shortest period that was well tolerated. To ensure complete delivery of bevacizumab, the IV infusion line must be flushed with 0.9% Sodium Chloride for Injection. Please note that this flush is not included in the infusion times. The following are two recommended methods for flushing the bevacizumab IV infusion line: When the bevacizumab infusion is complete, add an additional 50mL of 0.9% Sodium Chloride for Injection to the bevacizumab infusion bag. Continue the infusion until a volume equal to that of the volume contained in the tubing has been administered. Replace the empty bevacizumab infusion bag with a 50mL bag of 0.9% Sodium Chloride for Injection and infuse a volume equal to the volume contained in the tubing.

7.1.3 Accountability and Compliance

Doses and infusions will be documented in the patient's medical record.

7.2 Minocycline

7.2.1 How Supplied, Stored, Packaged and Labeled

Each minocycline hydrochloride capsule, USP for oral administration contains the equivalent of 50 mg, 75 mg or 100 mg of minocycline. In addition each capsule contains the following inactive ingredients: corn starch and magnesium stearate.

The 50 mg, 75 mg and 100 mg capsule shells contain: gelatin and titanium dioxide.

The 75 mg and 100 mg capsule shells also contain black iron oxide.

The imprinting ink contains: black iron oxide, potassium hydroxide, propylene glycol, and shellac

Minocycline Hydrochloride Capsules USP, 50 mg are Pink/Pink size '3' hard gelatin capsule filled with yellow granular powder and imprinted with 'C' on Pink cap and '76' on Pink body with black ink.

Minocycline Hydrochloride Capsules USP, 75 mg are White/Grey size '3' hard gelatin capsule filled with yellow granular powder and imprinted with 'C' on White cap and '77' on Grey body with black ink.

Minocycline Hydrochloride Capsules USP, 100 mg are Maroon/Pink size '2' hard gelatin capsule filled with yellow granular powder and imprinted with 'C' on Maroon cap and '78' on Pink body with black ink.

Minocycline should be stored at 20° to 25°C (68° to 77°F); excursions permitted to 15° to 30°C (59° to 86°F) [see USP Controlled Room Temperature]. Protect from light, moisture and excessive heat. Dispense in a tight, light-resistant container as defined in the USP.

Minocycline will be supplied by the Huntsman Cancer Institute Investigational Pharmacy.

7.2.2 Preparation and Administration

Minocycline hydrochloride capsules may be taken with or without food.
Ingestion of adequate amounts of fluids along with capsule and tablet forms of drugs in the tetracycline-class is recommended to reduce the risk of esophageal irritation and ulceration. The capsules should be swallowed whole.

7.2.3 Accountability and Compliance

Administrations and doses will be noted in the patient's medical record.

8.0 TOXICITIES AND DOSE MODIFICATION

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for adverse event and serious adverse event reporting. A copy of the CTCAE Version 4.0 can be downloaded:
(**http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx**).

8.1 Bevacizumab

Reduction/interruption of dosing for adverse events may take place at any time. Below are guidelines for dosage modification for bevacizumab–related toxicities as well as guidelines for their management.

Table 1: Dose Modifications

Event	CTCAE.v4.0 Grade	Action To Be Taken
Allergic reactions or Acute infusional reactions/ cytokine release syndrome	Grade 1-2	If infusion-related or allergic reactions occur, premedications should be given with the next dose and infusion time may not be reduced for the subsequent infusion.

	Grade 3	Bevacizumab infusion should be stopped and not restarted on the same day. At the physicians' discretion, bevacizumab may be permanently discontinued or re-instituted with premedications and at a rate of 90+15 min. **If bevacizumab is re-instituted, the patient should be closely monitored for a duration comparable to or longer than the duration of the previous reactions**.
	Grade 4	Discontinue bevacizumab
Arterial Thrombosis - Cardiac ischemia/ infraction - CNS ischemia (TIA, CVA) - any peripheral or visceral arterial ischemia/thrombosis	Grade 2 (if new or worsened since bevacizumab therapy)	Discontinue bevacizumab
	Grade 3-4	
Venous Thrombosis	Grade 3 OR asymptomatic grade 4	☐ Hold bevacizumab treatment. If the planned duration of full-dose anticoagulation is < 2 weeks, bevacizumab should be held until the full-dose anticoagulation period is over. ☐ If the planned duration of full-dose anticoagulation is >2 weeks, bevacizumab may be resumed during the period of full-dose anticoagulation **IF** all of the criteria below are met: - The subject must have an in-range INR (usually 2-3) on a stable dose of warfarin or be on a stable dose of heparin prior to restarting bevacizumab - The subject must not have pathological conditions that carry high risk of bleeding (eg, tumor involving major vessels or other conditions) - The subject must not have had hemorrhagic events while on study ☐ If thromboemboli worsen/recur upon resumption of study therapy, discontinue bevacizumab
	Grade 4 (symptomatic)	Discontinue bevacizumab
Hypertension*	[Treat with antihypertensive medication as needed. The goal of BP control should be consistent with general medical practice]	
	Grade 1	Consider increased BP monitoring
	Grade 2-3 asymptomatic	Begin or continue anti-hypertensive therapy and continue bevacizumab

	-Grade 2-3 Symptomatic	• Hold bevacizumab until symptoms resolve and hypertension is grade 2 or less • Begin or continue anti-hypertensive therapy
	Grade 4	Discontinue bevacizumab
Congestive Heart Failure	Grade 3 or 4	Discontinue bevacizumab
Proteinuria	UPC ratio < 3500mg/g	Continue bevacizumab
	UPC ratio > 3500mg/g	Hold bevacizumab until UPC <3500mg/g
	Grade 4 or nephrotic syndrome	Discontinue bevacizumab
Hemorrhage (CNS or pulmonary)	Grade 2-4	Discontinue bevacizumab
Hemorrhage (non-CNS; non-pulmonary)	Grade 3	• Patients receiving full-dose anticoagulation should discontinue bevacizumab • For patients not on full-dose anticoagulation, hold Bevacizumab until ALL of the following criteria are met: - the bleeding has resolved and Hb is stable - there is no bleeding diathesis that would increase the risk of therapy - there is no anatomic or pathologic condition that could increase the risk of hemorrhage recurrence. • Patients who experience recurrence of grade 3 hemorrhage should discontinue study therapy
	Grade 4	Discontinue bevacizumab
RPLS (reversible posterior leukoencephalopathy syndrome **or PRES** (posterior reversible encephalopathy syndrome)		**• Hold bevacizumab in patients with symptoms/signs suggestive of RPLS; subsequent management should include MRI scans and control of HTN** **• Discontinue bevacizumab upon diagnosis of RPLS**
Wound dehiscence requiring medical or surgical intervention		**Discontinue bevacizumab**
GI perforation, GI leak or fistula		Discontinue bevacizumab
Bowel obstruction	Grade 2 requiring medical intervention	Hold bevacizumab until complete resolution, with a minimum of 4 weeks after surgery.
	Grade 3-4	• Hold bevacizumab until complete resolution • If surgery is required, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion
Other unspecified bevacizumab-related AEs (except	Grade 3	Hold bevacizumab until symptoms resolve to < grade 1

ontrolled nausea/vomiting).	Grade 4	Discontinue bevacizumab • **Upon consultation with the principal investigator,** resumption of bevacizumab may be considered if a patient is benefiting from therapy and the grade 4 toxicity is transient, has recovered to < grade 1 and unlikely to recur with retreatment

***Current CTCAE definitions used by CTEP:**

- Grade 1: asymptomatic, transient (< 24 hours) increase by > 20 mmHg (diastolic) or to >150/100 if previously WNL; intervention not indicated
- Grade 2: recurrent or persistent (> 24 hours) or symptomatic increase by > 20 mmHg (diastolic) or to >150/100 if previously WNL; monotherapy may be indicated
- Grade 3: requiring more than one drug or more intensive therapy than previously
- Grade 4: life threatening (eg, hypertensive crisis)

Dose interruptions for Grade 2 non-hematologic toxicity for up to 2 weeks can be implemented at the discretion of the treating physician to manage intolerable or clinically significant toxicity. No dose reduction is required when resuming treatment.

8.2 Minocycline

Dose level	Dose
-1	75mg PO BID
0	100mg PO BID
1	200mg PO BID
2	400mg PO BID

During the expansion phase (step 2) or after the first post-radiation month during step 1, if a patient experiences a new grade 2 toxicity attributable to minocycline other than those specified next, minocycline must be interrupted until recovery to $\leq$ grade 1 and then reintroduced at one lower dose level. The following toxicities will require holding the minocycline only if grade 3 or higher toxicity is experienced: anemia, cholesterol. The following toxicities will not require holding the minocycline: lymphopenia, weight gain. If a patient requires a toxicity related dose delay of > 28 days, then the patient must be discontinued from the study. Minocycline will be stopped for a related grade 3 or higher toxicity, except as specified above, until the toxicity resolves to grade 1 or less, at which point the patient may resume minocycline at the next lowest dose level if agreed by the patient, the treating physician, and the PI. Dose reductions are

llowed until 100 mg PO BID (dose level 0), after which the patient must be removed from the study.

8.3 Discontinuation of Treatment and Withdrawal of Patients

The reasons a patient may discontinue or be withdrawn from the study include, but are not limited to, adverse event, disease progression, patient request, investigator decision, protocol violation, patient noncompliance, and study termination. Follow-up information every 3 months after the last day of radiation should be obtained if possible for patients who discontinue the study for reasons other than disease progression and are not started on another therapy for their recurrent glioma, in order to capture PFS3 data.

Patients withdrawn from the study for reasons other than toxicity or disease progression (e.g. protocol violation or noncompliance) may be replaced at the discretion of the investigator. To be considered evaluable, patients must experience a drug-related dose-limiting toxicity or receive at least 28 days of minocycline and 2 infusions of bevacizumab. During the dose-escalation portion of the study (step 1), unevaluable patients will be replaced. Treatment may be discontinued for an adverse event or at the discretion of the investigator.

.0 STUDY CALENDAR

STUDY DAY►	Screening (within 21 days of registration)	Radiation Treatment Period	D1 (+/- 7 days) of each monthly bevacizumab cycle	D15 (+/- 7 days) of each monthly bevacizumab cycle	28 (+/-14) days after the end of radiation	84 (+/-14) days after the end of radiation	182 (+/-28) days after the end of radiation
EVENT▼							
Informed Consent	X						
Medical History	X						
Height	X						
Weight	X		X		X	X	X
Vital Signs	X	X[5]	X	X	X	X	X
Physical Exam [1]	X	X[5]			X	X	X
Chem, Hem	X	X[5]	X		X	X	X
UA/UPC			X				
PT/INR	X						
Pregnancy Test [2]	X						
Karnofsky Performance Status Assessment	X				X	X	X
QOL by MDASI-BT	X				X	X	X
COGSTATE	X				X	X	X
MRI Brain Scan[3]	X				X	X	X
Concomitant Medications	X				X	X	X
Adverse Events [4]		X[5]	X	X	X	X	X
Minocycline Administration		X[6]	X	X	X	X	X
Radiation		X					
Bevacizumab infusions		X	X	X			

EXPLANATION OF SUPERSCRIPTS:

1. Complete physical exam (including neurological exam) at Screening, and neurological exam plus symptom-directed physical exam subsequently.
2. For women of child-bearing potential only; must be within 7 days of initiation of minocycline
3. Obtained within 21 days of registration and then 4, 12 and 26 weeks after radiation. For each patient, standard sequences will be obtained and research sequences may be obtained. Additional MRperfusion/spectroscopy scans may also be obtained.
4. Assessment of adverse events (dose limiting toxicities) for determination of MTD will end 4 weeks after the end of radiation; adverse events will continue to be monitored until all study treatment has been discontinued.
5. During the combined radiation, minocycline and bevacizumab treatment, patients will be seen weekly with a CBC, CMP, symptom directed physical exam, neurological exam, vital signs and adverse event monitoring.
6. Minocycline administration should be started the day prior to radiation; a window of 7 days prior is allowed.

10.0 STUDY PROCEDURES

10.1 Screening Evaluation

Within 21 days before registration, all patients will have a Screening evaluation that includes the following:

1. Sign and date an IRB-approved informed consent form before any study-specific screening procedures are performed
2. Medical history
3. Height, weight, vital signs (sitting blood pressure, pulse rate, respiratory rate, and temperature), and physical examination
4. Clinical laboratory evaluation [CBC with differential, CMP, PT/INR, serum pregnancy test (women of child-bearing potential)]
5. Karnofsky performance status assessment
6. MRI brain scan within 21 days of registration
7. Recording of concomitant medications

Patients who do not meet all inclusion and exclusion criteria will be noted as screen failures.

10.2 Baseline Evaluation

Between screening/consent and the first dose of radiation, QOL will be assessed by the MDASI-BT and cognition by the COGSTATE

10.3 Day 1 of subsequent cycles (± 7 Days)

On D1 of each cycle, patients will undergo the following procedures:

1. Weight, vital signs
2. CBC, CMP, and UA (urine protein to creatinine ratio if protein present on UA)
3. AE monitoring
4. Bevacizumab infusion

10.4 Day 15 of subsequent cycles (± 7 Days)

On D15 of each cycle, patients will undergo the following procedures:

1. Vital signs

2. Bevacizumab infusion
3. AE monitoring

10.5 Weekly during radiation

Once per week during radiation, patients will undergo the following procedures:

1. Vital signs
2. CBC
3. CMP
4. AE monitoring
5. Symptom-directed physical examination plus neurological exam

10.6 Four weeks post-radiation

Twenty-eight (+/-7) days after the end of radiation, patients will return to the clinic and undergo the following procedures:

1. Weight, vital signs, and symptom-directed physical examination plus neurological exam
2. CBC
3. CMP
4. Karnofsky performance status assessment
5. MRI brain scan
6. Recording of concomitant medications
7. AE monitoring
8. MDASI-BT
9. COGSTATE

10.7 Twelve weeks post-radiation

84 (+/- 14) days after the end of radiation, subjects are expected to return to the clinic and undergo the following procedures:

1. Weight, vital signs, and symptom-directed physical examination plus neurological exam

2. CBC
3. CMP
4. Karnofsky performance status assessment
5. Recording of concomitant medications
6. MRI brain scan
7. MDASI-BT
8. COGSTATE
9. AE monitoring

10.8 Twenty-six weeks post-radiation

182(+/-28) days after the end of radiation, subjects are expected to return to the clinic and undergo the following procedures:

1. Weight, vital signs, and symptom-directed plus neurological physical examination.
2. CBC
3. CMP
4. Karnofsky performance status assessment
5. Recording of concomitant medications
6. MRI brain scan
7. MDASI-BT
8. COGSTATE
9. AE monitoring

10.9 Continuously during trial

Minocycline oral administration (adverse event monitoring will continue until patient is removed from study treatment).

11.0 CRITERIA FOR EVALUATION AND ENDPOINT

11.1 Efficacy Evaluation

Response rate and PFS will be based on MRI using RANO criteria (see attachment 1). PFS will be based on all evaluable patients in step 1 and step 2. RR will be based on all evaluable patients in step 1 and step 2 who have measurable disease by RANO criteria.

Cognitive function will be assessed using Cogstate software, which is also being used in an RTOG trial open for low grade gliomas at HCI. Cogstate consists of 4 brief cognitive tests. The 4 tests involve pseudo-randomization of content to provide multiple alternate forms of the tests. There are nearly unlimited alternate test forms available for 3 of the tests, and the fourth contains 20 matched alternate forms. All tests are preceded by practice items. The total time of administration is about 22 minutes. Patients will have 60 minutes to complete the CogState test. If a patient has not completed the CogState test within 60 minutes, the patient may stop the testing at that time. Due to the CogState testing software, patients must complete all tests at each required time point in order to receive the test results, partially completed tests cannot be saved and therefore the data at that timepoint will not be used.

Subjects who cannot operate a computer or who cannot perform the practice test will not be required to complete the cognitive function evaluation.

11.2.2.1 *One Card Learning Test (OCLT) (visuoperceptual learning and memory)*
Standard playing cards appear in the center of the computer's screen one at a time, and the subject presses one mouse button if the card was presented previously and the other mouse button if it was not. Eighty-eight items are presented, in 8 trials of 11 cards. Learning and memory are operationally defined as the ability to discriminate between previously presented and novel (ie, distractors) information. Recognition paradigms are especially useful for assessing encoding and storage of newly learned information (Delis 2000). The test takes about 3 minutes.

11.2.2.2 *Detection Test (DET) (sensory registration, vigilance, and reaction time)*
A playing card appears in the center of the screen and turns face-up every 1 to 2 seconds. The subject presses one mouse button as quickly as possible after the card turns face-up. There are 35 items. The test takes about 3 minutes.

11.2.2.3 *Identification Test (IDN) (basic information processing/decision speed)*
A playing card appears in the center of the screen and turns face-up every 1 to 2 second; The subject pushes one mouse button if the card is red and the other mouse button if it is black. There are 30 items. The test takes about 8 minutes.

11.2.2.4 *Groton Maze Learning Test (GMLT) (spatial learning and executive functioning, including working memory, error monitoring, and ability to integrate feedback to modify problem solving)*
Subjects must learn a hidden pathway within a grid of tiles. Each time they click on a tile (using the mouse), they receive correct/incorrect feedback to help guide them through the pathway. Subjects must apply a set of simple rules to minimize errors. There are 28 steps and 11 turns to complete the maze. There are 5 trials of the same hidden pathway. The test takes about 8 minutes.

The instructions for the tests are given in attachment 2.

MDASI-BT will be given as a written questionnaire; it is given in attachment 3.

11.2 Safety Evaluation

Routine safety and tolerability will be evaluated from the results of reported signs and symptoms, scheduled physical examinations, vital sign measurements, and clinical laboratory test results.

More frequent safety evaluations may be performed if clinically indicated or at the discretion of the investigator. All AEs will be recorded from the time the patient receives the first dose of radiation up to 28 days after the last dose of radiation to assess the MTD. If the patient continues on minocycline, AE's will be assessed until discontinuation of treatment.

11.2.1 Physical Examination

Complete and symptom-directed physical examinations will be performed by a licensed physician (or physician's assistant or nurse practitioner).

11.2.2 Vital Signs

Vital signs (blood pressure, respiratory rate, pulse rate and temperature) will be obtained in the sitting position. Patients should be sitting for 3-5 minutes prior to obtaining vital signs.

11.2.3 Safety Laboratory Determinations

Laboratory evaluations will be performed as noted in the flow chart.

11.3 Stopping Rules

Refer to section 4.2 for definition and evaluation of Dose Limiting Toxicities and stopping rules.

12.0 STATISTICAL ANALYSIS

12.1 General Statistical Considerations

This is a Phase 1/2, single institution, open-label study with the primary objective to estimate the adverse event rate during reirradiation with concomitant bevacizumab and minocycline. Important secondary objectives are to estimate the PFS3, PFS6, response rate and cognitive effects with this regimen.

Since this is an open-label, single regimen clinical trial, descriptive statistics will be employed to analyze the data. Summary statistics for continuous variables will include the mean, standard deviation, median, and range (minimum/maximum). Categorical variables will be presented as

requency counts and percentages, and time-to-event variables will be summarized by Kaplan-Meier plots, medians and range.

The data will be tabulated and analyzed with respect to patient enrollment and disposition, demographic and baseline characteristics, prior and concomitant medications, efficacy, and safety measures.

Data listings will be created to support each table and to present all data collected.

For efficacy analyses, a 1-sided alpha of 0.2 without adjustment for multiple comparisons will be used

12.2 Sample Size and Power

12.2.1 Primary Objective: Rate of adverse events during and up to 4 weeks after radiation

12.2.1.1 Data needed

1. Adverse events at each cycle during radiation
2. Adverse events at 28 days post-radiation

Source documentation will capture adverse events through the end of all study treatment. Only those adverse events occurring through 28 days post-radiation will be recorded on the Case Report Forms.

12.2.1.2 Statistical analysis

Adverse event rates will be categorized as non-severe (CTCAE grade 1-2) or severe (CTCAE grade 3-4). The rate of non-severe and severe adverse events will be reported. In step 1, 3 patients per cohort will be used to define the maximum tolerated dose/maximum achieved dose to carry forward into step 2. All patients treated at the step 2 dose will be used in all analyses.

Fifteen patients at the final dose will give 80% power to detect any adverse event affecting at least 10.2% of patients. Margins of error, with alpha = 0.05, for estimates of any event affecting more than 1 patient in the trial will be less than 27%.

12.2.2 Secondary Objectives

12.2.2.1 PFS3

12.2.2.1.1 Data needed

1. Size of target lesion on MRI at baseline

2. Size of target lesion on MRI 12 weeks after the end of radiation

12.2.2.1.2 Statistical analysis

With bevacizumab continuation alone, the PFS3 would be expected to be <10%. Indeed, the median overall survival after progression on bevacizumab is 3 months. In prior studies in the bevacizumab-refractory population, treatments with PFS3 of 10-20% and PFS6 of 0-10% were considered not effective. (Reardon et al.) On the other hand, chemotherapy with PFS3 of ~40% and PFS6 of 16% was considered worthy of further study. (Reardon et al.) Assuming a 10% dropout/unevaluable rate, fifteen patients will give us 80 percent power to reject the null hypothesis of 10% if the true PFS3 is 30%.

12.2.2.2 PFS6

12.2.2.2.1 Data needed

1. Size of target lesion on MRI at baseline

2. Size of target lesion on MRI 26 weeks after the end of radiation

12.2.2.2.2 Statistical analysis

With bevacizumab continuation alone, the PFS6 would be expected to be <10%. In prior studies in the bevacizumab-refractory population, treatments with PFS3 of 10-20% and PFS6 of 0-10% were considered not effective. (Reardon et al.) On the other hand, chemotherapy with PFS3 of ~40% and PFS6 of 16% was considered worthy of further study. (Reardon et al.) Assuming a 10% dropout/unevaluable rate, fifteen patients will give us 80 percent power to reject the null hypothesis of 5% if the true PFS6 is 21%.

12.2.2.3 Response rate

12.2.2.3.1 Data needed

1. Size of target lesion on MRI at baseline

2. Size of target lesion on MRI 12 weeks after the end of radiation

12.2.2.3.2 Statistical analysis

In Fugh et al, the response rate to radiation alone was 10%. For drug studies in recurrent gliomas after bevacizumab, response rates over 10%

have been considered evidence of efficacy for proceeding to further trials. Assuming a 10% dropout/unevaluable rate, fifteen patients will give us 80 percent power to reject the null hypothesis of 10% response if the true response rate is at least 30%.

12.2.2.4 Quality of life change over time

12.2.2.4.1 Data needed

1. MDASI-BT at baseline, 4 weeks post-radiation, 12 weeks post-radiation, and 26 weeks post-radiation

12.2.2.4.2 Statistical analysis

MDASI-BT uses a 1-10 Likert scale for 22 symptoms. In high grade brain tumor patients, changes in MDASI_BT scores of 2 or more are considered clinically significant. The course of changes in MDASI-BT during and after progression has not been published. We assume that 16 patients are evaluable 4 weeks after radiation, 12 patients are alive and evaluable 12 weeks after radiation, and 8 patients are alive and evaluable 26 weeks after radiation. We will report the mean and standard deviation for each item, subscore, and total score at each time point.

12.2.2.5 Cognitive change over time

12.2.2.5.1 Data needed

1. COGSTATE at baseline, 4 weeks post-radiation, 12 weeks post-radiation, and 26 weeks post-radiation

12.2.2.5.2 Statistical analysis

The primary endpoint of cognitive function is measured by a battery of tests: the Detection Test (DET), the Identification Test (IDN), the One Card Learning Test (OCLT), and the Groton Maze Learning Test (GMLT). The primary outcome measure of the DET and IDN tests is speed of performance using the mean of the log10 transformed response times for correct responses (lower score = better performance). Accuracy of performance is the primary outcome measure for the OCLT, using the arcsine transformation of the square root of the proportion of correct responses across 8 rounds (higher score = better performance). Number of errors is the primary outcome measure for GMLT. Each of the battery's tests will be evaluated using the 2-sample t-test with a 2-sided significance level of 0.05 to determine if there is a clinically meaningful difference in the average change of NCF score from baseline to the time of assessment.

12.3 Analysis Populations

The primary population for the safety analysis will consist of patients who receive at least one dose of minocycline and have any follow-up data. The primary population for efficacy analyses will consist of all patients who receive at least one dose of minocycline.

12.4 Efficacy Analysis

Response to treatment will be evaluated using the Response Assessment in Neuro-Oncology (RANO) criteria (Wen 2010). The response criteria are summarized in Attachment 1. All patients who are eligible for efficacy analyses will be evaluable for PFS, calculated for each subject as the number of days from the first day of radiation to the date of the first documented disease progression or date of death, whichever occurs first. The Kaplan-Meier method will be used to estimate the median PFS. Those evaluable patients with measurable disease will be assessed for response.

For each subject with a response to therapy, duration of response will be calculated. The duration of response is defined as number of days from the date of initial response to the date of first documented disease progression or death, whichever occurs first. The Kaplan-Meier method will be used to estimate median duration of response. In the event no disease progression or death is documented prior to study termination, analysis cutoff, or the start of confounding anticancer therapy, PFS and duration of response will be censored at the date of last evaluable tumor assessments.

12.5 Safety Analysis

Safety variables to be analyzed are AEs, laboratory test results (hematology and clinical chemistry), weight, and vital signs.

Adverse event terms recorded on the eCRFs will be mapped to preferred terms using the Medical Dictionary for Drug Regulatory Activities (MedDRA®) version 10.1 or later. All AEs will be summarized according to the system organ class and preferred term within the organ class. Adverse events will be tallied for overall frequency (number and percentage of subjects), worst reported severity, and relationship to study drug for each preferred term per subject. Serious adverse events will be similarly summarized. Listings of deaths, SAEs, and AEs leading to early termination of study treatment or premature withdrawal from study will also be provided.

Laboratory values will be categorized according to their CTCAE (version 4) toxicity grade and tabulated by worst on-study toxicity grade.

13.0 REGISTRATION GUIDELINES

Patients must meet all of the eligibility requirements listed in Section 5 prior to registration.

Study related screening procedures can only begin once the patient has signed a consent form. Patients must not begin protocol treatment prior to registration.

Treatment should start within **five** working days after registration.

To register eligible patients on study, complete a Clinical Trials Office Patient Registration Form and submit to: CTORegistrations@hci.utah.edu.

14.0 DATA SUBMISSION SCHEDULE

The Case Report Forms (CRFs) are a set of electronic forms for each patient that provides a record of the data generated according to the protocol. CRF's should be created prior to the study being initiated and updated (if applicable) when amendments to the protocol are IRB approved. These forms will be completed on an on-going basis during the study. Data will be entered into the CRFs via OnCore (clinical research management system) by assigned study staff at the Huntsman Cancer Institute, as indicated on the Delegation of Duties log. The medical records will be source of verification of the data. During the study, the CRFs will be monitored for completeness, accuracy, legibility and attention to detail by a member of the Research Compliance Office. The data will be reviewed no less than annually by the Data and Safety Monitoring Committee. The Investigator will allow the Data and Safety Monitoring Committee or Research Compliance Office personnel access to the patient source documents, clinical supplies dispensing and storage area, and study documentation for the above-mentioned purpose. The Investigator further agrees to assist the site visitors in their activities.

15.0 ETHICAL AND REGULATORY CONSIDERATIONS

15.1 Informed consent

Informed consent will be obtained from all research participants prior to performing any study procedures using the most recent IRB approved version.

15.2 Institutional Review

Study will be approved by the Institutional Review Board of University of Utah.

15.3 Data and Safety Monitoring Plan

A Data and Safety Monitoring Committee (DSMC) is established at Huntsman Cancer Institute (HCI) and approved by the NCI to assure the well-being of patients enrolled on Investigator Initiated Trials that do not have an outside monitoring review. Roles and responsibilities of the DSMC are set forth in the NCI approved plan. The activities of this committee include a quarterly review of adverse events including SAEs, important medical events, significant revisions or amendments to the protocol, and approval of cohort/dose escalations. If the DSMC and/or the PI have concerns about unexpected safety issues, the study will be stopped and will not be resumed until the issues are resolved. The DSMC also reviews and approves audit reports generated by the Research Compliance Office.

- This is a **Phase I study**. For each phase I study using an agent of potential risk a member of the DSMC will be assigned as the primary medical monitor. The primary medical monitor will be notified immediately of all SAEs within 24 hours of knowledge of the event. A formal report should be submitted to the primary medical monitor within 10 days. All serious adverse events (SAEs) occurring in patients treated at HCI or its affiliates will also be reviewed by the full DSMC quarterly. Approval from the primary medical monitor is required for all dose escalations. The full committee will also review all grade 3 or greater toxicities for patients on treatment.

15.4 Adverse Events / Serious Adverse Events

This study will utilize the CTCAE (NCI Common Terminology Criteria for Adverse Events) Version 4.0 for AE and SAE reporting. An electronic copy of the CTCAE Version 4.0 can be downloaded from:**http://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx**

15.4.1 Adverse Events (AE)

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. For the purposes of this study, the terms toxicity and adverse event are used interchangeably. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

A patient's AE can occur from the time the patient receives the first dose of minocycline up to the last dose of study treatment. SAEs will continue to be monitored and captured for a 30 day period after the last dose of study treatment.

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit or phone contact during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. the severity grade based on CTCAE v.4 (grade 1-5)
2. its relationship to the study drug(s) (definite, probable, possible, unlikely, not related) For multi-drug regimens, relationship will be assessed for all treatments.
3. its duration (start and end dates or if continuing at final exam)

4. action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)
5. whether it constitutes an SAE

All adverse events will be treated appropriately. Such treatment may include changes in study drug treatment as listed in the dose modification section of this protocol (see section 8 for guidance). Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the commercial drug is described in the Drug Information (section 2) and the FDA-approved product labels. This information will be included in the patient informed consent and will be discussed with the patient during the study as needed.

All adverse events will be immediately recorded in the patient research chart.

15.4.2 Serious Adverse Event (SAE)

Information about all serious adverse events will be collected and recorded. A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- causes congenital anomaly or birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (procedures such as central line placements, paracentesis, pain control)
    - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
    - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - social reasons and respite care in the absence of any deterioration in the patient's general condition

Any death from any cause while a patient is receiving treatment on this protocol or up to 30 days after the last dose of protocol treatment, or any death which occurs more

than 30 days after protocol treatment has ended but which is felt to be treatment related, must be reported.

Toxicities which fall within the definitions listed above must be reported as an SAE regardless if they are felt to be treatment related or not. Toxicities unrelated to treatment that do NOT fall within the definitions above, must simply be documented as AEs in the patient research chart.

15.5 SAE Reporting Requirements

SAEs must be reported to the DSMC, the FDA, and the IRB according to the requirements described below:

DSMC Notifications:

- Email or provide verbal notification of event within 24 hours of knowledge to compliance@hci.utah.edu.
- Email completed MedWatch 3500A form within 10 days to compliance@hci.utah.edu.
- An HCI Research Compliance Officer will process and submit the SAE notification/MedWatch form to the proper DSMC member as necessary for each individual study.
- The RCO will summarize and present all reported SAEs according to the DSMP at the quarterly DSMC meeting.

FDA Notifications:

- Adverse events occurring during the course of a clinical study that meet the following criteria will be promptly reported to the FDA:
    - Serious
    - Unexpected
    - Definitely, Probably or Possibly Related to the investigational drug
- Fatal or life-threatening events that meet the criteria above will be reported within 7 calendar days after first knowledge of the event by the investigator; followed by as complete a report as possible within 8 additional calendar days.
- All other events that meet the criteria above will be reported within 15 calendar days after first knowledge of the event by the investigator.
- The CRC will complete a MedWatch 3500A form on the reportable SAE, gather other relevant supporting information and forward this to the RCO within 10 days of first knowledge of the event (5 days for fatal or life-threatening events).
- An RCO Compliance Officer will review the SAE information for completeness, accuracy and applicability to the regulatory reporting requirements.
- The Compliance Officer will ensure the complete, accurate and timely reporting of the event to the FDA.

- If the investigator of the IIT holds an investigational new drug application (IND), the Regulatory Coordinator will submit the report as an amendment to the application.
- For studies with an IND, all other adverse events and safety information not requiring expedited reporting that occur or are collected during the course of the study will be summarized and reported to the FDA through the IND Annual Report.

- IRB Notification:Events meeting the the University of Utah IRB reporting requirements (http://www.research.utah.edu/irb/) will be submitted through the IRB's electronic reporting system within 10 working days.
- The CRC will provide all necessary safety information to the Research Compliance Office for submission of the event.

*Medwatch 3500A form can be found at https://www.accessdata.fda.gov/scripts/medwatch/medwatch-online.htm

15.6 Protocol Amendments

Any amendments or administrative changes in the research protocol during the period, for which the IRB approval has already been given, will not be initiated without submission of an amendment for IRB review and approval.

These requirements for approval will in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial.

15.7 Protocol Deviations

A protocol deviation (or violation) is any departure from the defined procedures and treatment plans as outlined in the protocol version submitted and previously approved by the IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events.

Because some protocol deviations pose no conceivable threat to participant safety or scientific integrity, reporting is left to the discretion of the PI within the context of the guidelines below. The IRB requires the **prompt reporting** of protocol deviations which are:

- Exceptions to eligibility criteria.
- Intended to eliminate apparent immediate hazard to a research participant or
- Harmful (caused harm to participants or others, or place them at increased risk of harm - including physical, psychological, economic, or social harm), or
- Possible serious or continued noncompliance

15.8 FDA Annual Reporting

This study is IND exempt therefore there are no annual reporting requirements to the FDA.

15.9 Clinical Trials Data Bank

The study will be registered on **http://clinicaltrials.gov** and the NCI CTRP (Clinical Trials Reporting Program) by the Clinical Trials Office.

16.0 REFERENCES

(2008). "A pilot clinical trial of creatine and minocycline in early Parkinson disease: 18-month results." Clin Neuropharmacol **31**(3): 141-150.

Agustin, E. T., V. Gill, et al. (1994). "Mycoplasma pneumoniae meningoencephalitis complicated by diplopia." Heart Lung **23**(5): 436-437.

Anandharaj, A., S. Cinghu, et al. (2011). "Rapamycin-mediated mTOR inhibition attenuates survivin and sensitizes glioblastoma cells to radiation therapy." Acta Biochim Biophys Sin (Shanghai) **43**(4): 292-300.

Barza, M., R. B. Brown, et al. (1975). "Relation between lipophilicity and pharmacological behavior of minocycline, doxycycline, tetracycline, and oxytetracycline in dogs." Antimicrob Agents Chemother **8**(6): 713-720.

Bergers, G. and D. Hanahan (2008). "Modes of resistance to anti-angiogenic therapy." Nat Rev Cancer **8**(8): 592-603.

Bernardino, A. L., D. Kaushal, et al. (2009). "The antibiotics doxycycline and minocycline inhibit the inflammatory responses to the Lyme disease spirochete Borrelia burgdorferi." J Infect Dis **199**(9): 1379-1388.

Brem, S. S., P. J. Bierman, et al. (2011). "Central nervous system cancers." J Natl Compr Canc Netw **9**(4): 352-400.

Carney, S., R. A. Butcher, et al. (1974). "Minocycline excretion and distribution in relation to renal function in man." Clin Exp Pharmacol Physiol **1**(4): 299-308.

CBTRUS.

Chakravarti, A., G. G. Zhai, et al. (2004). "Survivin enhances radiation resistance in primary human glioblastoma cells via caspase-independent mechanisms." Oncogene **23**(45): 7494-7506.

Combs, S. E., C. Thilmann, et al. (2005). "Efficacy of fractionated stereotactic reirradiation in recurrent gliomas: long-term results in 172 patients treated in a single institution." J Clin Oncol **23**(34): 8863-8869.

D. A. Reardon, J. J. V., A. Desjardins, K. Peters, A. D. Coan, J. E. Herndon, H. S. Friedman; (2011). "Bevacizumab (BV) continuation following BV progression: Meta-analysis of five consecutive recurrent glioblastoma (GBM) trials." J Clin Oncol.

Fagan, S. C., J. L. Waller, et al. (2010). "Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study." Stroke **41**(10): 2283-2287.

Fang, C. T., W. F. Ferng, et al. (1997). "Life-threatening scrub typhus with meningoencephalitis and acute respiratory distress syndrome." J Formos Med Assoc **96**(3): 213-216.

Fogh, S. E., D. W. Andrews, et al. (2010). "Hypofractionated stereotactic radiation therapy: an effective therapy for recurrent high-grade gliomas." J Clin Oncol **28**(18): 3048-3053.

Geng, L., E. Donnelly, et al. (2001). "Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy." Cancer Res **61**(6): 2413-2419.

Gordon, P. H., D. H. Moore, et al. (2004). "Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis." Neurology **62**(10): 1845-1847.

Gutin, P. H., F. M. Iwamoto, et al. (2009). "Safety and efficacy of bevacizumab with hypofractionated stereotactic irradiation for recurrent malignant gliomas." Int J Radiat Oncol Biol Phys **75**(1): 156-163.

Hasan, T. and A. U. Khan (1986). "Phototoxicity of the tetracyclines: photosensitized emission of singlet delta dioxygen." Proc Natl Acad Sci U S A **83**(13): 4604-4606.

Hatanpaa, K. J., S. Burma, et al. (2010). "Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance." Neoplasia **12**(9): 675-684.

Hellstrom, N. A., O. R. Lindberg, et al. (2011). "Unique gene expression patterns indicate microglial contribution to neural stem cell recovery following irradiation." Mol Cell Neurosci **46**(4): 710-719.

Huang, W. C., Y. Qiao, et al. (2010). "Direct protection of cultured neurons from ischemia-like injury by minocycline." Anat Cell Biol **43**(4): 325-331.

Jiang, Z., N. Pore, et al. (2007). "Phosphatase and tensin homologue deficiency in glioblastoma confers resistance to radiation and temozolomide that is reversed by the protease inhibitor nelfinavir." Cancer Res **67**(9): 4467-4473.

Kelly, R. G. and L. A. Kanegis (1967). "Metabolism and tissue distribution of radioisotopically labelled minocycline." Toxicol Appl Pharmacol **11**(1): 171-183.

Kim, H. K., A. F. Thornton, et al. (1997). "Results of re-irradiation of primary intracranial neoplasms with three-dimensional conformal therapy." Am J Clin Oncol **20**(4): 358-363.

Kreisl, T. N., L. Kim, et al. (2009). "Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma." J Clin Oncol **27**(5): 740-745.

Lal, B., S. Xia, et al. (2005). "Targeting the c-Met pathway potentiates glioblastoma responses to gamma-radiation." Clin Cancer Res **11**(12): 4479-4486.

Lee, C. G., M. Heijn, et al. (2000). "Anti-Vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions." Cancer Res **60**(19): 5565-5570.

Lin, J., X. M. Zhang, et al. (2010). "gamma-secretase inhibitor-I enhances radiosensitivity of glioblastoma cell lines by depleting CD133+ tumor cells." Arch Med Res **41**(7): 519-529.

Liu, W. T., C. H. Lin, et al. (2011). "Minocycline inhibits the growth of glioma by inducing autophagy." Autophagy **7**(2): 166-175.

Lucio-Eterovic, A. K., Y. Piao, et al. (2009). "Mediators of glioblastoma resistance and invasion during antivascular endothelial growth factor therapy." Clin Cancer Res **15**(14): 4589-4599.

Macdonald, H., R. G. Kelly, et al. (1973). "Pharmacokinetic studies on minocycline in man." Clin Pharmacol Ther **14**(5): 852-861.

Markovic, D. S., K. Vinnakota, et al. (2011). "Minocycline reduces glioma expansion and invasion by attenuating microglial MT1-MMP expression." Brain Behav Immun **25**(4): 624-628.

Murat, A., E. Migliavacca, et al. (2008). "Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma." J Clin Oncol **26**(18): 3015-3024.

Niyazi, M., U. Ganswindt, et al. (2010). "Irradiation and Bevacizumab in High-Grade Glioma Retreatment Settings." Int J Radiat Oncol Biol Phys.

Jutile-McMenemy, N., A. Elfenbein, et al. (2007). "Minocycline decreases in vitro microglial motility, beta1-integrin, and Kv1.3 channel expression." J Neurochem **103**(5): 2035-2046.

Reardon, D. A., A. Desjardins, et al. (2011). "Phase II study of metronomic chemotherapy with bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy." J Neurooncol **103**(2): 371-379.

Reardon, D. A., A. Desjardins, et al. (2011). "Phase 2 study of carboplatin, irinotecan, and bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy." Cancer **117**(23): 5351-5358.

Rich, J. N. (2007). "Cancer stem cells in radiation resistance." Cancer Res **67**(19): 8980-8984.

Schnabel, L. V., M. G. Papich, et al. (2011). "Pharmacokinetics and distribution of minocycline in mature horses after oral administration of multiple doses and comparison with minimum inhibitory concentrations." Equine Vet J.

Smith, D. L., B. Woodman, et al. (2003). "Minocycline and doxycycline are not beneficial in a model of Huntington's disease." Ann Neurol **54**(2): 186-196.

Sotomayor, E. A., B. A. Teicher, et al. (1992). "Minocycline in combination with chemotherapy or radiation therapy in vitro and in vivo." Cancer Chemother Pharmacol **30**(5): 377-384.

Stupp, R., W. P. Mason, et al. (2005). "Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma." N Engl J Med **352**(10): 987-996.

Teicher, B. A., S. A. Holden, et al. (1995). "Potentiation of cytotoxic therapies by TNP-470 and minocycline in mice bearing EMT-6 mammary carcinoma." Breast Cancer Res Treat **36**(2): 227-236.

Tikka, T., B. L. Fiebich, et al. (2001). "Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia." J Neurosci **21**(8): 2580-2588.

Tikka, T. M., N. E. Vartiainen, et al. (2002). "Minocycline prevents neurotoxicity induced by cerebrospinal fluid from patients with motor neurone disease." Brain **125**(Pt 4): 722-731.

Tsai, Y. H., R. S. Hirth, et al. (1980). "A murine model for listerial meningitis and meningoencephalomyelitis: therapeutic evaluation of drugs in mice." Chemotherapy **26**(3): 196-206.

Vordermark, D., O. Kolbl, et al. (2005). "Hypofractionated stereotactic re-irradiation: treatment option in recurrent malignant glioma." BMC Cancer **5**: 55.

Weingart, J. D., E. P. Sipos, et al. (1995). "The role of minocycline in the treatment of intracranial 9L glioma." J Neurosurg **82**(4): 635-640.

Wen, P. Y., D. R. Macdonald, et al. (2010). "Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group." J Clin Oncol **28**(11): 1963-1972.

Zhou, H., R. Miki, et al. (2007). "Reciprocal regulation of SOCS 1 and SOCS3 enhances resistance to ionizing radiation in glioblastoma multiforme." Clin Cancer Res **13**(8): 2344-2353.

Zhuang, W., B. Li, et al. (2011). "Induction of autophagy promotes differentiation of glioma-initiating cells and their radiosensitivity." Int J Cancer **129**(11): 2720-2731.

ATTACHMENT 1: RANO RESPONSE CRITERIA

Patients will be assessed by the RANO (radiographic assessment in neurooncology) criteria.(Wen et al.). The response will be determined as outlined in the RANO criteria below.

Measurable Disease

a. Bidimensionally contrast-enhancing lesions with clearly defined margins by CT or MRI scan, with two perpendicular diameters of at least 10 mm, visible on two or more axial slices that are preferably, at most, 5 mm apart with 0-mm skip.

b. Measurement of tumor around a cyst or surgical cavity represents a particularly difficult challenge. In general, such lesions should be considered nonmeasurable unless there is a nodular component measuring ≥ 10 mm in diameter. The cystic or surgical cavity should not be measured in determining response.

Complete Response (requires all of the following):

a. Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.

b. No new lesions.

c. All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

d. Patients must be on no steroids.

e. Stable or improved non-enhancing (T2/FLAIR) lesions.

f. Stable or improved clinically.

Note: Patients with non-measurable disease cannot have a complete response. The best response possible is stable disease.

Partial Response (requires all of the following):

a. Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters (bi-dimensional measurements) of all measurable enhancing lesions sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.

b. No progression of non-measurable disease.

c. No new lesions.

d. All measurable and nonmeasurable lesions must be assessed using the same techniques as baseline.

e. The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.
f. Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.
g. Stable or improved clinically.

Note: Patients with non-measurable disease cannot have a partial response. The best response possible is stable disease.

Stable Disease (requires all of the following):

a. Does not qualify for CR, PR, or progression.
b. The designation of stable disease requires a minimum of 4-week duration.
c. All measurable and non-measurable sites must be assessed using the same techniques as baseline.
d. Stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan. In the event that the corticosteroid dose has been increased, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
e. Stable clinically.

Progressive Disease (defined by any of the following):

a. >25% increase in sum of the products of perpendicular diameters (bi-dimensional measurements) of enhancing lesions (over baseline if no decrease) on stable or increasing doses of corticosteroids. and/or
b. Significant increase in T2/FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids compared to baseline scan or best response following initiation of therapy, not due to co-morbid events (radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).
c. Any new lesion.
d. Clear clinical deterioration not attributable to other causes apart from the tumor (e.g., seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the treating physician, but it is recommended that a decrease in 20% of KPS or from any baseline to 50% or less be considered, unless attributable to comorbid events.
e. Failure to return for evaluation due to death or deteriorating condition.

Assessment of Response (Efficacy)

Timing of the Evaluation of Response: Assessment of response will begin with the previous MRI. All scans are to be compared to the smallest measurement scan to date.

ATTACHMENT 2: COGSTATE INSTRUCTIONS

TEST INSTRUCTIONS AND ADMINISTRATION PROCEDURES

Additional comments:

1. The computer used for the cognitive tests must be located in a quiet area relatively free of distractions and with reasonable lighting, comfortable seating, and a table of sufficient size for the computer. No family members or friends of the patient, or site colleagues, may be present during testing, even if they remain quiet or sit in the back of the room.
2. Testing must be completed in 1 session. Test instructions must be followed verbatim (test instruction scripts for each of the 4 tests are provided in the neurocognitive tests manual) with every patient at every study visit.
3. Tests are administered in the following order to every patient:

CogState tests instructions script

Important

Turn the computer screen so that it faces the subject. Ensure that the screen is positioned so that it is directly in front of the subject (not angled to the side) and at a comfortable distance from the subject. Adjust the screen pitch if necessary.

Detection Test (DET)

Before the Detection Test begins, a screen appears that allows the subject to practice entering "yes" and "no" responses via the mouse. The left mouse button is always "no" and the right mouse button is always "yes," regardless of the hand used. Ensure that the subject understands by practicing clicking the proper buttons several times. Do not permit the subject to use the keyboard to enter responses.

Click ***Enter*** to begin the Detection test.

The instructions appear on the screen. *The subject should read them silently as you read them aloud.*

Say, ***Has the card turned over? You are now going to do a practice. You will only need to use the "Yes" button for this task. In this task, a playing card will appear in the center of the screen. Press the "Yes" button when the card turns face-up as fast as you can. If you make a mistake you will hear an error sound. This means you have responded too soon. Try to make your response as accurate and fast as possible after a card turns face-up. Are you ready to start?***

Click ***Enter*** to begin the practice items. Remind the subject to press the "Yes" button as soon as the card turns
face-up.

If the subject appears confused or responds incorrectly, review the test requirements, including use of the right mouse button (the "Yes" button) for responses. Ensure the subject understands that in this test, all cards shown are the same Joker card. There is no need for the subject to examine details of the card closely.

When the practice items are complete, the instructions for the scored or "real" portion of the test appear.

Say, ***Has the card turned over? You are now going to do the real test. Are you ready to start?***

Click ***Enter*** to begin the scored items.

When presentation of items is complete, the Detection test ends and the Identification Test begins automatically.

Identification Test (IDN)

The instructions appear on the screen. *The subject should read them silently as you read them aloud.*

Say, ***Is the card red? You are now going to do a practice. You will need to use both the "Yes" and "No" buttons for this task. In this task, a playing card will appear in the center of the screen. As soon as it turns face-up you must decide: is the color of the card red? If it is red, press the "Yes" button. If it is not red, press the "No" button. If you make a mistake you will hear an error sound. Try to make your responses as accurate and fast as possible after a card turns face-up. Are you ready to start?***

Click ***Enter*** to begin the practice items. Remind the subject to press the "Yes" or "No" buttons as quickly and accurately as possible.

If the subject appears confused or responds incorrectly, review the test requirements, including use of the left and right mouse button (left button = "No" and right button = "Yes" for responses. Ensure the subject understands that in this test, all cards shown are the same Joker card, the only difference being color (red or black). There is no need for the subject to examine details of the card closely.

When the practice items are complete, the instructions for the scored or "real" portion of the test appear.

Say, ***Is the card red? You are now going to do the real test. Are you ready to start?***

Click ***Enter*** to begin the scored items.

When presentation of items is complete, the Identification test ends and the One Card Learning Test begins automatically.

One Card Learning Test (OCLT)

The instructions appear on the screen. *The subject should read them silently as you read them aloud.*

Say, ***Have you seen this card before in this task? You are now going to do a practice. You will need to use both the "Yes" and "No" buttons for this task.***

In this task, a playing card will appear face-down in the center of the screen and then turn face-up. As soon as a card turns face-up decide if you have seen it before in this task. Only a few of the face-up cards will repeat during the task. If you have seen the card before in this task, press the "Yes" button. If you have not seen the card before in this task, press the "No" button. If you make a mistake you will hear an error sound. Try to make your responses as accurate and fast as possible after the card turns face-up.

Click ***Enter*** to begin the practice items.

Ensure that the subject understands that now the stimuli are standard playing cards (no jokers, no tricks such as slightly altered cards) and when deciding whether the card in view was shown before, *color*, *number*, and *suit* must all be considered before responding. If the subject appears confused or responds incorrectly, review the test requirements, including use of the left and right mouse button (left button = "No" and right button = "Yes" for responses.

When the practice items are complete, the instructions for the scored or "real" portion of the test appear.

Say, ***Have you seen this card before in this task? Cards seen in the practice are not used again. You are now going to do the real test.***

Click ***Enter*** to begin the scored items.

If the subject appears confused, offer a single prompt. *Say,* ***Have you seen this card before in this task?***

When presentation of items is complete, OCLT ends and the Groton Maze Learning Test begins automatically.

Groton Maze Learning Test (GMLT)

Test administrators should expect to provide active assistance to subjects during the practice portions of this test, as it is somewhat complex. In brief, subjects must find a hidden pathway in a grid of tiles. The practice portion involves a small grid, presented three times, and each time the subject must try to find the same hidden pathway.

The scored portion involves a larger grid, presented five times, and each time the subject must try to find the same hidden pathway within the larger grid.

Say, ***Find the hidden pathway. This is a practice to help you learn the rules of this maze. To begin, you must tap the top left blue tile, and then find the hidden pathway by tapping on tiles one at a time until you get to the tile in the lower right corner. The first time you try to find the pathway, you need to guess each step. A green tick means you were correct, but a red cross means the move was wrong. After a wrong move, you need to go back to the last correct tile, and then try a move in a different direction. The rules of this task are:***

1. Only move to adjacent tiles (up, down, left, or right).

2. Do not move diagonally.

3. Do not move backwards along the pathway.

4. Do not tap twice on the same tile.

Tap as quickly and accurately as you can.

Click ***Enter*** to begin the practice maze.

If the subject appears confused or responds incorrectly, review the four rules, including, if needed, that the blue tile is the starting point and that after a red cross, go back to the last correct tile (green tick), click it, and try a move in a different direction.

When the target is reached, a new grid appears (the pathway is the same, however).

Say, ***You must now find the same hidden pathway. To begin, tap the top left blue tile, and then tap on the hidden pathway one tile at a time until you get to the target tile. The rules are the same as before. Tap as quickly and accurately as you can.***

If the subject appears confused or responds incorrectly, review the four rules, including, if needed, that the blue tile is the starting point and that after a red cross, go back to the last correct tile (green tick), click it, and try a move in a different direction. When the target is reached, a new grid appears (the pathway is the same, however).

This process occurs for a total of three practice maze trials, after which the instructions appear once again.

If the subject appears to be rushing and moving along the pathway so fast that gridlines are being clicked (in other words, insufficient care or time is taken to position the mouse pointer within a tile before clicking), remind the subject not to rush and to work carefully. If grout lines are clicked, the computer doesn't know which tile is selected and so will score the click (move) as an error.

When the instructions screen appears, say, ***Find the hidden pathway. You are now going to do the real test. To begin, you must tap the top left blue tile, and then find the hidden pathway by tapping on tiles one at a time until you get to the tile in the lower right corner. The first time you try to find the pathway, you need to guess each step. A green tick means you were correct,***

ut a red cross means the move was wrong. After a wrong move, you need to go back to the last correct tile, and then try a move in a different direction. The rules of this task are:

1. Only move to adjacent tiles (up, down, left, or right).

2. Do not move diagonally.

3. Do not move backwards along the pathway.

4. Do not tap twice on the same tile.

Tap as quickly and accurately as you can.

Click ***Enter*** to begin the maze. Do not review task rules during the scored portions of the test. If, *during the real test,* subjects ask you to review the instructions because they forgot them or don't know what to do, or ask you to clarify something, say, "*The rules won't let me help you*."

Encourage subjects to do their best. Encourage them to guess if they aren't sure of the correct next move.

When the target is reached, a new grid appears (the pathway is the same, however). This process occurs for a total of five scored maze trials.

Say, ***You must now find the same hidden pathway. To begin, tap the top left blue tile, and then tap on the hidden pathway one tile at a time until you get to the target tile. The rules are the same as before. Tap as quickly and accurately as you can.***

Click ***Enter*** to begin.

When GMLT is finished, the subject receives a cheer.

The CogState tests are complete.

 (The above instructions script was edited slightly for clarity.)

ATTACHMENT 3: 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?	○	○	○	○	○	○	○	○	○	○	○

MDASI-BT - 2006

	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