Protocol #: BMX-HGG-001

A Phase 1/2 Study

A Phase 1/2 Trial for Patients with Newly Diagnosed High Grade Glioma Treated with Concurrent Radiation Therapy, Temozolomide, and BMX-001

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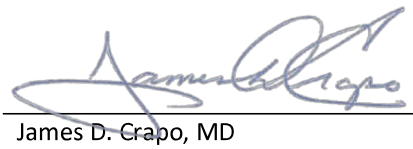

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Date

2 PRINCIPAL INVESTIGATOR SIGNATURE PAGE

I acknowledge that I have read and understand the BMX-HGG-001 protocol named above and agree to carry out all of its terms in accordance with applicable regulations and laws.
I assure that the study drug supplied by the sponsor will be used only as described in the protocol named above.

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4 LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AST	Aspartate Aminotransferase
BAC	Brief Assessment of Cognition
BDI-II	Beck Depression Inventory-II
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CPC	Cancer Protocol Committee
CR	Complete Response
CRM	Continual Reassessment Method
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
FACIT-F	Functional Assessment of Chronic Illness Therapy-Fatigue
FACT-Br	Functional Assessment of Cancer Therapy-Brain
FDA	Food and Drug Administration
GBM	Glioblastoma
Gy	Gray
HIF-1	Hypoxia-inducible factor-1
HGG	High Grade Glioma
H&P	History & Physical Exam
HRPP	Human Research Protections Program
HRQoL	Health-Related Quality of Life
ICS	Investigational Clinical Site
IRB	Institutional Review Board
KPS	Karnofsky Performance Scale
MDS	Myelodysplastic Syndrome
MnSOD	Manganese Superoxide Dismutase
MOS	Median Overall Survival
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NFκB	Nuclear Factor Kappa B
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PFS	Progression-free Survival
PFS-6	Progression-free Survival at 6 months
p.o.	per os/by mouth/orally
PR	Partial Response
PRO	Patient-Reported Outcome
PRT-BTC	Preston Robert Tisch Brain Tumor Center
QTc	Corrected QT Interval
RIO	Research Integrity Office
RT	Radiation Therapy
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase

SOC	Standard of Care
SOD	Superoxide Dismutase
SPGT	Serum Glutamic Pyruvic Transaminase
SQ	Subcutaneous
TdP	Torsades de pointes
TMZ	Temozolomide
WBC	White Blood Cells
WHO	World Health Organization
WIRB	Western Institutional Review Board

5 PROTOCOL SYNOPSIS

5.1 Purpose

This is a Phase 1/2 study of BMX-001 in combination with standard radiation therapy (RT) and temozolomide (TMZ) in the treatment of newly diagnosed high grade glioma (HGG) patients. Phase 1 was conducted as a single site study at the Preston Robert Tisch Brain Tumor Center at Duke (PRT-BTC). Phase 2 will be conducted as a multi-center study and will include up to 10 additional sites besides the PRT-BTC at Duke.

5.2 Phase 1

5.2.1 Primary Objective

1. To determine the maximum tolerated dose (MTD) of BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients.

5.2.2 Secondary Objectives

1. To assess the safety and tolerability of BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients.
2. To assess the efficacy of BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients based upon overall survival (OS) and progression free survival (PFS).
3. To examine the impact on cognition of BMX-001 in combination with standard RT and TMZ in treatment of newly diagnosed HGG patients.
4. To describe radiographic response in newly diagnosed HGG patients treated with BMX-001 in combination with standard RT and TMZ.
5. To characterize the pharmacokinetic profile of BMX-001 when delivered in combination with RT and TMZ in newly diagnosed HGG patients.

5.2.3 Exploratory Objectives

1. To describe patient-reported outcomes of health-related quality of life (HRQoL) in newly diagnosed HGG patients treated with BMX-001 in combination with standard RT and TMZ.
2. To describe changes in hair loss during BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients.

5.3 Phase 2

5.3.1 Primary Objective

1. To assess the effect on overall survival (OS) of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients.

5.3.2 Secondary Objectives

1. To assess the impact on cognition of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients.
2. To assess protection of bone marrow against chemotherapy-induced thrombocytopenia of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients.
3. To assess the safety and tolerability of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients.
4. To assess the effect on progression-free survival (PFS) of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients.

5. To assess radiographic response in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone.
6. To characterize the repeated-dose pharmacokinetic profile of BMX-001 when delivered in combination with RT and TMZ in newly diagnosed HGG patients.

5.3.3 Exploratory Objectives

1. To describe patient-reported outcomes of HRQoL in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone.
2. To describe changes in hair loss in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone.
3. To describe change in white matter integrity in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 in comparison to standard RT and TMZ alone.

5.4 Design and Procedure

5.4.1 Phase 1 (Enrollment Complete)

In the Phase 1 component of this protocol, we will conduct a dose-escalation study of the combination of BMX-001 with standard dosing of daily TMZ and RT in patients with newly diagnosed HGG (WHO grade III or IV). The dose escalation will be guided by a CRM design to determine the MTD of BMX-001 in combination with concurrent daily TMZ and RT. Subjects will be administered BMX-001 subcutaneously first as a loading dose given 0 to 4 days before the start of chemoradiation and then at a subsequent dose (50% of the loading dose) twice a week for eight weeks. There will only be two doses per week regardless of whether the dose is a loading or maintenance dose. TMZ will be dosed at 75 mg/m^2 orally daily for 42 days and RT will be delivered in daily fractions of 1.8-2 Gy given 5 days a week for 6 weeks for a total of 59-60 Gy.

Cohorts of 3 subjects will be accrued and treated until the MTD is defined. Following the treatment of the first 3 subjects at the initial dose level of 0.1 mg/kg dose level, the rules provided in Section 9.1.1.1 will be used to determine dose assignments for subsequent subjects and whether dose can be escalated. If a subject terminates protocol treatment without experiencing a DLT and before completing standard chemoradiation, then the subject will not be evaluable for the determination of DLT and will be replaced.

It is estimated that a maximum of 18 subjects will be required for Phase 1. The maximum tolerated dose (MTD), defined as the dose that has an estimated DLT rate nearest to 0.25, will be the dose used in the future Phase 2 study.

In order to evaluate the pharmacokinetics of BMX-001 in combination with current chemoradiation, blood samples will be drawn for analysis. The first dose of BMX-001 will be administered subcutaneously in the morning as a loading dose. Blood will be drawn on the following days: loading dose day, Day 8 or the next date on which drug is administered, and Day 36 or the next date on which drug is administered. Measures will be obtained at the following times: Predose, 0.5 hour, 1 hour, 2 hours, 6 hours, and 24 hours. Samples will be analyzed for BMX-001 using validated analytical methods at a laboratory identified by the sponsor. See Appendix B for details (Section 19.2).

With accrual in Phase I complete, a total of 17 subjects were enrolled. Enrollment numbers for Phase 1 broken down by dose escalation groups are as followed:

Dosing Group	Loading Dose	Maintenance Dose	Number of Subjects Treated
1	7 mg/subject	3.5 mg/subject b.i.w.	4
2	14 mg/subject	7 mg/subject b.i.w.	3
3	28 mg/subject	14 mg/subject b.i.w.	6
4	42 mg/subject	20 mg/subject b.i.w.	4

5.4.2 **Phase 2**

The Phase 2 portion of this study will be a randomized study comparing the impact of concurrent daily TMZ and RT with BMX-001 (Arm A) versus concurrent daily TMZ and RT alone (Arm B) on survival in patients with newly diagnosed HGG (WHO grade III and IV). The loading dose for Arm A determined from the Phase 1 component of this protocol is 28 mg/subject with a 14 mg/subject maintenance dose given twice weekly. Subjects will be randomized with a treatment arm allocation ratio of 1:1 and there will be 160 patients enrolled in this phase of the study (80 subjects per arm). Subjects in Arm A will be administered BMX-001 subcutaneously first as a loading dose given 0 to 4 days before the start of chemoradiation and then at subsequent dose twice a week for eight weeks. Subjects in Arm A and Arm B will receive TMZ dosed at 75 mg/m^2 orally daily for 42 days and RT delivered in daily fractions of 1.8-2 Gy given 5 days a week for 6 weeks for a total of 59.4-60 Gy. Cognitive performance will be measured at the time of enrollment and 2 weeks after the completion of RT, and standard of care (SOC) clinic visits (approximately 8 weeks apart +/- 2 weeks) until death, or patient's choice to discontinue study measures. Cognitive testing should continue after noted progression until the 12 month follow up timepoint is reached (testing may be done at a visit following the 12 month timepoint).

In order to characterize the repeated dose pharmacokinetic profiles of BMX-001 in combination with RT and TMZ in newly diagnosed HGG patients, pharmacokinetic blood samples will be drawn from six patients in the Phase 2 portion of the study on Days 8 and 36 only (see Section 19.2.2). The first 6 patients enrolled and assigned to Arm A at PRT-BTC at Duke will participate in the PK portion. Measures will be obtained at approximately the following times: Pre-dose, then 0.5 hour, 1 hour, 2 hours, 6 hours and 24 hours post-dose. Samples will be analyzed for BMX-001 using validated analytical methods at a laboratory identified by the sponsor. See Appendix B for details (Section 19.2).

5.5 Selection of Subjects

The inclusion/exclusion criteria for the Phase 1 and 2 component of this protocol are identical.

5.5.1 **Inclusion Criteria**

1. Subjects must have histologically confirmed diagnosis of WHO grade III or IV malignant glioma
2. Subjects must be planning to start standard of care radiation therapy and chemotherapy (temozolomide)
3. Subjects must be within 12 weeks of last major neurosurgical procedure for the high-grade glioma (craniotomy, open biopsy, or stereotactic biopsy)
4. Subjects must have had a definitive resection with residual radiographic contrast enhancement on post-resection CT or MRI of less than or equal to 3 cm in any two perpendicular planes on any images
5. Age $\geq$ 18 years
6. Karnofsky Performance Status (KPS) $\geq$ 70%
7. Hemoglobin $\geq$ 9.0 g/dl, ANC $\geq$ 1,500 cells/µl, platelets $\geq$ 125,000 cells/µl
8. Serum creatinine $\leq$ 1.5 mg/dl, serum SGOT and bilirubin $\leq$ 1.5 times upper limit of normal
9. Signed informed consent approved by the Institutional Review Board

10. If sexually active, patients must agree to use appropriate contraceptive measures for the duration of the study and for 12 months afterwards as stated in the informed consent
11. If using corticosteroids: stable and/or decreasing dose of corticosteroids for greater than or equal to 7 days
12. Subjects must be fluent in English (due to the neurocognitive testing and the Brief Assessment of Cognition (BAC) application only being available in English)

5.5.2 **Exclusion Criteria**

1. Pregnancy or breast-feeding
2. Active infection requiring IV antibiotics within 7 days before enrollment
3. Signs of wound-healing problems or infection at the craniotomy/biopsy site
4. Prior, unrelated malignancy requiring current active treatment with the exception of cervical carcinoma in situ and adequately treated basal cell or squamous cell carcinoma of the skin
5. Co-medication that may interfere with study results; e.g. immuno-suppressive agents other than corticosteroids
6. Prior treatment with radiotherapy for a brain tumor, irrespective of the grade of the tumor
7. Prior allergy to temozolomide
8. Prior progression on adjuvant temozolomide dosed at 150 mg/m^2 to 200 mg/m^2 on Days 1-5 of a 28-day cycle
9. Evidence of > grade 1 CNS hemorrhage on MRI or CT scan
10. Systemic treatment with inducers or strong inhibitors of cytochrome P450 within four days before enrollment or planned treatment during the study drug treatment period of the study (see Appendix D).
11. Metal in the body that is not compatible with MRI
12. Severe allergy to contrast agents

5.5.3 **BMX-001 Specific Concerns**

Subjects meeting any of the following criteria are **ineligible** for study entry:

1. Inadequately controlled hypertension (defined as systolic blood pressure >150 mmHg and/or diastolic blood pressure > 100 mmHg) within 28 days of first study treatment
2. Active or history of postural hypotension and autonomic dysfunction
3. Clinically significant (i.e. active) cardiovascular disease or cerebrovascular disease, for example cerebrovascular accidents ≤ 6 months prior to study enrollment, myocardial infarction ≤ 6 months prior to study enrollment, unstable angina, New York Heart Association (NYHA) Grade II or greater congestive heart failure (CHF), or serious cardiac arrhythmia uncontrolled by medication or potentially interfering with protocol treatment
4. History or evidence upon physical/neurological examination of central nervous system disease (e.g. seizures) unrelated to cancer unless adequately controlled by medication or potentially interfering with protocol treatment
5. Significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months prior to start of study treatment
6. A marked baseline prolongation of QT/QTc interval (e.g., repeated demonstration of a QTc interval >480 milliseconds (ms) (CTCAE grade 1) using the specific/usual choice by clinical center for correction factor.
7. A known history of additional risk factors for Torsades de Pointes (TdP) (e.g., congestive heart failure, hypokalemia, known family history of Long QT Syndrome).

For inclusion criteria (Section 5.5.1) #4 and exclusion criteria (Section 5.5.2) #9, final review and determination will be made centrally. The baseline CT or MRI scan will be uploaded to the Duke coordinating center for final confirmation of eligibility. Review the sponsor provided Imaging Manual

for more details about this transfer. Once eligibility is confirmed the site will be notified. Subjects will not be randomized until final eligibility is confirmed.

5.6 Risk/Benefit Assessment

5.6.1 **Potential Benefits**

The potential benefits may include protection against cognitive decline caused by RT and/or reduction and/or remission of the subject's HGG. Additionally, a potential benefit may include protection against development of thrombocytopenia caused by treatment with TMZ. From prior study of HGG, roughly 15% of patients will develop grade 3 or 4 thrombocytopenia [1]. These subjects are at risk for a bleeding complication and may have their cancer therapy interrupted to allow recovery of the bone marrow. If this can be prevented it would be a major advance in therapy for HGG.

Because this clinical protocol is experimental, it cannot be guaranteed that subjects will receive any benefit as a result of participating in this research study. The information collected in this research may help scientists better understand the mechanisms involved in oxidative stress as it pertains to treatment of brain cancer. If such an understanding emerges from this research, it may benefit society by furthering the development of improved treatment methods for newly diagnosed HGG in the future.

5.6.2 **Risks of BMX-001**

Studies have been performed using intravenous infusions of a related metalloporphyrin in multiple models including rats, mice, dogs, guinea pigs, and baboons with the finding of dose-related hypotension [2]. Metalloporphyrin compounds being evaluated are potent, catalytic antioxidants and have the potential to scavenge superoxide within the vascular system and thereby change the balance of superoxide and nitric oxide in the microvasculature. This would augment the vasodilating activity of nitric oxide and lead to hypotension [2]. This has been found in animal models to be a significant, but transient side effect and therefore blood pressure monitoring is essential in this clinical trial of a metalloporphyrin compound. Studies of rats, mice, and monkeys have been performed with BMX-001 and have shown transient, dose-related hypotension. Hypotension is related to plasma Cmax levels and subcutaneous administration significantly reduces the risk of this toxicity in mice and primates (unpublished data per BioMimetix JV, LLC). This hypotensive effect was also found in primates. We have designed this protocol to monitor blood pressure closely.

A summary of the possible adverse side effects that could be associated with BMX-001 administration is below. These possible side effects are dose-dependent, and the relatively low doses of this drug planned in this study have not been associated with side effects in animals other than those related to the color of the injected drug.

<u>The most common side effects (expected to occur in more than 30% of subjects) are</u>:

- Red to brown discoloration of the skin at the injection site which may take up to several weeks to resolve
- Irritation at the site of the injection of the drug under the skin
- Transient tachycardia from study drug

<u>Less common side effects (expected to occur in 10-30% of subjects) are:</u>

- Transient pain at the injection site. This is expected to be related to the concentration of administered drug and the volume administered, rather than subject body size
- Local histamine release which could be caused by study drug. This could cause pruritus (itching), erythema (redness), edema (swelling), urticaria (welts). This is expected to resolve within a couple of hours of injection

Rare side effects (expected to occur in less than 10% of subjects) are:

- Temporary hypotension (low blood pressure)
- Malaise or "not feeling well" for a few hours
- Prolongation of the QTc interval

Additional possible side effects:

- Light-activated skin rash in response to sun exposure (not yet reported in humans)
- Red to dark color of urine (not yet reported in humans)
- It is also possible that previously unobserved and unexpected side effects could occur.

Human safety data for BMX-001 in combination with TMZ and RT has been studied in Phase 1 of this clinical trial. BMX-001 at 42 mg/subject loading dose and 20 mg/subject subsequent doses given twice weekly was the maximum administered dose and 28 mg/subject loading dose and 14 mg /subject for subsequent twice weekly doses was the MTD. Sinus tachycardia (grade 3) was the dose-limiting toxicity at 42 mg/subject loading dose (n=1). The only other related grade ≥ 3 event seen was hypotension (grade 3) (n=1). The most common related toxicity was grade 1 injection site reaction (n=7). There is no apparent toxicity to end organ tissues or bone marrow.

5.6.3 **Risks of TMZ**

Most common side effects (occurring in more than 30% of subjects):

- Fatigue
- Nausea
- Hair loss

Less common side effects (occurring in 10-30% of subjects):

- Thrombocytopenia (low platelets), which may result in easy bruising or bleeding for a longer time.
- Loss of appetite
- Headache
- Constipation
- Diarrhea
- Vomiting
- Rash
- Swelling in extremities

Rare side effects (occurring in less than 10% of subjects):

- Abdominal and/or breast pain
- Dry skin, skin redness, and/or itching
- Inflammation of the mouth, throat and/or sinuses
- Dizziness, weakness
- Confusion, memory impairment
- Anxiety, depression
- Joint and muscle pain
- Trouble sleeping
- Change in sense of taste
- Blurred vision
- Coughing or shortness of breath
- Urinary incontinence/frequency, urinary tract infection
- Weight increase
- Seizures/convulsions
- Adrenal hypercorticisim (elevated hormone levels)

- Low white blood cell (WBC) count, which may lead to infection.
- Allergic reaction, sometimes severe
- Anemia (decreased number of red blood cells), which may cause symptoms of shortness of breath, weakness, and fatigue.

Rarely, unusual ("opportunistic") infections have occurred. Rare cases of erythema multiforme (skin condition) have been reported, which got better after TMZ was stopped and, in some cases, recurred upon restarting treatment with TMZ.

Very rare side effects have included secondary cancers including leukemia and myelodysplastic syndrome (MDS). MDS is a disorder of the bone marrow in which blood cells that do not function normally are produced.

There have been reports of hepatotoxicity or liver injury in patients receiving TMZ. TMZ may cause elevations in liver enzymes, which measure liver function, elevation of bilirubin levels, which may lead to jaundice or a yellowing of the skin and mucous membranes, cholestasis, which results from a decreased flow of bile from the liver, and hepatitis, an inflammation of the liver.

Reproductive studies have not been done with TMZ. Immature sperm and testicular atrophy (wasting away) occurred in studies with rats and dogs, using doses of TMZ 1/4 and 5/8 of the recommended human doses. In animal studies, TMZ caused death and multiple malformations in rats and rabbits exposed during pregnancy.

5.6.4 **Radiation Treatment Side Effects**

Possible side effects include swelling of the brain, hair loss, localized skin irritation, low blood counts, fatigue, memory loss, hearing loss, nausea and/or vomiting, loss of appetite, headaches, RT necrosis (death of tissue or skin), and secondary cancer.

5.6.5 **Risks of Phlebotomy**

Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the site of the needle puncture, light headedness, fainting and very rarely, local infection, which may be severe. These risks are reduced by the fact that the blood will be drawn by a qualified physician, nurse or phlebotomist (a professional trained to draw blood).

5.6.6 **Risks of MRI**

Risks and/or discomforts associated with MRI scans include anxiety produced from being in a tight, enclosed space (claustrophobia). In addition, the machine operates using a large and powerful magnet, which attracts certain metals. Therefore, people with metals in their bodies that are not compatible with MRI will be excluded from the study. Patients will also be checked to make sure that they do not bring any metal objects into the MRI facility. Dental fillings are less affected by the magnetic fields generated and are therefore permitted. It will be asked that patients let the physicians conducting this study know of any metal in their bodies other than dental fillings. During the MRI, patients will be given a contrast agent. The agent is given routinely to obtain enhanced MRI scans of the brain. The agent is administered through the vein and requires the placement of an IV catheter. The catheter placement is similar to drawing blood except that the catheter remains in the vein during the time the agent is actively delivered. The risks of a blood draw and insertion of a catheter are similar. There have been a few, rare cases of allergies to the agent used in MRI contrast enhanced scans. Patients with any known severe allergies to contrast agents will be excluded from the study. Patients with mild allergies (i.e., rash only) may be pretreated with Tylenol and Benadryl prior to injection of the contrast agent. A rare but serious adverse reaction has been observed in patients that received a gadolinium-based contrast material during MRI examinations, a reaction called nephrogenic systemic fibrosis (NSF). Patients with kidney disease are at increased risk of

developing NSF. NSF may cause skin thickening, joint pain and/or swelling. In rare cases NSF can lead to lung and heart problems and cause death.

5.6.7 **Unknown Risks**

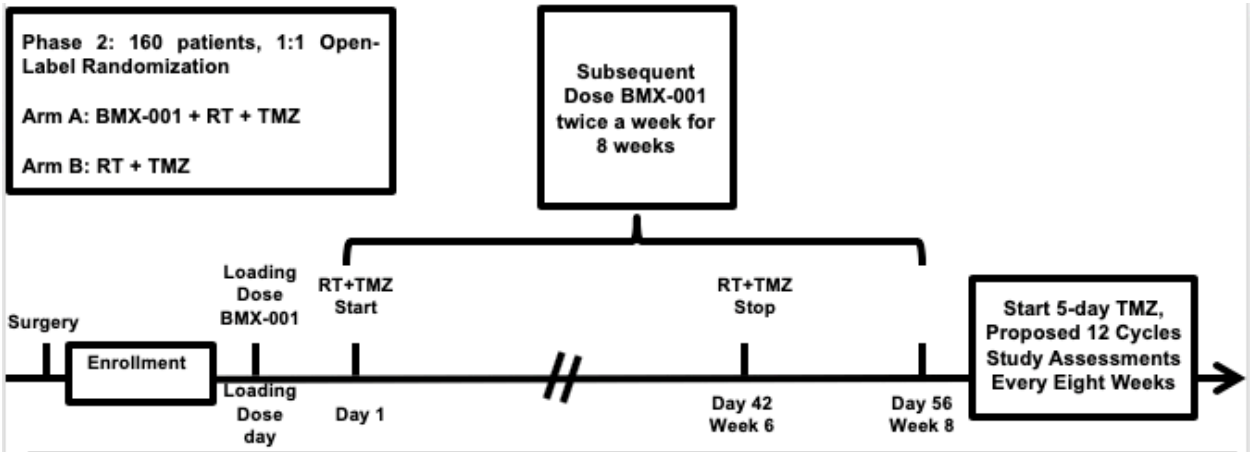
The overall risk classification of this research is unknown.

5.7 Data Analysis and Statistical Considerations

Within the Phase 1 portion of this study, the MTD for BMX-001 administered in conjunction with temozolomide and radiation treatment will be determined for patients newly diagnosed with high grade glioma. A CRM model as described in section 16.4.1.2 will be used to determine the dose with the estimated DLT rate closest to the target DLT rate of 0.25. This Phase 1 study will be conducted in two stages. The first stage involves dose escalation in successive cohorts of 3 patients until a DLT is observed. The second stage commences with the observation of a DLT. Within the second stage, all accumulated data will be used within the context of a one-parameter model to determine the appropriate dose for each subsequent cohort of 3 patients. When the toxicity outcomes associated with patients within a cohort within the second stage are known, the one-parameter logistic model will be re-estimated by the study's statistical team using all available data. Based upon this re-estimated model, the dose level that results in a DLT rate nearest to the target DLT rate will be determined, and used to treat the next patients, subject to a dose escalation restriction. That restriction requires that the dose for the next patient cannot be more than one level higher than that of the current patient.

In Phase 2, the study's primary endpoint is overall survival, defined as the time between randomization and death. A logrank test will be used to compare the survival experience of the two treatment arms. An intent-to-treat philosophy will be used in the analysis of survival by including all patients who are randomized in the analysis. Among the secondary endpoints are two that are of particular interest: cognitive function and thrombocytopenia. The mean change in cognitive functioning between baseline (enrollment) and week 24 within each treatment arm will be computed, and analysis of covariance will be conducted to compare groups with respect to that change. Treatment group assignment will be included in the model as a predictor, and the baseline measure of cognitive function will be included as a covariate. The proportion of patients who experience grade 3 or 4 thrombocytopenia during concurrent temozolomide and radiation, a period that terminates 2 weeks after completion of radiation treatment, will be summarized. A chi-square test will be conducted to compare the prevalence of such thrombocytopenia observed in patients with and without BMX-001 [1].

6 STUDY SCHEMA



Assessment Timing	Study Assessment
Enrollment	History, Demographics, Physical/Neurological Exam, CBC with differential, CMP, MRI Brain, Cognitive Testing, HRQoL PRO, Hair Assessment, MRI Diffusion Tensor Imaging
Loading Dose Day	History, Physical/Neurological Exam
RT+TMZ (6 weeks)	History, Physical/Neurological Exam Every Two Weeks, CBC with differential Weekly, CMP Weekly, Pharmocokinetics (if applicable)
Day 56, Week 8	History, Physical/Neurological Exam, MRI Brain, Cognitive Testing, HRQoL PRO, Hair Assessment, MRI Diffusion Tensor Imaging
TMZ Adjuvant Phase, Every 8 Weeks	History, Physical/Neurological Exam, CBC with differential, CMP, MRI Brain, Cognitive Testing, HRQoL PRO, Hair Assessment
24 Weeks After Start of RT+TMZ	MRI Diffusion Tensor Imaging
Ongoing	Safety and Toxicity Assessment and Evaluation by CTCAE Version 4.0, Platelet Counts

Figure 1: Study Schema for Phase 2 Portion of the Study. Review the table in Section 13 for a complete list of study procedures.

7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

Primary brain tumors represent 1% of all diagnosed cancers, but are among the most lethal [3]. Median survival for adults with HGG (WHO grade III and IV) is poor and ranges from 9-15 months after diagnosis. Most high grade CNS tumors are highly resistant to current available therapies. The standard of care for newly diagnosed high-grade gliomas (HGGs) involves surgical resection, followed by standard RT with concurrent TMZ, and subsequent adjuvant TMZ. Patients with HGG not only contend with oncologic diagnosis but also a neurologic one that can lead to cognitive dysfunction.

7.2 Radiation-Induced Damage during Brain Tumor Treatment

During the disease treatment course, the nervous system can be damaged by all parts of the multimodal approach. In particular, RT can lead to the generation of reactive oxygen species which in turn leads to oxidative stress [4]. The oxidative stress is not only responsible for the damaging of the DNA and killing of the tumor cells but also leads to damage of the white matter [5]. Central nervous system toxicity from RT is defined as acute, early delayed, and late delayed [6]. Acute form can occur during RT and is more apparent with doses > 60 Gy. This is rarely seen with the modern approach to standard chemoradiation in adult glioma patients. Early delayed encephalopathy and late delayed encephalopathy are still seen with some frequency in the HGG population. Early delayed encephalopathy is characterized clinically by somnolence, attention deficits, and gait impairment. This occurs between one to six months after RT and is thought to be due to transient demyelination. Late delayed encephalopathy occurs months to years after RT and, clinically, patients demonstrate a pattern of subcortical dementia (short-term memory loss, problems with gait and attention). Mechanism of action involves damage to the supporting vasculature, which in turn leads to demyelination and, in severe cases, RT necrosis. The impact to patients is cognitive impairment and subsequent degradation of HRQoL. In fact, 50-90% of brain tumor patients that received RT demonstrated cognitive impairment, if they survived >6 months from RT [7-10]. In summary, the oxidative stress produced by RT in brain tumor patients leads to white matter damage, subsequent cognitive impairment, and finally quality of life impairment.

7.3 Cognition and HRQoL in HGG

Cognitive dysfunction, whether it develops due to the underlying tumor, treatment, or use of medications such as anti-epileptics and corticosteroids, usually comes in concert with a reduction in HRQoL. In both newly diagnosed and recurrent HGGs, reduction in cognitive function was associated with a reduction in reported HRQoL [11-13]. When compared to healthy controls or patients with other malignancies, high-grade glioma patients experience a great degree of cognitive impairment and related reduction in HRQoL [13].

Before and during treatment for HGG, adult patients experience a decline in perceived HRQoL[14,15]. Liu defines HRQoL as “a concept that encompasses the multidimensional well-being of a person and reflects an individual’s overall satisfaction with life” [15]. For brain tumor patients, focal neurological dysfunction, whether cognitive or physical, fatigue associated with treatment, symptoms such as nausea and anorexia associated with treatment, and other common symptoms such as insomnia, seizures, and headaches all have negative impacts on HRQoL. Overall, functionality and HRQoL are particularly important in brain tumor patients since these patients have more dysfunction in these areas than age-matched controls with non-small lung cancer [13]. In a large prospective study by Budrukkar and colleagues of HRQoL in brain tumor patients, they found that HRQoL, as rated by scores from questionnaires, was low before starting any type of treatment, and factors associated with poorer HRQoL included patients with poor performance status, illiteracy, and a more aggressive nature of the tumors [16]. Moreover, HRQoL is dependent upon the physical function and cognition of the brain tumor patient such that patients with better physical function and better cognitive

function reported having a higher level of HRQoL [11]. Liu and colleagues discuss how these symptoms and signs are interrelated and should not be studied alone because cognitive decline/ dysfunction were associated with increased fatigue and poorer performance status [15,17,18]. During concurrent chemoradiation, brain tumor patients experience a decline in relative HRQoL particularly due to fatigue and changes in cognition [7,19].

In order to capture fully the many aspects of cognition and HRQoL, we have utilized both objective and subjective measures to evaluate issues with cognition, fatigue, HRQoL, and mood. Cognition can be measured objectively with standardized cognitive testing. One testing model is CNS Vital Signs®, a computerized battery. CNS Vital Signs® has been validated and is being used extensively in our research, not only primary brain tumor patients, but also patients with other forms of cancer [20]. Modalities within CNS Vital Signs® assess cognitive flexibility, complex attention, executive functioning, psychomotor speed, processing speed, verbal and visual memory. We published the cognitive performance of newly diagnosed HGG using this battery and showed that they had measurable cognitive dysfunction in all domains [21,22]. After diagnosis, newly diagnosed HGG patients exhibit deficits in all tested cognitive domains with standardized scores ranging from 90.1 to 72.7 (a score of 100 represents mean standardized score for normative age-matched subjects). These deficits continue throughout the treatment and disease trajectory with lower performances present after radiotherapy and 24 weeks after initial diagnosis. Importantly, performance on neurocognitive testing is independent of education, grade, gender, and tumor location, but Karnofsky Performance Scale (KPS) and surgery extent are important predictors of neurocognitive performance. Moreover, long-term survivors of primary HGG (WHO grade IV) continued to exhibit cognitive dysfunction despite having achieved an improved survival [23]. Therefore, there is an unmet need to improve the cognitive outcomes in patients with newly diagnosed HGG, irrespective of survival outcomes. Another testing model called The Brief Assessment of Cognition (BAC) is a battery of tests is implemented through an iPad-based app (VeraSci). The BAC was created by Richard Keefe, Jim Gold, Terry Goldberg, and Philip Harvey as a pen-and-paper measure for the repeated assessment of cognition in clinical trials for schizophrenia (referred to as BACS) [24]. With published normative data [25] and over a decade of use, the BACS is the subject of approximately 100 peer-reviewed papers and has been used in over 30 multisite clinical trials across a range of patient groups. To reduce rater burden and promote standardization of neurocognitive testing in clinical trials, the BAC is now implemented as an iPad-based app [26]. The BAC App assists raters in administration and scoring of all subtests included in the original pen-and-paper instrument.

Subjectively, HRQoL is measured using patient-reported outcome questionnaires known as PROs. A commonly used instrument is the Functional Assessment of Cancer Therapy-Brain (FACT-Br) which has been used extensively and has been documented to identify key QoL problems for brain tumor patients [27]. Other standardized questionnaires will include Functional Assessment of Cancer Therapy-Fatigue, Functional Assessment of Cancer Therapy-Cognition, and Beck Depression Inventory.

RT in patients with HGG causes oxidative stress in brain tissue, which can lead to white matter damage, subsequent cognitive impairment, and HRQoL impairment. White matter damage due to RT can be detected using serial MRI diffusion tensor/susceptibility imaging [28-30]. This damage is evident and detectable using serial MRI diffusion tensor/susceptibility imaging both immediately and subsequently after the completion of RT in HGG patients [31-33]. Therefore, MRI diffusion tensor/ susceptibility imaging can serve as a biomarker of white matter damage caused by RT in HGG patients. In fact, early changes in the parahippocampal white matter seen on MRI diffusion tensor/ susceptibility imaging have been linked to late decline in verbal memory in patient that received RT [34].

Interventions to improve cognition and HRQoL in brain tumor patients have included stimulants such as methylphenidate and modafinil. Clinical trials with methylphenidate in brain tumor patients

undergoing RT were discontinued in early stages because interim analysis did not show any evidence of effectiveness [35]. Studies with modafinil did show some positive effects on HRQoL in brain tumor patients, but the study was not placebo-controlled [36]. Medications to treat Alzheimer's disease are being explored in the glioma population. Donepezil, a reversible acetylcholinesterase inhibitor used in mild to moderate Alzheimer's dementia, has been studied in primary brain tumor patients. In this Phase 2 study, donepezil improved cognition, mood, and HRQoL in irradiated brain tumor patients [37]. More rigorous Phase 2 studies are needed to evaluate the effectiveness of anti-Alzheimer's agents in glioma patients and side effects from these medications have limited their usefulness. Therefore, new approaches and more clinical studies are needed to evaluate interventions in regards to cognition and HRQoL in primary brain tumor patients.

In Phase 1, all fifteen subjects have completed quality of life evaluations and cognitive evaluations for the time points before concurrent chemoradiation and after concurrent chemoradiation. Cognitive testing included Hopkins Verbal Learning Test-Revised (HVLT-R), Trails Making A and B Tests, and Controlled Oral Wood Association Test (COWA). While it is early to assume cognitive protection of BMX-001, we have early findings that there is improvement of verbal memory as demonstrated by improved scores on HVLT-R, and no degradation in performance on Trails Making B Test (Figure 2) and COWA. This is notable because these arethe primary cognitive tests that reveal poorer performance in HGG patients after RT.

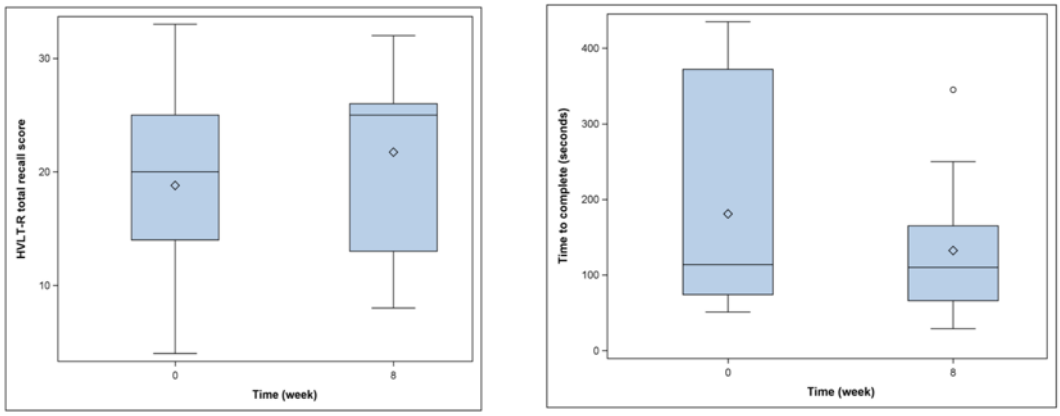


Figure 2. Performance on cognitive testing in 15 subjects in Phase 1 study of newly diagnosed high-grade glioma utilizing BMX-001 in combination with concurrent chemoradiation. Left panel: HVLT-R (higher scores = better performance). Right panel: Trails Making B Test (faster/shorter times = better performance) (note, only 13 subjects completed the second Trails Making B Test)

While promising, these results, along with the quality of life data, will need to be considered more fully after more subjects are treated with BMX-001 in Phase 2.

7.4 Thrombocytopenia

Another key observation made in the Phase 1 portion of this study is that none of our patients have experienced bone marrow suppression secondary to radiation and temozolomide outside of two instances of grade 1 thrombocytopenia in week 6 of radiation and chemotherapy.

In Phase 1, marked bone marrow protection has been observed as shown in Figure 3 below. In patients receiving temozolomide the standard of care is to stop therapy for patients developing a platelet count below 100,000. None of the fifteen evaluable subjects receiving BMX-001 + temozolomide developed platelet counts below 100,000. We compared this to patients that were not on protocol but had a diagnosis of glioblastoma (GBM) and were receiving radiation and TMZ and were being treated at the Duke Cancer Institute. In this control cohort, 9 out of 20 patients who

received concurrent TMZ and radiation therapy developed thrombocytopenia with platelet counts below 100,000. While these groups cannot be directly compared, the observation that subjects that received BMX-001 along with concurrent chemoradiation did not develop platelet counts below 100,000/mm^3 is compelling.

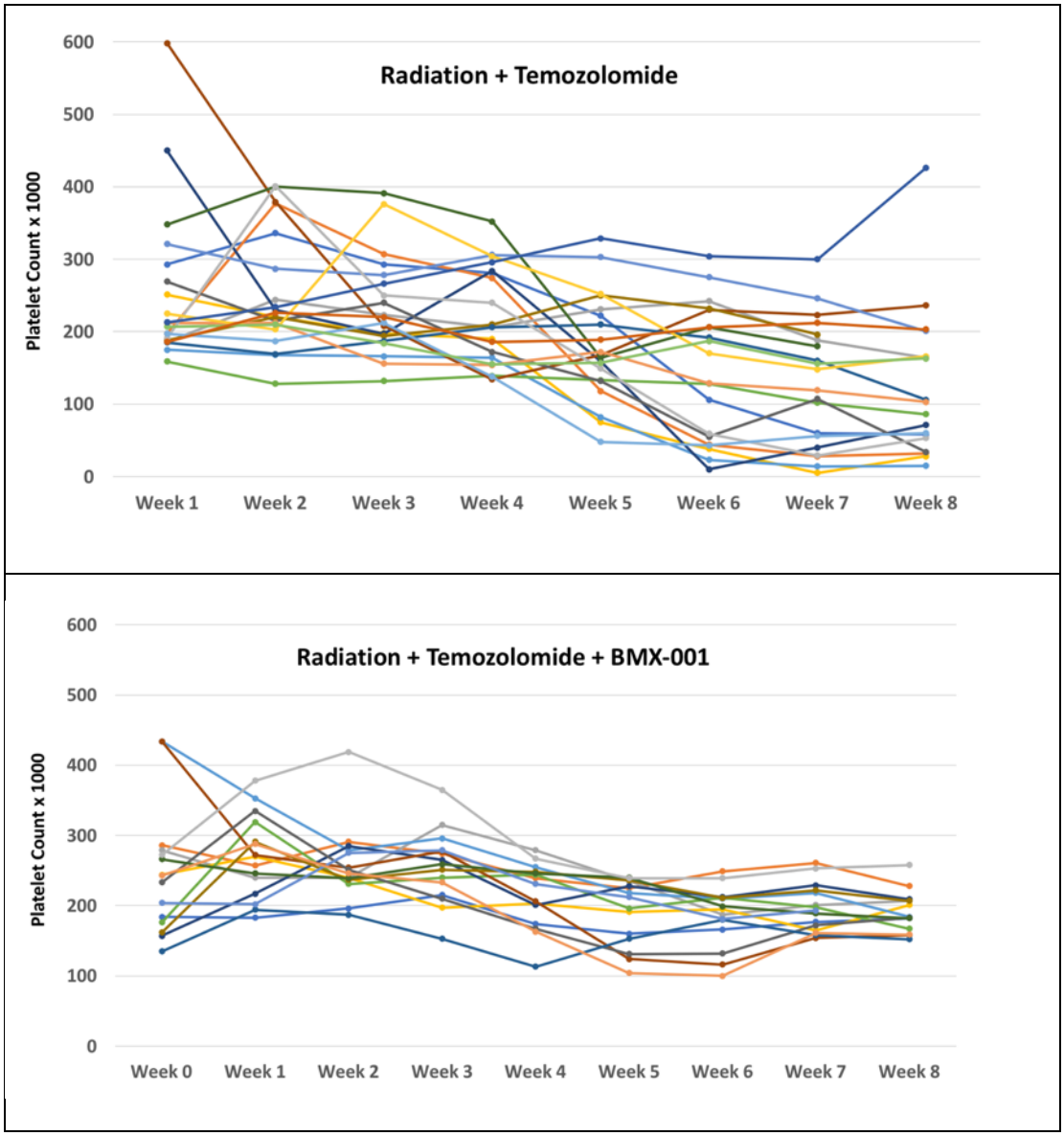


Figure 3. Platelet counts in high-grade glioma patients receiving temozolomide with and without BMX-001. Each line represents a separate patient. N=20 for patients receiving chemoradiation alone (left panel). N=15 for patients receiving BMX-001 + chemoradiation (right panel).

The data supports the conclusion that BMX-001 may be beneficial in preventing chemotherapy-induced bone marrow suppression at administered doses between 7 mg and 42 mg/subject load followed by one-half the loading dose administered biweekly.

Of note, there are now current studies underway with BMX-001 in radiation protection for mucositis and xerostomia in irradiated head and neck cancer patients, as well as a therapeutic for patients with

multiple brain metastases. With the information from these studies, we are confident in our toxicity pattern for BMX-001 in cancer patients and in the doses chosen for Phase 2 evaluation.

7.5 BMX-001

It is well-established that induction of oxidative stress after RT and chemotherapy plays a major role in tumor and normal tissue therapy responses [38,39]. Ironically, the responses in tumor tend to protect vascular endothelium and tumor cells from RT damage [40], whereas these same stresses after RT promote normal tissue damage [38,41]. A variety of approaches have been investigated to mitigate oxidative stress post irradiation, including the free radical scavenger, amifostine [42-44]. Although this agent is approved for this use, its daily use is limited by side effects [42,44]. Other agents that have been tested pre-clinically and clinically include nitroxides and genistein, but these are not currently approved for protection against normal tissue injury post irradiation, and neither provides catalytic inactivation of reactive species responsible for oxidative stress [42]. Over the past 20 years, Dr. Ines Batinic-Haberle and colleagues have developed a class of manganese (Mn) porphyrin-based compounds that: 1) are potent anti-inflammatory agents and 2) catalytically inactivate a range of reactive oxygen species, including peroxynitrite and superoxide anion [45-47]. These properties give this class of compound the unique function of protecting normal tissues while also augmenting tumor killing in patients undergoing radiation and chemotherapy. In addition to inactivation of reactive oxygen species, these drugs inhibit transcriptional activity of stress-induced transcription pathways by either eliminating reactive oxygen species that activate stress responses or by indirectly preventing activation. Transcription factors known to be inhibited by this class of metalloporphyrin include HIF-1 [48,49], NFκB [50], SP-1 [51] and AP-1 [45]. The most recently developed compound, BMX-001 ($MnTnBuOE\text{-}2\text{-}PyP^{5+}$), is among the most highly potent in a series of well over two dozen metalloporphyrins that have been evaluated thus far [52]. BMX-001 is lipophilic and crosses the blood brain barrier. This drug has been licensed for commercial development and will be the lead compound for this project.

In normal brain, oxidative stress incites inflammatory responses that contribute to white matter degradation and cognitive deficits. Metalloporphyrins have been shown to protect against a range of CNS conditions associated with inflammation, including stroke [53] and spinal cord damage [54-56]. Furthermore, metalloporphyrins have been shown to protect mice from total body irradiation toxicity [57] and xerostomia following parotid gland irradiation [58]. Radiation-induced lung injury was protected against by administration of metalloporphyrins in rhesus macaques [59]. Relevant to this application, Weitzel and colleagues showed protection against hippocampal stem cell loss and white matter degradation after whole brain RT therapy [58]. In this study, C57BL/6J mice were exposed to 8 Gy of whole brain irradiation with and without concurrent BMX-001. In mice that had been treated with BMX-001 and RT, there was preservation of myelin in the corpus callosum and anterior commissure in comparison to mice that received only RT without BMX-001. Moreover, the mice that received BMX-001 and RT performed better on rotorod tests and running wheel tests, both tests of cognitive function, in comparison to the RT alone group. It is well-established that oxidative stress and cognitive losses are a consequence of brain irradiation [6]. Therefore, there is ample evidence to suggest that BMX-001 will reduce the magnitude of oxidative stress after RT therapy and TMZ; this will lead to protection against cognitive losses and improvement in HRQoL.

In tumors, Dr. Dewhirst and colleagues showed that RT and chemotherapy increase oxidative stress, driving upregulation of HIF-1 [40,60], a transcription factor that promotes endothelial and tumor cell survival and increases tumor aggressiveness [61]. Radiation creates persistent oxidative stress in surviving cells, which upregulates NFkB [62]; this transcription factor also drives treatment resistance [63]. Treatment with this new class of metalloporphyrin prevents HIF-1 and NFkB upregulation in tumors after RT and chemotherapy and improves tumor treatment responses [57]. Using LN-18 and LN-229 HGG cell lines, BMX-001 showed no significant protective effect on clonogenic survival when

cells were also exposed to RT [64]. The effect of BMX-001 has been studied on mouse flank HGG xenografts. In this study by Weitzel and colleagues, while RT did lead to a reduction in GBM tumor growth in comparison to saline alone, the combination of BMX-001 and RT demonstrated a greater and significant degree of tumor reduction [64]. This demonstrated that BMX-001 has a differing effect in tumor tissue in comparison to normal tissues with BMX-001 showing potential to protect normal tissues and sensitize tumors to RT. Thus, the rationale for this protocol is based on the demonstrated dual activity of BMX-001 to protect normal tissue while sensitizing tumors to therapy.

7.6 Study Rationale

BMX-001 added to RT and TMZ has the potential not only to enhance the survival of HGG patients but also to protect against deterioration of cognition, impairment of HRQoL, and bone marrow suppression. We have performed a Phase 1 trial of BMX-001 at escalating doses in combination with standard RT, 33 fractions to 59-60 Gy, plus daily TMZ at the standard dosing of 75 mg/m^2. We have confirmed that BMX-001, when added to standard RT and TMZ, will be safe at pharmacologically relevant doses in patients with newly diagnosed HGG. Safety and MTD have been determined in the Phase 1 portion of this study, thus we will now move to a Phase 2 study comparing concurrent daily TMZ and standard RT alone to the same regimen in combination with BMX-001 to assess the impact of BMX-001 on the survival of newly diagnosed HGG patients. We hypothesize that BMX-001, when added to standard RT and TMZ, will be safe and will sensitize the malignancy resulting in enhanced patient survival. In addition, BMX-001 will protect normal brain tissue from the effects of radiation and chemotherapy and will protect bone marrow from chemotherapy-induced thrombocytopenia.

8 OBJECTIVES AND ENDPOINTS

Table 1: Phase 1 Objectives and Endpoints

	Objective	Endpoint	Analysis
Primary	To determine the maximum tolerated dose (MTD) of BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients	MTD is defined as the dose level that has an estimated DLT rate nearest to 0.25	See Section 16.4.1
Secondary	To assess the safety and tolerability of BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients	Proportion of patients with DLT	See Section 16.4.1.4
Secondary	To examine the impact on cognition of BMX-001 in combination with standard RT and TMZ in treatment of newly diagnosed HGG patients	Mean change between baseline and week 24 in normalized scores for cognition using standardized cognitive tests and computerized battery CNS Vital Signs®. This includes Hopkins Verbal Learning Test-Revised, Trails Making Test Parts A and B, and Controlled Word Association Test and CNS Vital Signs® battery consisting of seven tests that measure verbal and visual memory, finger tapping, symbol digit coding, the Stroop Test, a test of shifting attention, and continuous performance test.	See Section 16.4.1.4
Secondary	To assess the efficacy of BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients based upon overall survival (OS) and progression free survival (PFS)	Median OS, where OS is defined as the time to death from the time of protocol treatment is initiated Median PFS, where PFS is defined as the time between initiation of protocol treatment and the first recurrence of disease or death	See Section 16.4.1.4
Secondary	To describe radiographic response in newly diagnosed HGG patients treated with BMX-001 in combination with standard RT and TMZ	Proportion of patients with radiographic response	See Section 16.4.1.4
Secondary	To characterize the pharmacokinetic profile of BMX-001 when delivered in combination with RT and TMZ in newly diagnosed HGG patients	Plasma concentrations and calculated pharmacokinetic parameters (maximum concentration, time to reach maximum concentration, area under the curve, half-life, total body clearance, and volume of distribution) will be determine for BMX-001	See Section 19.1
Exploratory	To describe patient-reported outcomes of HRQoL in newly diagnosed HGG patients treated with BMX-001 in combination with standard RT and TMZ	Mean change from baseline at each follow-up assessment for subscales of the following PROs include Functional Assessment of Cancer Therapy-Brain Cancer (FACT-BR) version 4, Functional Assessment of Cancer Therapy-Fatigue (FACIT-Fatigue) subscale, version 4, Functional Assessment of Cancer Therapy-Cognition (FACT-Cog) subscale, Beck depression inventory (BDI-II), revised version, Godin Leisure questionnaire	See Section 16.4.1.5
Exploratory	To describe changes in hair loss during BMX-001 in combination with standard RT and TMZ in newly diagnosed HGG patients	Subjective investigator and patient assessment, photographs	See Section 16.4.1.5

Table 2: Phase 2 Objectives and Endpoints

	Objective	Endpoint	Analysis
Primary	To assess the efficacy of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients based upon overall survival (OS).	Median OS, where OS is defined as the time to death from randomization.	See Section 16.5.1
Secondary	To examine the impact on cognition of BMX-001 in combination with standard RT and TMZ in treatment of newly diagnosed HGG patients.	Mean change between baseline and week 24 in normalized scores for cognition using standardized cognitive tests and BAC This includes Hopkins Verbal Learning Test-Revised, Trails Making Test Parts A and B, and Controlled Word Association Test and BAC (VeraSci) consisting of seven tests that measure verbal memory and learning, working memory, motor function, verbal fluency, speed of processing, and executive function.	See Section 16.5.2
Secondary	To assess protection of bone marrow against chemotherapy induced thrombocytopenia in patients being treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients.	Proportion of patients with thrombocytopenia grade 3 or 4 adverse event during standard RT and TMZ treatment. Proportion of patients with a platelet count below 100,00 during standard RT and TMZ treatment and who had suspension of TMZ therapy.	See Section 16.5.2.2
Secondary	To assess the safety and tolerability of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients.	Proportion of subjects who experience grade 3 or 4 adverse event during standard RT and TMZ treatment. Proportion of subjects who experience grade 3 or 4 adverse event that are definitely, possibly, or probably related to BMX-001 treatment.	See Section 16.5.2
Secondary	To assess the efficacy of standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone in newly diagnosed HGG patients based upon progression-free survival (PFS).	Median PFS, where PFS is defined as the time between initiation of protocol treatment and the first recurrence of disease or death.	See Section 16.5.2
Secondary	To assess radiographic response in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone.	Proportion of subjects with complete or partial radiographic response.	See Section 16.5.2
Secondary	To characterize the repeated-dose pharmacokinetic profile of BMX-001 when delivered in combination with RT and TMZ in newly diagnosed HGG patients.	Plasma concentrations and calculated pharmacokinetic parameters (maximum concentration, time to reach maximum concentration, area under the curve, half-life, total body clearance, and volume of distribution) will be determined for BMX-001.	See Section 16.5.2
Exploratory	To describe patient-reported outcomes of HRQoL in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone.	Mean change from baseline at each follow-up assessment for subscales of the following PROs include Functional Assessment of Cancer Therapy-Brain Cancer (FACT-BR) version 4, Functional Assessment of Cancer Therapy-Fatigue (FACIT-Fatigue) subscale, version 4, Functional Assessment of Cancer Therapy-Cognition (FACT-Cog) subscale, Beck depression inventory (BDI-II), revised version, Godin Leisure questionnaire.	See Section 16.5.3

Exploratory	To describe changes in hair loss in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone.	Subjective investigator and patient assessment, photographs.	See Section 16.5.3
Exploratory	To describe change in white matter integrity in newly diagnosed HGG patients treated with standard RT and TMZ in combination with BMX-001 compared to standard RT and TMZ alone.	MRI Brain Diffusion Tensor /Susceptibility Imaging (white matter integrity).	See Section 16.5.3

9 INVESTIGATIONAL PLAN

9.1 Study Design

As this study is a Phase 1/2 study, this protocol includes a Phase 1 Dose Escalation portion and a randomized Phase 2 portion. In the Phase 1 Dose Escalation portion of the study, the dose of BMX-001 will be escalated, while the dose of daily TMZ and RT (chemoradiation phase) will remain fixed and will be followed by adjuvant TMZ (adjuvant phase). The randomized Phase 2 study component will assess the impact of BMX-001 in combination with daily TMZ and standard RT in comparison to daily TMZ and standard RT in newly diagnosed HGG patients on overall survival and, as secondary endpoints, impact on cognition and on bone marrow suppression.

A clinical research coordinator will schedule all of the appointments and the calendar will be provided to the patients. For study schema, please see Section 6 and Figure 1.

9.1.1 **Phase 1 Specific Design**

This Phase 1 study will be a single-site study of escalating doses of BMX-001 in combination with RT and TMZ (chemoradiation phase), followed by adjuvant TMZ (adjuvant phase). In the chemoradiation phase, external beam RT therapy will be given over less than seven weeks in 30-33 fractions for a total dose of 59-60 Gy. TMZ (75 mg/m^2 po daily) will be started on the first day of RT and will be administered for 42 days. TMZ will be given before RT daily. In line with Brain Tumor Center standard of care, adjuvant TMZ therapy will begin 2 to 4 weeks after the completion of RT. Adjuvant TMZ could be administered for up to 12 scheduled 28-day cycles. TMZ will be given orally at night on days 1-5 of 28-day cycle. During the chemoradiation phase and 2 weeks thereafter, CBC with differential and a CMP will be obtained weekly. A minimum of 3 subjects and a maximum of 18 subjects with newly diagnosed HGG who are patients at The Preston Robert Tisch Brain Tumor Center clinic at Duke University Medical Center will be enrolled into this study during a 12-month period. We will aim to enroll 3 subjects every eight to ten weeks. Once 3 patients have undergone treatment without experiencing a DLT after a monitoring period of four weeks following completion of all doses of BMX-001, the dose of the subsequent group of 3 patients will be escalated.

9.1.1.1 **Dose Escalation for BMX-001 in Phase 1**

BMX-001 will be administered subcutaneously as a loading dose before starting concurrent chemoradiation (days -5 through 0 depending on patient/radiotherapy scheduling). After the loading dose, the dose levels will be given two times per week for 8 weeks. The starting dose level will be 7 mg loading dose. BMX-001 dose escalation will be 7 mg loading dose, 14 mg loading dose, 28 mg loading dose, and 42 mg loading dose in four dose levels. After the loading dose, the BMX-001 dose will be administered at the amounts listed below twice a week (see Table 3 below). The corresponding volumes of BMX-001, Injection Solution 10 mg/mL associated with the loading doses are as follows (the equivalent dose for a 70 kg adult is also given):

0.7 mL/subject loading dose = 7 mg/subject and is equivalent to 0.1 mg/kg for 70 kg subject
1.4 mL/subject loading dose = 14 mg/subject and is equivalent to 0.2 mg/kg for 70 kg subject
2.8 mL/subject loading dose = 28 mg/subject and is equivalent to 0.4 mg/kg for 70 kg subject
4.2 mL/subject loading dose = 42 mg/subject and is equivalent to 0.6 mg/kg for 70 kg subject

The corresponding maintenance doses would be equivalent to the following:

0.35 mL/subject dose = 3.5 mg/subject and is equivalent to 0.05 mg/kg for 70 kg subject
0.7 mL/subject dose = 7 mg/subject and is equivalent to 0.1 mg/kg for 70 kg subject
1.4 mL/subject dose = 14 mg/subject and is equivalent to 0.2 mg/kg for 70 kg subject
2 mL/subject dose = 20 mg/subject and is equivalent to 0.3 mg/kg for 70 kg subject

BMX-001 will be administered by subcutaneous injection of a sterile 10 mg/mL solution in saline. Syringes will be either 1 mL or 3 mL with a 25 gauge X 5/8 inch needle depending on the volume of injection. The subcutaneous injections may be at any optimum site on the torso, the upper leg or upper arm. Sites of injection should be alternated. Subsequent (maintenance) doses will be one-half the size of the loading dose (except Group IV where the subsequent dose is rounded down to 20 mg) and should be administered twice weekly. The first dose should be delivered 3 to 5 days after the loading dose and further doses delivered at 3 to 4 day intervals to average 2 doses per week. The BMX-001 doses other than the loading dose will be delivered within 12 hours before or after RT. Schedule of subsequent doses will be dependent on the day of the loading dose and radiation schedule. For each week the doses are given, the days will either be Monday and Thursday for injections or Tuesday and Friday for injections. BMX-001 will not be administered on Saturday or Sunday.

Table 3: Loading Doses and Subsequent Doses for BMX-001 in Phase 1 Study

	Subject Group			
	I	**II**	**III**	**IV**
	Loading Dose[1]			
	7 mg	14 mg	28 mg	42 mg
Week	**Subsequent Doses[1]**			
1	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
2	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
3	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
4	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
5	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
6	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
7	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
8	3.5 mg b.i.w.	7 mg b.i.w.	14 mg b.i.w.	20 mg b.i.w.
[1]Dose of BMX-001 for Injection, 10 mg/mL administered to subject.				

9.1.1.2 **Definition of Dose-Limiting Toxicity (DLT)**

Toxicities will be graded according to the NCI CTCAE version 4.03 criteria. DLTs will be defined as any of the following events that are possibly, probably, or definitely attributable to BMX-001 during dose escalation.

A dose limiting toxicity is defined as an adverse event or laboratory abnormality that is: a. assessed as unrelated to disease progression, intercurrent illness, or concomitant medications; b. occurs during and/or following the first dose of BMX-001; and c. meets any of the following criteria:

9.1.1.2.1 Non-hematologic

1. The occurrence of non-hematologic grade 3 or greater adverse events considered to be possibly, probably, or definitely related to BMX-001 during treatment with BMX-001, excluding grade $\geq$ 3 alopecia and elevation in alkaline phosphatase, grade $\geq$ 3 nausea or vomiting unless occurring despite the use of standard anti-emetics; and grade 3 hypertriglyceridemia or hypercholesterolemia unless occurring despite standard lipid-lowering agents.
2. > 14 day delay to re-treat due to failure to resolve any grade 3 or greater non-hematologic drug-related toxicity to re-treatment criteria or pre-treatment baseline.
3. Grade ≥ 2 neurological toxicity that impairs activities of daily living and persists ≥ 7 days.
4. Inability to complete > 75% of the planned RT treatments.

9.1.1.2.2 Hematologic

Occurrence of any of the following hematologic adverse events will be defined as a DLT:

1. Grade 4 neutropenia (ANC, including bands, ≤0.5 x 109 /l);

2. Grade 3 thrombocytopenia (platelet count of ≤ 50x109/l);
3. > 14 day delay to re-treat due to failure to resolve hematologic toxicity to re-treatment criteria.

9.1.1.2.3 Other Considerations
Not applicable

9.1.1.3 **Dose Modification**

There are no reductions in the BMX-001. If adverse events occur that require holding BMX-001, the dose will remain the same once treatment resumes.

Any toxicity possibly, probably, or definitely associated with BMX-001 should be managed according to standard medical practice. There is no available antidote for BMX-001.

Subjects should be assessed clinically for toxicity prior to, during, and after each injection. If an unmanageable toxicity, i.e. in the opinion of the subject and treating physician, occurs because of BMX-001 at any time during the study, treatment with BMX-001 should be discontinued.

Injection of BMX-001 should be interrupted for subjects who develop dyspnea or clinically significant hypotension and it may be re-started at the following scheduled injection, if the treating physician considers it safe for the subject to proceed on study. Subjects who experience a Grade 3 or 4 allergic reaction / hypersensitivity, adult respiratory distress syndrome, or bronchospasm (regardless of grade) will be discontinued from BMX-001 treatment.

Regardless of the reason for holding study drug treatment, the maximum allowable length of treatment interruption is 3 weeks. If BMX-001 is held for greater than 3 weeks, BMX-001 will be discontinued.

9.1.2 **Phase 2 Specific Design**

The Phase 2 portion of this study will be a randomized, multi-center study comparing Arms A and B. Arm A will consist of daily TMZ and RT with BMX-001 and Arm B will include daily TMZ and RT alone.

For the Phase 2 Study, BMX-001 will be administered subcutaneously as a loading dose before starting concurrent chemoradiation (days -5 through 0 depending on patient/MRI scheduling), during treatment, and after treatment concludes. After the loading dose, the subsequent doses will be given two times per week for 8 weeks. The BMX-001 doses other than the loading dose will be delivered within 12 hours before or after RT. Patients will be randomized with a treatment arm allocation ratio of 1:1 to receive BMX-001 or to proceed with RT and TMZ alone. There will be 160 patients enrolled in this phase of the study (80 subjects per arm). The Phase 1 trial has confirmed the safe RP2D for BMX-001. The loading dose determined safe from Phase 1 and being studied in Phase 2 is 28 mg/subject and the maintenance doses are each 14 mg/subject.

BMX-001 will be administered by subcutaneous injection of a sterile 10 mg/mL solution in saline. The subcutaneous injections may be at any optimum site on the torso, the upper leg or upper arm. The first subsequent dose should be delivered 1 to 5 days after the loading dose and subsequent doses delivered at 3 to 4 day intervals to average 2 doses per week. The Phase 2 criteria for holding, re-treating and discontinuing BMX-001 is captured in Section 13.4 BMX-001 Treatment Criteria.

9.1.3 **Safety Considerations**

In Phase 1, no hypotension was found in the dose chosen for Phase 2. However, because of the development of transient hypotension in rat models, patients will undergo monitoring before and at 5 (+/-2), 15 (+/-3), 30 (+/-5) , and 60 minutes (+/- 5) minutes after the BMX-001 injections on the loading dose day and 5 (+/-2), 15 (+/-3), 30 (+/-5) on all other days of BMX-001 injections. This will

include the following vital signs: heart rate, oxygen saturation, and blood pressure. Injection site reaction will also be reviewed. In summary, patients will be observed for at least 60 (+/-5) minutes after the subcutaneous injection of BMX-001 on the loading dose day and at least 30 (+/- 5) minutes on the maintenance dose days. This observation will include collection of heart rate, oxygen saturation, blood pressure, as well as injection site reaction.

In primate studies, the primary toxicity seen was skin reaction/irritation and/or transient skin discoloration due to subcutaneous injection. Excretion of the drug was also associated with a red/purple color of urine. Care will be taken to limit the volume of the injections to no more than 1.4 mL per site. Thus a 28 mg/subject (2.8 mL @ 10 mg/mL) or 20 mg/subject (2 mL @ 10 mg/mL) dose will be administered in two equal portions at two different sites. A 42 mg/subject (4.2 mL @ 10 mg/mL) dose will be administered in three equal portions at three different sites. A separate drug product vial, syringe and needle will be used for each injection. Before patients are initiated on any treatments (radiation, TMZ, or BMX-001), patient must be at least 2 weeks from neurosurgical procedure (craniotomy, open biopsy, or stereotactic biopsy). Moreover, there can be no signs of wound-healing problems or infection at the craniotomy/biopsy site.

9.1.4 **Prolongation of QTc Interval and Management**

There have been reports of human subjects with a QT prolongation present after a BMX-001 loading dose (28 mg/subject) that was not present at baseline. Prolongation of QTc interval has not been observed in preclinical studies of BMX-001.

In all cases Electrocardiogram QT corrected interval prolonged did not exceed a grade 2 (average QTc 481 - 500 ms) according to CTCAE.

No action with regards to BMX-001 was taken due to these events and subjects continued on the study and the QT prolongation appears to have resolved. These cases have been reviewed in detail by BioMimetix.

If a subject develops prolonged QT after receiving a dose of BMX-001, the case should be reviewed with the BioMimetix Medical Monitor to determine further dosing of BMX-001 and inclusion in the study. Additionally, levels of electrolytes (potassium, calcium, and magnesium) should be checked and supplementation given to correct any values outside the normal range. Finally, concomitant therapies should be reviewed and adjusted as appropriate for medications with known QT-prolonging effects.

If the cause is not identified and the investigator believes it is appropriate, particularly if QTc remains elevated, study drug may be interrupted, and an ECG should be re-checked in approximately 1 week after the QTc prolongation was first observed or more frequently as clinically indicated. If QTc has recovered or improved and the investigator believes it is safe to do so, continuing BMX-001 therapy should be considered if previously held.

9.1.5 **Missed Doses**

Regardless of the reason for withholding study drug treatment, the maximum allowable length of treatment interruption is 3 weeks. If BMX-001 is withheld for greater than 3 weeks, BMX-001 will be discontinued. If BMX-001 is discontinued for missed doses, the patient will remain on the study and all planned study endpoints will be assessed. Any missed doses should be made up at the end of treatment, so that the subjects complete the planned number of doses (which is 17). Any missed doses of BMX-001 should be documented with the reason included.

9.1.6 **Concomitant Medications**

Because corticosteroids are anti-inflammatory and could interrupt oxidative stress, patients will be required to be on stable or decreasing corticosteroids dose at the time of the study enrollment. If a patient is required to have additional systemic steroids such as to treat an allergic reaction, adrenal insufficiency, increased neurological symptoms, and/or increased intracranial pressure, then systemic corticosteroids can be added to control symptoms at the discretion of the treating physician and at the minimal effective dose deemed medically appropriate. Dexamethasone use is allowed in this study. Topical steroids are not a contraindication.

Standard anti-emetics, such as ondansetron, will be able to be used during TMZ therapy. Ondansetron is typically administered 30 minutes before TMZ.

Use of drugs known to lengthen QT intervals should be avoided or minimized when possible.

9.1.7 **TMZ Treatment Plan**

TMZ is an oral alkylating agent which has demonstrated anti-tumor activity as a single agent in the treatment of recurrent glioma. As stated previously, Stupp et al.[65] have demonstrated an increase in efficacy of TMZ in combination with RT in the adjuvant treatment of primary malignant gliomas. In addition, the regimen was considered safe. Non-hematological grade 2 toxicities included: Fatigue (26%), other constitutional symptoms (7%), rash and dermatologic side effects (9%), infection (1%), vision (14%), and nausea and vomiting (13%). Grade 3 / 4 non-hematological toxicities occurred <10%: Fatigue (7%), other constitutional symptoms (2%), rash and dermatologic side effects (1%), infection (3%), vision (1%), and nausea and vomiting with a $5HT_3$-RA (<1%). Thus, this safe and standard regimen will be utilized in this protocol. During the chemoradiation phase, daily TMZ therapy will be calculated at a dose of 75 mg per square meter of body surface area per day and be administered 7 days a week from the first until the last day of RT therapy or a total of 42 days. Weekly CBC with differential and CMP will begin at week 2 and continue through week 8. Platelet count is part of CBC with differential count. Additional clinical and laboratory assessments will be at the discretion of the RT therapist. During the adjuvant phase, 5-day TMZ therapy will be calculated at a dose of 150 or 200 mg/m^2 and be administered the first 5 days of a 28 day cycle. One cycle will consist of 28 days. If a subject cannot tolerate the 5-day TMZ and is switched to metronomic TMZ (low-dose, continuous TMZ), they will remain on the study and will complete all planned study endpoints. Use of Optune device will be allowed during the adjuvant phase with TMZ per NCCN guidelines and subjects will complete all planned study endpoints. If a subject completes only 6 cycles of temozolomide (vs. 12 cycles), they will remain in the study and subjects will complete all planned study endpoints through the planned 12 cycle timeframe. After that they will be continued to be followed for vital status.

9.1.8 **Radiation Treatment Plan**

As detailed by the Stupp [65] regimen, RT therapy will be administered as fractionated focal irradiation in daily fractions of 1.8-2 Gy given 5 days a week for 6 weeks for a total of 59-60 Gy. Patients will be followed closely by the RT therapist throughout treatment. The RT therapist and oncologist will conduct the standard physical exams and obtain the lab work necessary for the eligibility. Additional clinical and laboratory assessments will be at the discretion of the RT therapist and oncologist. All RT will be planned and performed at the clinical center as part of standard of care.

9.1.8.1 **Dose Definition and Schedule**

The external beam RT therapy (RT) plan will be determined at the discretion of the local RT therapist dependent on tumor size and location. It should begin 2-12 weeks after surgery. One treatment of 1.8-2.0 Gy will be given daily, 5 days per week, (30-33 fractions over less than seven weeks) for a total

of 59-60 Gy. All portals shall be treated during each treatment session. Doses are prescribed at the maximum dose line encompassing ≥ 95% of the target volume.

9.1.8.2 **Physical Factors**

Treatment shall be delivered on megavoltage machines of energy ranging from 4 to 18 MV photons. Selection of the appropriate photon energy(ies) should be based on optimizing the RT dose distribution within the target volume and minimizing dose to non-target normal tissue. Photon energies > 10 MV should be utilized only in dual energy beam arrangements using at least one beam with energy < 10 MV. Source skin distance for SSD techniques or source axis distance for SAD techniques must be at least 80 cm.

9.1.8.3 **Localization, Simulation, and Immobilization**

The patient shall be treated in the supine or another appropriate position for the location of the lesion. A head-holding device that is transparent to x-rays and provides adequate immobilization must be utilized at all times during planning and therapy to ensure reproducibility.

The initial target volume shall include the contrast-enhancing lesion and surrounding edema (if it exists) demonstrated on the pre-operative MRI plus a 2.0-cm margin. If no surrounding edema is present, the initial target volume should include the contrast-enhancing lesion plus a 2.5-cm margin. This primary target volume will be treated to 45-46 Gy in 23-25 daily fractions, at 1.8-2.0 Gy per fraction.

The boost volume will be based on the post-operative MRI performed during treatment planning. After 45-46 Gy, the boost volume will include the contrast-enhancing lesion plus a 1.5-cm margin or, if minimal contrast-enhancing lesion is present at a portion of the resection cavity on MRI, the surgical defect plus a 2.0-cm margin, whichever is greater at that segment of the MRI image. The boost volume will be treated to an additional 14-14.4 Gy in 7-8 daily fractions, 1.8-2.0 Gy per fraction. This will bring the total target dose to 59-60 Gy in 30-33 fractions. All parts of the target volumes are to receive at least 100% but no more than 110% of the dose at the prescription isodose line.

All RT will be planned and performed by the clinical center as part of standard of care.

9.1.8.4 **Treatment Planning and Safety**

Treatment plans may include opposed lateral fields, a wedge pair of fields, rotation, or multiple-field techniques, including intensity-modulated radiotherapy (IMRT) and volumetric-modulated arc therapy (VMAT). MRI-guided 3D treatment planning is necessary to assure accuracy in the selection of field arrangements. Isodose distributions for the primary and boost target volume are required on all patients, including those treated with parallel-opposed fields. A composite plan is required showing the respective target volumes. The inhomogeneity across the target volume shall be kept to a minimum. The maximum dose should be no higher than 112% of the prescription. Possible side effects include swelling of the brain, hair loss, localized skin irritation, low blood counts, fatigue, memory loss, hearing loss, nausea and/or vomiting, loss of appetite, headaches, RT necrosis (death of tissue or skin), and secondary cancer.

9.1.8.5 **Dose Limitations to Critical Structures**

The lens and cervical spine must be shielded from the direct beam at all times. The maximum dose to the optic apparatus (optic chiasm, optic nerves, eyes) must be limited to 54 Gy, and the brain stem limited to 60 Gy.

9.1.9 **Randomization**

Within the Phase 2 portion of this study, patients will be randomized using a 1:1 allocation ratio to receive standard of care treatment consisting of TMZ and RT with BMX-001 (Arm A) or without BMX-

001 (Arm B). A permuted block randomization algorithm stratified by grade (III / IV) and by institution will be used.

9.2 Rationale for Selection of Dose and Regimen

We have additional safety data from the completion of this Phase 1 trial in humans. In Phase 1, the DLT for BMX-001 administered at 42 mg loading dose and 20 mg maintenance dose was sinus tachycardia (grade 3) in one subject. The only other toxicity related grade $\geq$ 3 event seen was hypotension (grade 3) in one patient. The most common related toxicity was grade 1 injection site reaction in 7 patients. The volume of subcutaneous injection will be limited to 1.4 mL per site. Thus a 28 mg/subject (2.8 mL @ 10 mg/mL) dose will be administered in two equal portions at two different sites. A separate drug product vial, syringe and needle will be used for each injection.

9.3 Rationale for Correlative Studies

Not applicable.

9.4 Definition of Evaluable Subjects

Within the Phase 1 portion of this protocol, a patient who terminates protocol treatment before completion of the standard RT and TMZ without experiencing DLT will not be considered evaluable for the purpose of determining the MTD.

Within the Phase 2 portion of this protocol, all patients who are randomized will be included in primary analyses for the primary objective of survival regardless of treatment compliance. An intent-to treat approach will be used in statistical analyses.

9.5 Data collection for subjects unable to come to clinical center

In the event that subjects are unable to visit the clinical center we request that the clinical center staff either mail, email, or conduct the following HrQoL assessments by phone:

-FACT-BR
-FACT-Cog
-FACIT-Fatigue
-Beck Depression Inventory
-Godin Leisure Questionnaire

Mailed questionnaire will be sent with a cover letter and will be pre-labeled with the Subject's Study ID Number. Subjects will be asked not to include their names or return addresses on the package in order to limit the PHI sent by mail.

9.6 Early Study Termination

This study can be terminated at any time for any reason by the sponsor. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 13.7.1, which describes procedures and processes for prematurely withdrawn patients and in accordance with Section 13.7.2.

10 STUDY DRUG (BMX-001)

10.1 Names, Classification, and Mechanism of Action

BMX-001, also known as MnTnBuOE-2-PyP^{5+} (manganese butoxyethyl pyridyl porphyrin), is a metalloporphyrin antioxidant that has anti-inflammatory, anti-oxidant and anti-tumor functions. A series of metalloporphyrins have undergone preclinical evaluation and BMX-001 represents one with increased catalytic potency along with high lipophilicity. This class of metalloporphyrins leads to inactivation of reactive oxygen species and in turn leads to reduction of oxidative stress. The lipophilicity of BMX-001 leads to its accumulation in the brain parenchyma and it has been shown to cross the blood-brain barrier. Because the vasculature of most mammalian species is highly sensitive to an oxidative shift, there is a concern for blood pressure monitoring in studies of the metalloporphyrin compounds. Studies in Sprague-Dawley rats showed that intravenous delivery of metalloporphyrin could lead to transient hypotension [2]. In this same study, mice, guinea pigs, dogs, and baboons receive intravenous delivery of metalloporphyrins with less effect on blood pressure. BMX-001 was patented by Duke University and has been licensed to BioMimetix JV, LLC. Molecular structure is depicted in Figure 4.

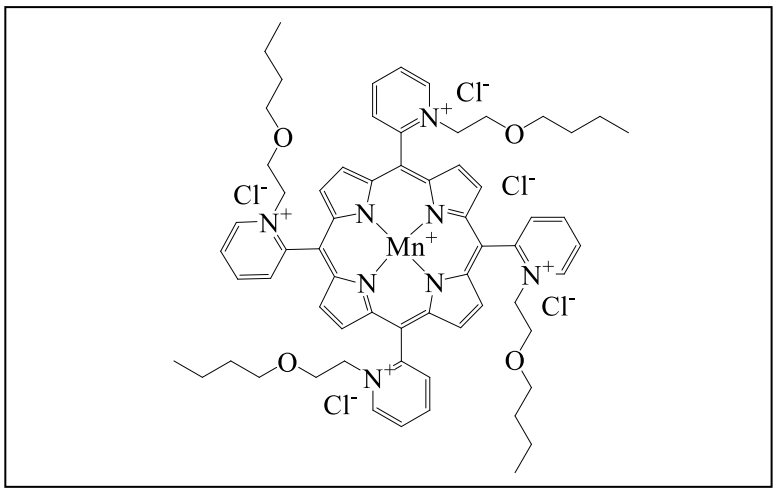


Figure 4: BMX-001 Structure

10.2 Packaging and Labeling

10.2.1 Drug Product Formulation and Manufacture

The drug will be supplied by BioMimetix JV, LLC. The drug substance (1.2 kg of solid) was manufactured and stored according to cGMP by Albany Molecular Research, Inc. (AMRI).

BioMimetix has contracted with AMRI (Glasgow) Ltd. to manufacture single dose vials of sterile BMX-001 for injection. Manufacturing will be conducted on AMRI's Flexicon FPC50W filling machine, which is dedicated for aseptic GMP manufacturing within a clean room suite. Manufacturing will include batch pre-filtration and aseptic filtration and filling in accordance with AMRI Glasgow's validated process.

BMX-001 will be formulated as a solution in 0.9% saline at 10 mg/mL, aseptically filtered and filled to 2 mL in a 5 mL Type 1 clear glass vial, sealed with a 20 mm Flurotec serum stopper and flip off aluminum overseal. A nitrogen overlay will be applied to each vial following filling and prior to stoppering.

In-process testing will include pre-filtration bioburden, post-use filter integrity test, and in-process weight checking. Release tests and specifications for BMX-001 for injection are currently under development, but will be established before manufacturing of the clinical batch commences. These will include sterility (USP <71>), bacterial endotoxins (USP <85>), particulate matter (USP <788>), appearance, color and clarity, pH, osmolality, assay and purity.

The clinical batch of drug product will be placed on stability and tested for the duration of its use during the clinical trials.

10.2.2 **Labels for BMX-001 for Injection, 10 mg/mL**

An exemplary label for the BMX-001 for Injection, 10 mg/mL is reproduced in Figure 5.

BMX-001 for Injection, 10 mg/mL

Lot # P07715

Administer SQ, ≤1.4 mL/site.

Caution: New Drug – Limited by Federal Law to Investigational Use

Emergency contact: Dr. Shayne Gad, cell 919-618-0523

Store at Room Temperature

Figure 5: Exemplary Label for BMX-001 for Injection, 10 mg/mL

10.2.3 **Syringe and Label for Administration of BMX-001 for Injection, 10 mg/mL**

A pharmacist will prepare the loaded syringe(s) and needle(s) that will be used for subcutaneous administration of the appropriate dose of BMX-001 for Injection, 10 mg/mL to the clinical subject. In Phase 2, the loading dose is 28 mg/subject and the maintenance doses are 14 mg/subject biw. The drug product is shipped to the clinical centers as a sterile solution of BMX-001 in saline packaged in single use vials. The pharmacist at the clinical center needs to draw the appropriate volume into a syringe.

The syringe should be stored at room temperature until use, which will occur within 4 hours of preparation. Appropriate procedures for drug accountability must be followed.

No more than 1.4 mL is to be administered at a single injection site. Thus a 28 mg or 20 mg dose will be administered in two equal portions (14 mg or 10 mg, respectively), taken from different vials and injected via separate syringes and needles at two different sites. A 42 mg dose will be administered in three 14 mg portions taken from three different vials and injected via separate syringes and needles at three different sites.

10.2.4 **Multi-Dose Kits**

Multi-dose kits containing vials labeled as described in Section 10.2.2 are packaged in paperboard cartons. The carton label includes the information indicated in Figure 6, where "XXX" is replaced by the number of vials contained in the carton.

Lot # P07715 Store at Room Temperature

BMX-001 for Injection, 10 mg/mL Multi-Dose Kit

Contents: XXX Single-Dose Units @ 20 mg each

Caution: New Drug – Limited by Federal Law to Investigational Use

Emergency contact: Dr. Shayne Gad, cell 919-618-0523

Figure 6: Exemplary Label for Multi-Dose Kits

10.3 Supply, Receipt, and Storage

The multi-dose kits described in Section 10.2.4 will be shipped by overnight delivery to the clinical center pharmacy. These kits will be stored at room temperature per the Certificate of Analysis provided in the BMX-001 Investigator Brochure, in a locked, physically secure environment, according to the pharmacy's standard procedures for storage of investigational drug products. Drug accountability records will be maintained by the clinical center for all clinical trial supplies.

10.4 Compliance and Accountability

Drug accountability records will be maintained for all clinical trial supplies.

10.5 Disposal and Destruction

All empty and partially used clinical trial supplies will be destroyed in accordance with the institution’s requirements for an investigational agent. Unused supplies must not be destroyed until after drug accountability is performed by the sponsor and instructions are provided regarding destruction or return. Disposition of all unused boxes of study drug will be carried out according to instructions provided by BioMimetix JV, LLC, at the end of the study after drug accountability is performed. The pharmacy will maintain detailed documentation of the number and identification of BMX-001 vials which are destroyed, and copies of these documents will be provided to BioMimetix JV, LLC.

11 STUDY DRUG (TMZ)

11.1 Description

8-Carbamoyl-3-methylimidazol[5,1-d]-1,2,3,5-tetrazine-4-(3H)-one, CCRG 81045, M&B 39831)-Temozolomide is commercially available and not provided in this study.

11.2 Formulation

TMZ is available in 250 mg, 180 mg, 140 mg, 100 mg, 20 mg, and 5 mg strengths. Refer to package insert for contents of the formulation.

11.3 Storage

TMZ capsules should be stored between 2^{o}C to 30^{o}C. Commercial supply and packaging will be used.

11.4 Administration

TMZ is administered orally on an empty stomach. The drug is approximately 100% bioavailable. The dose will be rounded to the nearest 5 mg when possible. Final dose will be determined by the subject's primary neuro-oncologist and the following can be taken into account: number of pills per day and number of co-payments required. Effects of food on product absorption are not yet known. **DO NOT OPEN CAPSULES. DO NOT MIX WITH FOOD. DO NOT CHEW CAPSULES.**

TMZ at a dose of 75mg/m^2 po daily will be started with external beam RT (+/- 5 days). TMZ will be held according to standard of care for the institution (typically when platelets drop below 100,000 cells/uL), grade 4 neutropenia, or grade 4 non-hematologic toxicity secondary to TMZ. TMZ will be re-started at 50 mg/m^2 after resolution of the toxicity, or $\leq$ grade 1. If the grade 3 thrombocytopenia, grade 4 neutropenia, or grade 4 non-hematologic toxicity secondary to TMZ recurs at 50 mg/m^2, TMZ will be held during RT therapy, but may resume after RT therapy if the patient meets the treatment criteria.

11.5 Toxicities

TMZ has been well tolerated by both adults and children with the most common toxicity being mild myelosuppression. Other, less likely, potential toxicities include nausea and vomiting, constipation, headache, alopecia, rash, burning sensation of skin, esophagitis, pain, diarrhea, lethargy, and hepatotoxicity. Hypersensitivity reactions have not yet been noted with TMZ. As is the case with many anti-cancer drugs, TMZ may be carcinogenic. Rats given TMZ have developed breast cancer. The significance of this finding for humans is not presently known.

11.6 Packaging and labeling

TMZ is manufactured by multiple sources and commercial drug will be used is in this study.

12 SUBJECT ELIGIBILITY

Please see Section 5.5 for a complete list of inclusion and exclusion criteria.

13 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Table 4: Screening and On-Study Tests and Procedures

On-Study Tests and Procedures											
			Week 1 Arm A only [r]	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7** [q]	**Week 8** [q]	**Weeks 9 and Forward**
Evaluation	Screening[a]	Day -5 to 0 [p]									Adjuvant Phase[b, g, v]
Informed consent and Demographics [w]	X										
Randomization [o]	X										
History and physical exam, including full neurologic exam & KPS [c]	X	X		X		X		X		X	X[b]
Vital signs, including blood pressure [m]	X	X[l]	X[l]	X	X[l]	X	X[l]	X	X[l]	X	X[b]
CBC with differential [d]	X			X	X	X	X	X	X	X	X[b]
CMP	X			X	X	X	X	X	X	X	X[b]
PT, aPTT	X										
Beta-HCG, if applicable, within 48 hours prior to start of study treatment	X										
ECG	X	X[e]	X[e]			X[e]					
PK sampling serum [f]		X		X			X				
MRI of the brain	X										X[b]
MRI (DTI/ Susceptibility Imaging) in Phase 2 only		X									X[h]
BAC App Cognitive Testing [s]	X (baseline)										X[b, s]
Additional Cognitive Testing[g, t]	X (baseline)										X[b]
HRQoL Measurements[g]	X (baseline)										X[b]
Hair Loss Evaluation[u]	X (baseline)										X[b]
Toxicity Assessment	X	Ongoing									X[l]
Radiation			Continuous Dosing Monday-Friday for 6 weeks								
TMZ			Continuous Dosing at 75 mg/m^2 for 42 days								Will start adjuvant[b]
BMX-001		X[j]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	X[k]	

[a] Within 3 weeks of starting BMX-001.

[b] Will transition to 5-day TMZ dosed at 150-200 mg/m^2 po daily for the 1st 5 days in 28-day cycle (Adjuvant Phase). Study assessments for HRQoL Measurements, and Hair Loss Evaluation will be obtained approximately every 8 weeks (+/- 2 weeks) when a subject returns for a Standard of Care visit, until tumor progression, death, or patient comes off of study. Cognitive testing will also be completed every 8 weeks and at SOC visits; Cognitive testing will continue after tumor progression. CBC with differential will be obtained approximately on Days 21 or 28 of each 28-day cycle, and CMP will be obtained approximately on Day 21 of each 28-day cycle, or per the ordering physicians Standard of care practice, during the adjuvant phase, until the subject comes off the study. If subject is transitioned to metronomic temozolomide because of poor tolerance to 5-day temozolomide, then they will continue to be on study. Moreover, if Optune is used during the adjuvant phase, then they will continue to be on study. Adjuvant temozolomide can start up to 4 weeks after radiation therapy has completed.

[c] Starting at Loading dose day or Day -5 to 0, this will occur once every other week during weeks 1-8. This may be modified to meet standard of care scheduling for patient visits.

[d] Weekly CBCs and CMPs will begin at week 2 and continue through week 8. Platelet count is part of CBC with differential count.
[e] ECG will be done at screening for all subjects. For subjects in Arm A, ECG will also be obtained before the loading dose is administered (within 5 to 60 minutes before the dose), and at 60 (+/- 15 min) minutes after the loading dose. Pre-dose (within 5 to 60 minutes before the dose), and Post-dose (60 +/- 15 min) ECGs will also be collected at the Week 1 first maintenance dose and on either one of the BMX-001 injection days in Week 4.
[f] See Appendix B for timing of PK sampling, Day 8 and Day 36 only. Phase 1- all subjects, Phase 2- 6 subjects at Duke.
[g] HRQoL measurements will be completed during Screening as a baseline and then approximately every 8 weeks when a subject returns for a Standard of Care visit during the Adjuvant Phase. These assessments should be completed prior to other study-related evaluations at study visits, if at all possible.
[h] DTI/Susceptibility Imaging is for clinical centers selected by the sponsor and completed at baseline, during the Adjuvant Phase before the start of the first cycle of adjuvant temozolomide, as well as 6 months (approximately 24 weeks, +/- 2 weeks) after radiotherapy/chemotherapy
[i] Toxicity assessment will continue to be obtained at every SOC clinic visit during the Adjuvant Phase for a minimum of 30 days after last BMX-001 treatment for subjects receiving BMX-001 or last chemo/RT for subjects not receiving BMX-001, and also followed for any unresolved adverse events considered related to study therapy.
[j] For BMX-001, this dose will be the loading dose. In Phase 2- Applicable only for patients in Arm A.
[k] For BMX-001, doses (50% of the loading dose) will be twice a week subcutaneously weeks 1-8. There will only be two doses per week regardless of being a loading or maintenance dose. In Phase 2- Applicable only for patients in Arm A.
[l] For patients in Phase 2 Arm B (standard RT + TMZ without BMX-001), vital sign evaluation will only be required on days of the History and Physical assessment.
[m] Arm A: Patients will be monitored at drug administration with vital signs and injection site reaction at pre dose, at 5 (+/- 2), 15 (+/-3), 30 (+/-5), and 60 (+/- 5) minutes post administration on the BMX-001 loading dose. Patients will be monitored at drug administration with vital signs and injection site reaction at pre dose, at 5 (+/-2), 15 (+/-3) and 30 (+/-5) minutes post administration on BMX-001 maintenance injection days. See Section 0.
[o] Phase 2 only, subjects will be randomized after all eligibility criteria is confirmed.
[p] For Phase 2: For subjects in Arm A, this column is the day of the BMX-001 Loading Dose. For Subjects in Arm B, these items should occur -5 to 0 days prior to the start of RT. The procedures for this visit should not take place until final eligibility is confirmed and randomization has occurred.
[q] In Phase 2, subjects randomized to Arm B will NOT have a Week 7 or Week 8 research study visit.
[r] In Phase 2, subjects randomized to Arm B do not have a Week 1 study visit, although they will be in the clinic for the start of RT and TMZ.
[s] These assessments should be completed prior to other study-related evaluations at study visits, if at all possible. CNS Vital Signs®(Phase 1) /NeuroCog BAC App (Phase 2) will be done screening (baseline), and then approximately every 8 weeks when a subject returns for a Standard of Care visit during the Adjuvant Phase.
[t.] Hopkins Verbal Learning Test-Revised (Forms 1, 2, and 3), Trails Making Test Parts A and B, and Controlled Word Association Test will be performed only at baseline, the standard of care visit before the start of the first cycle of adjuvant temozolomide, and the standard of care visit that is 6 and 12 months from the completion of radiotherapy/chemotherapy. See appendices for appropriate versions of these forms to use.
[u] Hair loss evaluation includes subjective description and photos.
[v] Patients will continue to complete visits in the adjuvant cycle for up to the 12 planned cycles, as long as they do not have progression on MRI (even if they stop taking adjuvant TMZ). Cognitive testing will be completed even after progression is noted.
[w] Collection of Gender, Race, Ethnicity, Education completed, employment, and smoking status to be obtained at screening.

13.1 Screening Examination

The screening examination will take place between Day -21 and -1. An informed consent must be signed by the patient before any study specific screening procedures take place. Information collected prior to the signing of informed consent according to standard of care for procedures such as KPS, labs, history and physical, and MRI, etc. may be used to determine eligibility. Subject data to be collected at the Screening Examination is listed in the table above and includes physical and neurological examination; vital signs and weight; ECG, laboratory studies (complete blood count with differential, complete metabolic panel, PT/aPTT, and beta-HCG); past medical history; and concomitant medications (including but not limited to steroids and anticonvulsants). Additional

baseline testing will be completed for the following: cognitive testing, HRQoL Measurements, and hair loss evaluation.

The screening MRI or CT scan will be uploaded to the Duke central core for final review of eligibility. This will be required for all subjects. The clinical centers will be notified of the final determination of eligibility prior to the subject completing any study visit.

The pathology report used to determine eligibility, which includes any tumor markers will be redacted and uploaded to the sponsor electronic data capture system.

If a subject is found to be ineligible to participate in the study, the patient will not be randomized to a treatment arm and minimal records regarding the subject and the reason for screen failure will be retained.

13.2 Run-In Period

Not applicable.

13.3 Chemoradiation Treatment Period (with and without BMX-001)

The treatment period for BMX-001 is eight weeks. This consists of treatment prior to start of chemotherapy- RT, during the six weeks of concurrent daily TMZ and standard RT with BMX-001 (Phase 1 and Phase 2 (Arm A)) followed by 2 weeks of BMX-001 (Phase 1 and Phase 2 (Arm A)). For subjects in Arm B, this period is six weeks of concurrent daily TMZ with standard RT followed by 2 weeks of no treatment. For subjects in Arm B, they do not need to return to the clinical center for a study visit once TMZ and RT is complete for visits at Week 7 and Week 8.

13.4 BMX-001 Treatment Criteria

If adverse events occur that require holding BMX-001, the dose will remain the same once treatment resumes. Missed doses should be made up at the end of treatment to achieve the total number of planned doses (17).

13.4.1 Phase 2 - Criteria for holding BMX-001

Non-Hematologic Criteria

1. The occurrence of non-hematologic grade 3 or greater adverse events considered to be possibly, probably, or definitely related to BMX-001 during treatment with BMX-001, excluding grade ≥ 3 alopecia and elevation in alkaline phosphatase, grade ≥ 3 nausea or vomiting unless occurring despite the use of standard anti-emetics; and grade ≥ 3 hypertriglyceridemia or hypercholesterolemia unless occurring despite standard lipid lowering agents.
2. Intolerable ≥ Grade 2 Injection Site Reactions
3. ≥ Grade 2 electrocardiogram prolonged QTc interval

Hematologic Criteria

1. Grade 4 neutropenia (ANC, including bands, ≤ 500 cells /µl);
2. Grade 3 thrombocytopenia (platelet count of ≤ 50,000 cells/µl);

13.4.2 Phase 2- Re-treatment Criteria for BMX-001

Non-Hematologic

1. Resolution of any grade 3 or greater toxicity felt to be possibly, probably or definitely attributable to BMX-001 to grade 1 or pre-treatment baseline.
2. Resolution of Intolerable ≥ Grade 2 Injection Site Reactions to ≤ Grade 1
3. Resolution of Grade 2 electrocardiogram prolonged QTc interval to baseline, or after review by sponsor.

Hematologic

1. ANC ≥ 1,000 cells/µl
2. Platelets ≥ 50,000 cells/µl

13.4.3 Phase 2- Discontinuation Criteria for BMX-001

Inability to resolve any grade 3 or greater toxicity felt to be possibly, probably or definitely attributable to BMX-001 to grade 1 or pre-treatment baseline within 21 days.

For both non-hematologic and hematologic events, if any event requires holding BMX-001 > 21 days without retreatment, BMX-001 would not be started again. In these cases, subjects should continue to be followed for survival without the completion of study treatment visits.

13.5 End of Study

The end of study for a subject will occur when the subject comes off study for the following reasons: completion of all study tests/procedures, progressive disease, unacceptable toxicity, allergic reaction to TMZ, allergic reactions to BMX-001, non-compliance with study follow-up, or withdrawal of consent. Patient death due to progressive disease will not be reported as a SAE. It is the intent that all patients will be followed as long as possible for survival. See Section 13.7.2 for follow-up requirements after the patient comes off study.

13.6 Overall Study Completion

The study will be considered complete once enrollment has been met, follow-up procedures on all subjects have been conducted, and data analysis is concluded. The study may also be terminated early for any reason by the sponsor. In order to terminate the study with the external IRB, all data extraction and analysis must be complete. Therefore, if any articles for publication are derived from the current study, they must be submitted and accepted with no further need for additional data review prior to termination with the IRB.

Subjects that are lost to follow-up will be documented in the patient record and in the database.

13.7 Withdrawal of Subject(s)

13.7.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time. The PI may also withdraw a subject from the study at any time based on his/her discretion. Reasons for PI-initiated withdrawal may include, but is not limited to the following:

- Adverse events
- Abnormal laboratory values
- Abnormal test procedure results
- Protocol deviation
- Administrative issues
- Disease progression- Patients will still complete cognitive testing (HVLT-R, COWA, TMT, BAC App) after coming off for disease progression. All other assessments will stop at the point of

progression. If a patient came off study for disease progression prior to this protocol amendment, and is still having study visits or is within 3 months from their 12 month visit, they should be contacted and asked to complete the cognitive testing at their next visit.

- Pregnancy

13.7.2 Follow-up Requirements for Early Withdrawal

Subjects who are withdrawn due to toxicity or progressive disease will be followed for as long as they are receiving treatment for their HGG at the clinical center. Study visits will cease, however data collection for survival should continue at a minimum of three month intervals. For subjects who voluntarily withdraw, there is not an end of study visit, unless deemed appropriate by the PI. Early withdrawal subjects will be followed for survival where possible, except for subjects that specifically request not to have any further follow up.

13.8 Study Assessments

13.8.1 Medical History

Standard medical history assessments will be conducted and recorded per institutional and sponsor guidelines.

13.8.2 Physical and Neurological Examinations

Physical and neurologic examinations, vital signs (temperature, respiratory rate, blood pressure and pulse), height (screening only), weight and KPS must be performed at:

- screening
- day of loading dose (Arm A in Phase 2)
- two weeks after the completion of RT and TMZ
- every standard of care (SOC) clinic visit (approximately every 8 weeks) during adjuvant TMZ
- at the time of study completion

Additional physical and neurological examination may be performed every two weeks during standard RT and TMZ per the standard of care practice. More frequent examinations may be performed at the investigator's discretion, if medically indicated.

a. Physical examination, vital signs, weight and height
A complete Physical Examination (PE) will include a general physical examination of major body systems, temperature, respiratory rate, blood pressure, heart rate, body weight and height.

Height will be measured and documented at screening only.

Blood pressure should be measured per institutional guidelines.

b. Neurologic examination
A complete neurologic examination will include assessment of level of consciousness, mental status, speech, cranial nerves, motor, sensory, coordination, reflexes, and gait. Follow-up evaluation of the neurologic examination will be based on any changes in the neurologic clinical examination from the previous exam. Changes should be unrelated to postictal state or other unrelated events such as infection.

13.8.3 Electrocardiogram (ECG)

ECG will be done for screening purposes for all subjects. For subjects in Arm A, a baseline ECG prior to BMX-001 administration will be completed and at 60 (±15) minutes after dosing on the loading day of BMX-001 and on the first BMX-001 maintenance dose administration day during Week 1 and any

dose in Week 4 pre-dose and at 60 (±15) minutes after dosing. Results will be filed in the source for each subject and redacted tracings will be uploaded to the sponsor electronic database.

13.8.4 **Radiographic Response**

The guidelines and criteria for radiographic response will be based on the RANO criteria. MRI brain with and without contrast will be obtained at baseline after standard RT and TMZ (within 2 to 4 weeks) and every standard of care (SOC) clinic visit (approximately every 8 weeks) during adjuvant TMZ. Institutional standard operating procedure will be followed (see Protocol Appendix A, Section 19.1). The clinical centers will be provided with a Clinical Trial Radiographic Assessment Form (CTRAF) to aid them with the data collection required for the study. A different form may be used with approval from the sponsor. RANO criteria will be utilizing based on the modifications from Ellingson et al. [66]. In these criteria, particularly in regards to newly diagnosed subjects, the areas considered in determining comprehensive objective status in RANO criteria are target lesions (enhancing disease), new sites of measurable disease, neurological examination status, and corticosteroid usage and dose.

Complete Response (CR): Requires all of the following:

1. Disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks. The first scan exhibiting disappearance of all enhancing measurable and non-measurable disease is considered “preliminary CR”. If the second scan exhibits measurable enhancing disease with respect to the “preliminary CR” scan, then the response is not sustained, noted as “pseudoresponse”, PsR, and is now considered “preliminary PD” (note confirmed PD requires at least two sequential increases in tumor volume). If the second scan continues to exhibit disappearance of enhancing disease or emergence of non-measurable disease (less than 10mm bidimensional product), it is considered a durable CR and the patient should continue on trial until confirmed PD is observed.
2. Patients should be either off of corticosteroids or on physiologic replacement doses only.
3. Stable or improved neurological examinations.

Partial Response (PR): Requires all of the following

1. Greater than or equal to a 50% reduction in the size (products of the largest perpendicular diameters) for all enhancing lesions compared with baseline, sustained for at least 4 weeks. The first scan exhibiting greater than or equal to a 50% decrease in the sum of the products of the perpendicular diameters of all measurable enhancing lesions compared with the baseline is considered “preliminary PR”. If the second scan exhibits PD with respect to the “preliminary PR” scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered “preliminary PD” (note confirmed PD requires at least two sequential increases in tumor volume.) If the second scan exhibits SD, PR, or CR, it is considered a durable PR and the patient should continue on trial until confirmed PD is observed.
2. Patients should have either the same corticosteroid doses or on lower corticosteroid dose compared with baseline scan.
3. Stable or improved neurological examinations

Stable Disease: Evaluations that do not meet criteria for CR, PR or PD.

Progressive Disease: Defined as the following

1. Greater than or equal to a 25% increase in the product of the largest perpendicular diameters of any enhancing lesion or any new enhancing tumor on MRI scans. The first scan exhibiting greater than or equal to a 25% increase in the sum of the products of perpendicular diameter of the enhancing lesions should be compared to the smallest tumor measurement obtained either at baseline (if no decrease) or best response (on stable or increasing corticosteroid dose) and is

noted as “preliminary PD”. If the second scan at least 4 weeks later exhibits a subseqeuent greater than or equal to 25% increase in the sum of products of perpendicular diameters of the enhancing lesions relative to the “preliminary PD” scan, it is considered “confirmed PD” and the patient should be discontinued from the study. If the second scan at least 4 weeks later exhibits SD or, PR/CR, this scan showing “preliminary PD” is noted as “pseudoprogression”, PsP, and the subject should remain on study until a second increase in tumor size relative to the PsP scan is observed. Of note, any new measurable (> 10 mm X 10 mm) enhancing lesion should not be immediately considered PD, but instead should be added to the sum of the bidimensional products representing the entire enhancing tumor burden.

2. In the case where the baseline or best response demonstrates no measurable enhancing disease (visible or not visible), then any new measurable (>10mm x 10mm) enhancing lesions are considered PD after confirmed by a subsequent scan ≥4 weeks exhibiting greater than or equal to a 25% increase in sum of products of perpendicular diameters relative to the scan first illustrating new measurable disease. The first scan exhibiting new measurable disease is noted as “preliminary PD”. If the second scan at least 4 weeks later exhibits a subsequent greater than or equal to a 25% increase in sum of products of perpendicular of enhancing lesions relative to the “preliminary PD” scan it is considered “confirmed PD” and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD, CR, PR, or becomes non-measurable, this scan showing “preliminary PD” is noted as “pseudoprogression”, PsP, and the subject should continue on study until a second increase in tumor size relative to the “preliminary PD”, or PsP, scan is observed. Note that any new measurable (>10mm x 10mm) enhancing lesions on the subsequent scan following the “preliminary PD” scan should not be immediately considered “confirmed PD”, but instead should be added to the sum of bidimensional products representing the entire enhancing tumor burden.
3. Unequivocal and significant worsening neurological examination not attributable to other causes.
4. Of note, if there has been a major reduction in steroid dosage in the interval and the patient is felt to be clinically stable or improved, the proper assessment may be “indeterminate” and the therapy could be continued pending the next evaluation.
5. Failure to return for evaluation as a result of death or deteriorating condition.

Not Evaluable (NE): Progression has not been documented and one or more sites have not been assessed.

13.8.5 **HRQoL**

HRQoL will be assessed by the Functional Assessment of Cancer Therapy-Brain (FACT-BR) scale. The FACT-BR (version 4) contains subscales for physical (7-items), functional (7-items), emotional (6-items), and social/family (7-items) well-being. In addition, this instrument contains an 23-item brain cancer subscale (BCS) which assess symptoms commonly reported by brain cancer patients [27]. Cancer-related fatigue will be assessed by the 13-item Fatigue Scale using the Functional Assessment of Cancer Therapy-Fatigue (FACIT-Fatigue) subscale, version 4 [67]. Cognitive problems will be assessed using version 3 of the Functional Assessment of Cancer Therapy-Cognition (FACT-Cog) subscale [68]. This includes subscales for perceived cognitive impairments (20 items), comments from others (4 items), perceived cognitive abilities (9 items), and impact of quality of life (4 items). Beck depression inventory (BDI) will be used to evaluate for underlying depressive symptoms. We will use the revised version (BDI-II) and the scores will range from 0 (no depression) to 63 (severe depression) [69]. The BDI contains 21 questions in regards to mood symptoms and is multiple-choice and self-reported. Exercise behavior will be assessed using the Godin Leisure questionnaire [70]. HRQoL PROs questionnaires will be obtained at screening, after standard RT and TMZ (within 2 to 4 weeks), and approximately every 8 weeks when a subject returns for a Standard of Care visit during adjuvant TMZ.

13.8.6 **Cognitive Testing**

Cognitive testing will include the performance on the following testing using a computerized battery CNS Vital Signs® [22] (Phase 1) and Brief Assessment of Cognition (BAC) through the NeuroCog app (Phase 2). The CNS Vital Signs® battery consists of seven tests that measure verbal and visual memory, finger tapping, symbol digit coding, the Stroop Test, a test of shifting attention, continuous performance test. Verbal memory test will assess verbal learning, memory for words, immediate recall, and delayed recall. Visual memory tests will assess visual learning, memory for geometric shapes, immediate recall, and delayed recall. Finger tapping test will assess motor speed and fine motor control. Symbol digit coding test will evaluate complex attention, visual-perceptual speed, and information processing speed. Stroop Test will assess executive function, simple and choice reaction time, speed-accuracy trade-off, information processing speed, and inhibition/disinhibition. Shifting attention test will assess executive function, reaction time, and information processing speed. Continuous performance test will assess sustained attention and choice reaction time. Normative data is available for this testing through CNS Vital Signs® and patients' performance will be compared to this normative data. In Phase 2, the BAC battery of tests is implemented through an iPad based app called the BAC App (the vendor that provides this is called VeraSci, previously called NeuroCog) [22]. The table below shows a description of the BAC App Subtests. Examples are included in Appendix E. The verbal responses provided during the tests conducted with the iPad will be audio recorded. These audio recordings will be used by the representatives of the site and sponsor to make sure that your responses are recorded correctly. The audio recordings will be stored and van be viewed later using VeraSci's proprietary portal, which appropriate credentials are needed to access. They will be retained for the period of time specified for all other study data or as required by law. Cognitive testing with the BAC App will be obtained at screening (for the baseline assessment), after standard RT and TMZ (within 2 to 4 weeks), and approximately every 8 weeks when a subject returns for a Standard of Care visit during adjuvant TMZ. If a subject comes off study for disease progression before the approximate 6 and 12 months post completion of treatment timepoint, cognitive testing should still be collected at those timepoints. Additional cognitive testing (HVLT-R, COWA, and TMT) will be performed at these selected times: baseline, standard of care visit before the start of the first cycle of adjuvant temozolomide, and standard of care visits that are 6 and 12 months from the completion of radiotherapy/chemotherapy. These tests will be performed by a trained neuropsychologist or delegate at the clinical center. The tests will include Hopkins Verbal Learning Test-Revised to assess learning, total recall, and memory [71], Trails Making Test Parts A and B to assess attention and executive function [72], and Controlled Oral Word Association Test to assess verbal fluency [73].

Table 5: Description of the BAC App Subtests. Traditional BAC App Battery. List of tests, the domains of cognition they assess, a description of the methodology for assessment and the total time for test administration.

Domain	BAC Subtest	Summary	Duration (mins)
Verbal Memory & Learning	Verbal Memory	Subject is presented with 15 words and asked to recall as many as possible. This procedure is repeated 5 times. *Outcome measure*: Number of words recalled	7
Working Memory	Digit Sequencing	Subject is presented with auditory clusters of numbers (e.g. 9, 3, 6) of increasing length and asked to tell the rater the numbers in order from lowest to highest. *Outcome measure*: Number of correct responses	5
Motor Function	Token Motor	Subject is presented with tokens and asked to drag them to a center container as quickly as possible for 60 seconds. *Outcome measure*: Number of tokens correctly dragged into the container	3
Verbal Fluency	Semantic Fluency	Subject is given 60 seconds to verbally generate as many words as possible in a given category. *Outcome measure:* Number of words generated	5
	Letter Fluency	In two separate trials, subject is given 60 seconds to verbally generate as many words as possible beginning with a given letter of the alphabet. *Outcome measure:* Number of words generated	
Speed of Processing	Symbol Coding	Subject is provided a key and asked to fill in the corresponding numbers beneath a series of symbols as quickly as possible within 90 seconds. *Outcome measure:* Number of correct items	3
Executive Function	Tower of London	Subject is asked to give the minimum number of times the balls in one picture would need to be moved in order to make the arrangement of balls identical to that in the opposing picture. *Outcome measure:* Number of correct responses	7

13.8.7 Hair Integrity and Hair Loss

Subjective assessments of hair loss will be obtained by the treating provider for subjects at screening (baseline), after standard RT and TMZ finishes (within 2 to 4 weeks), and standard of care (SOC) clinic visit (approximately every 8 weeks) during adjuvant TMZ.

The photographs will be taken of the craniotomy scar (surgical incision site) and can be taken at any angle or background approximately 6-48 inches from the scalp. There will be no identifiers on the photographs. Up to 6 photographs may be taken at any one time point. Photographs will not include the subjects entire face (will not include eyes, nose, mouth). The photographs will be taken by the PI or trained staff and may be uploaded to the electronic medical record, kept in a separate electronic research file or on paper. One photo per timepoint will also be uploaded to the sponsor electronic database. Participation in these pictures is optional for the patient and they can decline the picture taking at any time. Hair loss will be subjectively described and evaluated clinically by the providers treating the patient and later by the sponsor. If the patient is bald, the photos and descriptions may be omitted.

13.8.8 Medical and Demographic Information

Medical and demographic information will consist of general demographic information (e.g., race, ethnicity, education, employment, smoking status). Demographic information should be collected at the time of screening. Medical information will be abstracted from medical records and study questionnaires.

13.8.9 Bone Marrow Assessments

Bone marrow function will be assessed by CBC with differential (which includes platelet counts) as described in the table in Section 13 Screening and On-Study Tests and Procedures.

13.8.10 MRI Diffusion Tensor/Susceptibility Imaging

MRI diffusion tensor/susceptibility imaging during Phase 2 involves the application of standard MRI imaging of the brain to evaluate white matter integrity. This will be done before contrast injection on subjects enrolled at clinical centers as indicated by the sponsor in Phase 2. All images will be acquired on state-of-the-art 3T scanners.

Imaging should include T1-weighted 3D imaging before and after single dose contrast agent injection as specified in the standardized brain tumor imaging protocol (BTIP, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4588759/, Table 1). T2 and FLAIR imaging should also be performed as specified in this publication.

For susceptibility images, images will be acquired using a multi-echo 3D gradient echo sequence.

For **Siemens** scanners, use the following parameters:

TE1	=min
TR	=60 ms
flip angle	= 12º
FOV	= 220x220 mm^2
matrix size	= 256x256, 2mm slice thickness with no gaps, parallel imaging acceleration factor 2.

For **GE scanners**, use the following parameters:

TE1	=min
TR	=45 ms
flip angle	= 12º
FOV	= 220x220 mm^2
matrix size	= 256x256, 2mm slice thickness with no gaps, parallel imaging acceleration factor 2.

Obtain maximum number of echoes (at least 9). Sites should reconstruct and save both magnitude and unfiltered phase images from this sequence.

Diffusion tensor images will be acquired before contrast injection using a multi-channel head coil (at least 8-channels) and a standard single-shot EPI sequence with a parallel-imaging acceleration factor of 2. The parameters will be as follows: TE = 82 ms, TR = 8 s, FOV = 220x220 mm^2, matrix size = 128x128, slice thickness = 2 mm without gap, b-value = 1000 s/mm^2, 5 non-diffusion weighted images and 25 diffusion encoding directions. 75 slices will be acquired to cover the whole brain.

These images will be obtained three times during the study: on day -5 to 0, after standard RT and TMZ (within 2 to 4 weeks), and 24 weeks after the start of standard RT and TMZ (within 2 to 4 weeks).

Images will be cleaned of identifiers (except for the date of the scan which is needed for analysis), encrypted and transferred to the Duke Imaging center for analysis. Please see the sponsor provided Imaging Manual for specifics.

13.8.11 **Drug Levels and Pharmacokinetic Assessment**

See Appendix B, Section 19.2. In Phase 2, only 6 subjects enrolled in Arm A (at Duke) will have PK samples drawn.

14 SAFETY MONITORING AND REPORTING

The Principal Investigator is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. The Principal Investigator will review and sign off on all adverse events and problems as they occur and will report them to the IRB in accordance with HRPP policies and to the Sponsor, BioMimetix JV, LLC. At each study visit, the Principal Investigator or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

14.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a study subject which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease that occurs in any subject enrolled in this clinical trial. It may or may not be related to use of the BMX-001. Abnormal laboratory findings without clinical significance (based on the Principal Investigator's judgment) should not be recorded as AEs. But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events.

From the time the subject signs the informed consent form through 30 days after completion of the final BMX-001 treatment (for subjects in Arm A, this will be 30 days after the last chemo/RT for subjects in Arm B), all AEs must be recorded in the subject medical record and adverse events case report form.

AEs will be assessed according to the CTCAE version 4.03. If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:
- Definite: The AE is clearly related to the BMX-001
- Probably: The AE is likely related to the BMX-001
- Possible: The AE may be related to the BMX-001
- Unlikely: The AE is doubtfully related to the BMX-001
- Unrelated: The AE is clearly NOT related to the BMX-001

14.1.1 **Reporting of AEs**

BioMimetix JV, LLC should be notified of all treatment-related adverse events on a regular basis (to be agreed upon by the study team and BioMimetix JV, LLC).

14.2 Serious Adverse Events

An AE is considered "serious" if in the opinion of the investigator it is one of the following outcomes:
- Fatal
- Life-threatening
- Constitutes a congenital anomaly or birth defect
- A medically significant condition (defined as an event that compromises subject safety or may require medical or surgical intervention to prevent one of the three outcomes above).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption to conduct normal life functions.

14.2.1 **Reporting of SAEs**

Only adverse events that the Sponsor and/or Principal Investigator determines to be serious, unanticipated, and related or possibly/probably (i.e., more likely than not) related to the research must be reported to the sponsor. Those adverse events will be submitted, according the following guidelines:

- Report within 24 hours of learning about any subject's death that was unanticipated and more likely related to the research than unrelated;
- Report within 5 business days of learning about any serious, unanticipated, and related or possibly/probably related adverse event;
  or other unanticipated problem or event that was more likely related to the research than unrelated.
- Report within 10 business days of learning about any other SAE not meeting the above criteria.

Refer to sponsor Standard Operating Procedures for SAE Reporting specifics.

The Principal Investigator is responsible for compliance with all applicable regulations pertaining to the reporting of adverse events to the IRB. Follow-up information on these events shall also be reported to BioMimetix JV, LLC, and the IRB as required as soon as follow up information is available. Follow-up information may include hospital admission records, discharge summaries and autopsy reports, where applicable.

The Sponsor must report to the FDA, in an IND safety report, any suspected adverse reaction that is both serious and unexpected. Before submitting this report, the sponsor needs to ensure that the event meets all three of the definitions contained in the requirement:

- Suspected adverse reaction (i.e. there is a reasonable possibility that the drug caused the adverse event)
- Serious
- Unexpected

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The Sponsor is required to report to the FDA all IND Safety reports in writing within 15 days (7 days for unexpected fatal or life-threatening suspected adverse reaction). The FDA Form 3500A can be found on the FDA website, www.fda.gov. All other adverse events will be reported to the FDA in the IND Annual Report.

In addition to the above mentioned reporting responsibilities, after discontinuation of the study, all SAEs considered to be drug related will be reported to BioMimetix JV, LLC, by the Principal Investigator.

14.3 External Data and Safety Monitoring Board (DSMB)

The DSMB-Plus established for Phase 1 will be used for Phase 2 during the period that Duke Cancer Center is the only participating clinical center. When additional clinical centers are added to the Phase 2 study, the DSMB-Plus will be modified to become a DSMB-Plus which has oversight for all clinical centers. This will involve approval of a new charter.

The sponsor and Principal Investigators are responsible for overseeing the safety and efficacy of the trial, executing the DSMB plan, and complying with all reporting requirements to local and federal authorities. Additional oversight will be carried out by an external DSMB In Phase 2.

An external DSMB-Plus will be utilized to monitor Phase 2 when this becomes a multi-center study. This DSMB will be organized to meet requirements of Western IRB, the institutional IRBs, and the NCI. We anticipate a charter with a minimum of 3 members, which may include: a radiation oncologist with experience in high grade glioma, a medical oncologist with expertise in high grade glioma, a biostatistician, and/or an ethicist or patient advocate. The responsibilities of the DSMB will be to review safety of study procedures, to maintain study integrity, to review adverse events and to review efficacy of the outcome data. The DSMB will meet either face to face or by teleconference or with electronic communication at least semi-annually and on an ad hoc basis as indicated by any adverse events. Minutes will be kept of both open and closed meetings of the DSMB. At the end of the study, all minutes taken will be available to the clinical trial principal investigators, the NCI, and BioMimetix JV, LLC.

The DSMB's responsibilities will include evaluation of treatment outcome for evidence of tumor protection by the study drug. The DSMB will have authority to recommend closure of the clinical trial for either efficacy or futility.

15 QUALITY CONTROL AND QUALITY ASSURANCE

15.1 Monitoring

BioMimetix JV, LLC personnel will monitor this study. The Clinical Center Principal Investigator and Study Coordinator should be available to the clinical trial monitor during monitoring visits and ensure that access to all documents and records is provided. This local Principal Investigator agrees to cooperate with the clinical trial monitor to ensure that any problems detected in the course of these monitoring visits are resolved. For this study, the expected average monitoring frequency is at least one visit approximately every 10 weeks, while the study is enrolling and subjects are receiving treatment.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of the clinical center institution, the Sponsor, the Clinical Center Principal Investigator, or the IRB. All study documents must be made available upon request to the clinical trial monitor and other authorized regulatory authorities, including but not limited to the National Institute of Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

15.1.1 **DSMB-Plus**

Per the recommendation of the Duke School of Medicine Research Integrity Office (RIO), a data safety monitoring board DSMB-Plus has been formed and a charter has been established for periodic review and assessment of data and outcomes to assure continuing safety of research participants in this trial. The committee will participate in decisions regarding reports or publications to ensure there is no bias as a result of conflict of interest questions. Because of Duke University's potential for conflict of interest with BMX-001, the Duke RIO has recommended use of a DSMB-Plus for review of the study for bias in the protocol design, performance of the study, and assessment of endpoints and ongoing periodic assessment of adverse events and study conduct.

Members of the DSMB-Plus will be approved by the ROI office and a DSMB-Plus charter established. For Phase 1 narrative summaries will be prepared by the study coordinator or research nurse and reviewed and signed by the PI. Of note, this DSMB-Plus will be involved in the Phase 1 portion of this study. Narrative summaries of subjects from each dose level will be prepared four weeks after the last dose of BMX-001. These summaries will include a review of safety data, Adverse Event assessments with attention paid to endpoint assessments and data in the protocol. PK data may also be reviewed at this timepoint. The DSMB-Plus committee will review narrative summaries and offer a recommendation about dose escalation or discontinuation of enrollment to the PI. Policies of the DSMB-Plus will be described in the DSMB-Plus Charter, which will be signed by the DSMB-Plus members and PI.

In Phase 1 DSMB-Plus meetings will be convened after the 3rd subject on a cohort (dose level) has reached 4 weeks after the last dose of BMX-001. DSMB-Plus review will be prior to each dose escalation. In Phase 2, the DSMB-Plus will meet twice a year. The DSMB-Plus will review safety and data reports which will be provided by the sponsor prior to the meetings. Review of data can be via WEBEX or teleconference. Copies of DSMB-Plus meeting minutes will be provided to RIO office (Dr. Gregory Samsa) within 30 days of the DSMB-Plus review.

This DSMB-Plus committee will also consider conflict of interest questions related to this research. The DSMB-Plus will evaluate whether financially-linked biases have affected the design, conduct, or reporting of the research related to BMX-001.

Policies of the DSMB-Plus will be described in the DSMB-Plus Charter, and signed by the DSMB-Plus members.

15.2 Audits

The study may be audited by an audit team originating from the sponsor or its designee. The Investigator agrees to cooperate with the auditor to ensure than any problems detected in the course of the audit visits are resolved.

15.3 Data Management and Processing

15.3.1 **Study Documentation**

In Phase 1- Duke Brain Tumor Center has developed the study database. Phase 1 data will be provided to BioMimetix JV, LLC in either paper format or downloaded documents from Title 21 CFR Part II Compliant Clinical database. All documentation transmitted to BioMimetix JV, LLC will be de-identified in the appropriate manner required to be HIPAA compliant.

In Phase 2, BioMimetix JV, LLC, will develop the electronic Data Capture system and database for the study. Electronic records of subject data will be maintained using a Title 21 CFR Part 11 Compliant Clinical database called REDCap Cloud. Access to electronic databases will be limited to the Principal Investigator, biostatisticians, data manager and key personnel.

15.3.2 **Data Management Procedures and Data Verification**

The Clinical Center Principal Investigator is responsible for maintaining adequate records to enable the conduct of the study to be fully documented. Subsequent electronic review of the data may result in queries being generated that will be forwarded simultaneously to the Investigator or designee for resolution. All data modifications resulting from review or querying of the data will be electronically tracked. Any errors detected by either the monitor or the Investigator should be communicated to the Sponsor.

Copies of all regulatory documents such as the protocol, study approval letters, all CRFs, drug dispensing and accountability logs, all original patient consent forms, and all correspondence pertaining to the conduct of the study should be kept by the Clinical Center Principal Investigator for the maximum period of time permitted by local regulations.

15.3.3 **Coding**

All medical terms will be coded using CTCAE v.4.03, which has been harmonized to MedDRA (Medical Dictionary for Regulatory Activities) coding.

15.3.4 **Study Closure**

Following completion of the study, the Clinical Center Principal Investigator will be responsible for ensuring the following activities:

- Data clarification and/or resolution of queries
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to the designated laboratories

16 STATISTICAL METHODS AND DATA ANALYSIS

All statistical analysis will be performed under the direction of the statistician designated in key personnel. Any data analysis carried out independently by the investigator must be approved by the statistician before publication or presentation.

16.1 Analysis Sets

MTD Analysis Set: Patients who complete concurrent chemoradiation with scheduled treatments with BMX-001 or terminate treatment during that period of time due to DLT will be considered evaluable and included in the analysis of MTD.

Adverse Experience Analysis Set: All patients who receive BMX-001 will be included in any overall summary of adverse experienced.

Survival Analysis Data Set: All patients who receive BMX-001 will be included in analyses of survival and progression-free survival.

PRO / Cognition Analysis Set: All patients who receive BMX-001 and provide a follow-up assessment will be included in statistical analyses of HRQoL and cognition.

The MTD Analysis dataset as described above is applicable to the Phase 1 portion of this study; whereas, the other datasets are applicable to both the Phase 1 and Phase 2 portions of this study.

16.2 Patient Demographics and Other Baseline Characteristics

For each phase of this study, the clinical and socio-demographic characteristics of all patients treated will be summarized using descriptive statistics (e.g. means/standard deviations, percentiles, frequencies). Patient characteristics within patient subgroups defined by dose level may also be generated for the Phase 1 portion of this protocol. Within the Phase 2 portion, patient characteristics will be summarized by treatment group assignment.

16.3 Treatments

Within the Phase 1 portion of this study, the number of patients treated at each dose level will be summarized.

Within the Phase 2 portion of this study, a CONSORT diagram will be generated that displays the number of patients treated within each treatment arm, as well as the number of cycles of adjuvant treatment received.

16.4 Phase 1 Dose Escalation

16.4.1 **Primary Objective**

The primary objective of this Phase 1 study is to determine the maximum tolerated dose (MTD) of BMX-001 administered with standard radiotherapy and daily TMZ among patients with newly diagnosed HGG. Four dose levels are planned and are described in Section 9.1.1.

16.4.1.1 **Variable**

Based upon all available data and the CRM model described in Section 16.4.1.2, the MTD will be the dose with the estimated DLT rate closest to the target DLT rate of 0.25.

16.4.1.2 CRM Design Considerations

Though 3+3 study designs are commonly used to determine MTD, we propose to adopt a Phase 1 dose escalation strategy that avoids the following shortcomings of the standard 3+3 design. Specifically, the 3+3 design suffers from the problem of "memory loss" that occurs as dose is escalated. In addition, the 3+3 algorithm allows many patients to be treated at low and ineffective doses due to conservative dose escalation, does not have a statistical justification, and does not involve an explicit target DLT rate [74].

A two-stage CRM design will be used to determine the MTD of BMX-001 where the first stage involves dose escalation in successive patients until an initial dose-limiting toxicity (DLT) is observed. Within the second stage, all accumulated data will be used within the context of a one-parameter logistic model to determine the appropriate dose for each subsequent patient. Maximum likelihood estimation will be used. This modeling process will determine the dose with a DLT rate closest to the target DLT rate of 0.25. Cohorts of 3 patients will be accrued to this study within both stages of the trial.

The remaining portion of this section provides information about the development of the CRM model that is to be used specifically for this study. R software developed by Cheung [75] aided in its development.

MTD: No dose-limiting toxicities (DLTs) were observed in monkey toxicity studies for BMX-001 within the range of doses being planned for these human studies. Hence, we do not expect DLTs to occur within the range of doses that will be used in this Phase 1 study.

One-stage versus two-stage CRM: With no expectation that DLTs will be observed among the doses that will be investigated in this study, the use of a one-parameter CRM model is inappropriate as such a model assumes that the initial patient is treated at the prior or expected MTD. Rather, a two-stage CRM with dose escalation restriction will be used. The first stage involves a predetermined dose escalation scheme that starts at dose level 1 and terminates once the first DLT is observed. Thereafter, a model-based CRM will be used in the second stage to define patient dose assignment.

Whenever a dose assignment is needed during the second stage, all available data will be used with maximum likelihood estimation to generate the CRM model. The dose that results in a DLT rate nearest to the target rate will be determined from this model, and used to treat the next patient subject to a dose escalation restriction. That restriction requires that the dose for the next patient cannot be more than one level higher than that of the current patient.

Target DLT rate (θ)*:* CRM designs used for oncology therapeutic trials are often designed with a target DLT rate (θ) between 0.2 and 0.3. This study is designed to detect a target DLT rate of 0.25.

Sample size: Cheung [75] suggests that the fixed sample size used in one-stage CRM designs be constrained so that

$$\frac{N-3\ (\#\ dose\ levels-2)}{2} > \frac{1}{Target\ DLT\ Rate}$$

If this study was a one-stage study, the implication of this constraint is that N > 14. For the two-stage design, Cheung [75] recommends that the number of observations reserved for the highest dose level in case of no toxicity should be greater than 1.5/target DLT rate, or 6 for this study. The simulations described below consider a fixed sample size ranging between 16 and 20 patients.

Functional form of model: The logistic and empiric models are commonly used as the underlying functional form describing the relationship between the probability of DLT and dose. The one-parameter logistic model has the following form where β is an unknown parameter to be estimated:

$$\text{Probability of DLT} = \frac{\exp(3+\exp(\beta)dose)}{1+\exp(3+\exp(\beta)dose)}$$

The one-parameter model has the following functional form:

$$\text{Probability of DLT} = \text{dose}^{\exp(\beta)}$$

Simulations reported below examine the impact of functional form (logistic and empiric) on model inference.

Initial guess of toxicity probability: Within the second stage of the CRM model, there is a need to specify a set of initial guesses for the dose-specific probability of DLT. Referred to as the skeleton of the CRM, this initial guess at toxicity probability is increasing with dose. Even with good clinical guesses for the skeleton, the resulting CRM model may fail regularity conditions required for the study to have good operating characteristics. As an alternative, an initial toxicity probability distribution can be generated so that the CRM is δ-sensitive. A δ-sensitive CRM will eventually select a dose as the MTD with toxicity falling between θ±δ [74].

The GETPRIOR function available within the R-package DFCRM was used to estimate the skeleton assuming δ was 0.03, 0.05, 0.07 [74,76,77]. Though we expect that DLT will not be encountered at any dose level, we will assume that the prior MTD is dose level 5. Tabulated below are the initial guesses generated by the GETPRIOR function regardless of underlying functional form (logistic or empiric).

	Initial Guess of Toxicity Probability with Empiric Model			
Delta (δ)	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
0.03	0.097	0.141	0.192	0.250
0.05	0.036	0.084	0.157	0.250
0.07	0.009	0.043	0.124	0.250

	Initial Guess of Toxicity Probability with Logistic Model			
Delta (δ)	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
0.03	0.101	0.143	0.193	0.250
0.05	0.044	0.089	0.158	0.250
0.07	0.016	0.051	0.127	0.250

Initial design: The GETINIT function within the R-package DFCRM was used to identify compatible and coherent initial designs given the identified initial guesses of toxicity probability and the model's functional form (logistic or empiric) [74,75]. The algorithm started the search for an initial design with the most aggressive initial design that started the trial at the second highest dose. More conservative designs were subsequently tested for compatibility if the current design compatible.

Compatible designs exist that involve treating cohorts of 2 or 3 patients with increasing dose until at least one DLT is observed. Given the expectation that DLT will not be observed, a rapid escalation would be appropriate with the treatment of cohorts of 3 patients. However, an important secondary outcome of the Phase 1 study is pilot data concerning the impact of dose on cognition. Hence, an initial design with cohorts of 3 patients will be used to garner additional information about the cognitive effects of BMX-001 treatment.

Cohort size during the second stage: With an accrual rate of approximately 3 patients per month, the observation period for the determination of DLT is 4 weeks. Cohort sizes of 3 will be considered for the second stage.

Study Simulations: The performance of the two-stage design CRM design was examined under a variety of conditions, including functional form [logistic vs empiric], initial guess of DLT toxicity probabilities, cohort size, overall sample size [17,19,21]), and various true underlying toxicity probability profiles using the CRMSIM function within the R-package DFCRM [74,75,78]. Results based upon 500 simulations are summarized in Appendix C. These simulations suggest the following:

- The majority of simulations identify the correct MTD dose, or a contiguous dose.
- The probability of selecting a dose as the MTD is comparable with the two different functional forms.
- When the MTD is at a higher dose, the probability of declaring that dose as the MTD is greatest when the initial guess of toxicity probability assumes δ=0.03.
- Increasing the maximum number of subjects from 16 to 20 does not increase the probability of correctly choosing the MTD substantially.

Therefore, the following design elements will be used in the conduct of this study:

- Logistic functional form
- Initial guess of toxicity probability that assumes δ=0.03, i.e. (0.101, 0.143, 0.193, 0.250)
- The maximum number of patients evaluable for the determination of MTD that will be enrolled is 18.
- Cohorts of 3 patients will be accrued to this study within both stages of the trial.

16.4.1.3 Implementation of Phase 1 Study

Initial Design / Stage 1: As described above, this Phase 1 study will be conducted in two stages. In the first stage, cohorts of 3 patients will be treated starting with dose level #1. Once 3 patients have been undergone treatment without experiencing DLT after a monitoring period of four weeks after completion of all doses of BMX-001, the dose of the subsequent group of 3 patients will be escalated. This process will continue until a patient experiences a DLT.

Model-Based Escalation / Stage 2: Within stage 2, patients will be accrued to the study in cohorts of 3 patients. Whenever the toxicity outcomes associated with patients within a cohort are known, the one-parameter logistic model will be re-estimated by the study's statistical team using all available data. Based upon this re-estimated model, the dose level that results in a DLT rate nearest to the target DLT rate will be determined, and used to treat the next patient subject to a dose escalation restriction. That restriction requires that the dose for the next patient cannot be more than one level higher than that of the current patient.

Study Termination: A maximum of 18 patients evaluable for the determination of MTD will be accrued to the study. Though simulations demonstrating properties of this design assumed a fixed sample size, accrual termination will also be allowed once 6 or more consecutive patients have been treated at a dose level without any indication that the dose should be escalated or de-escalated [74].

16.4.1.4 Secondary Objectives of Phase 1 Study

Adverse events will be tabulated in a different manner for the manuscript summarizing the results of this study, annual reports for the Safety Oversight Committee, and the final report included within ClinicalTrials.gov.

For the manuscript, adverse events that are possibly, probably, and definitely treatment-related will be summarized. For each type of toxicity, the maximum grade experienced by each patient will be summarized with frequency distributions within each treatment group.

Two tabulations will be generated for review by the Duke Cancer Institute Safety Oversight Committee including one that includes all toxicities regardless of attribution, and another that includes only toxicities that are possibly, probably, and definitely related to study regimen. For each of these tabulations, the maximum grade of each type of toxicity experienced by each patient will be summarized with frequency distributions within each treatment group.

The mean change between baseline and each follow-up assessment for cognitive function will be computed. Baseline will be the last assessment obtained before initiation of standard of care treatment consisting of temozolomide and RT. Analyses will be sub-divided in terms of WHO grade and histological subtype.

Kaplan-Meier methods will be used to graphically describe the distribution of overall survival (OS) and progression-free survival. OS is defined as the time interval between initiation of protocol treatment and death, with overall survival censored at last follow-up if the patient remained alive. PFS is defined as the time between initiation of protocol treatment and disease progression or death. If the patient remains alive without disease progression, then PFS will be censored at last follow-up. Median OS and PFS, as well as 6- and 12-month estimates of OS and PFS will be estimated from the Kaplan-Meier curve. Because OS and PFS can differ in HGG based on grade (WHO grade III vs IV) and histological subtype (e.g. anaplastic oligodendroglioma vs. anaplastic astrocytoma), analyses will be sub-divided in terms of WHO grade and histological subtype.

The radiographic response rate will be calculated as the proportion of patients with a complete or partial response for all patients and within subgroup of patients defined by grade. Exact binomial confidence intervals will be computed.

Pharmacokinetics will be done as described in Appendix B (Section 19.2).

16.4.1.5 Exploratory Objectives of Phase 1 Study

The mean change between baseline and each follow-up assessment for HRQoL will be computed as was computed for cognitive function outcome variables. Analyses will be sub-divided in terms of WHO grade and histological subtype. Hair loss is a descriptive measure and will be defined and analyzed using pictures.

16.4.1.6 Interim Analysis of Phase 1 Study

See Sections 16.4.1.2 and 16.4.1.3.

16.4.1.7 Sample Size Calculation

See Section 16.4.1.2.

16.5 Phase 2

16.5.1 Primary Objective for Phase 2

The primary goal of this randomized open-label Phase 2 study is to examine the impact of BMX-001 on overall survival, among patients newly diagnosed with HGG who are receiving standard of care (SOC) treatment consisting of daily TMZ and RT.

16.5.1.1 Variable

The study's primary endpoint is OS as defined by the time between randomization and death, or the last follow-up if the patient remains alive.

16.5.1.2 Statistical Hypothesis, Model, and Method of Analysis

As stated in section 7.6, this study will assess the hypothesis that BMX-001, when added to standard RT and TMZ, will sensitize the malignancy and result in enhanced patient survival.

All randomized patients will be included in the analysis of survival and included in their assigned treatment arm regardless of compliance. This approach to analysis is consistent with an intent-to-treat analysis approach.

Kaplan-Meier methods will be used to graphically describe the distribution of overall survival (OS) among patients within the BMX group and within the control group. Median OS and PFS, as well as 6- and 12-month estimates of OS and PFS will be estimated from the Kaplan-Meier curve. A logrank test will compare arms with respect to the survival outcome.

Because OS can differ in HGG based on grade (WHO grade III vs IV) and histological subtype (e.g. anaplastic oligodendroglioma vs. anaplastic astrocytoma), the survival of patient subgroups of patients defined by grade and histological subtype will be described.

16.5.2 Secondary Objectives for Phase 2

This study has 6 secondary objectives that include an assessment the impact of BMX-001 on cognition, thrombocytopenia, adverse events, progression free-survival, radiographic response, and pharmacokinetics.

16.5.2.1 Secondary Objective #1 for Phase 2: Cognition

The key secondary objective includes an evaluation and comparison of cognition among patients newly diagnosed with HGG and whether the addition of BMX-001 to standard RT and TMZ impacts cognition. This secondary endpoint is the mean change in cognitive functioning between baseline and week 24 within each treatment arm. The baseline measure is the last assessment obtained before initiation of standard chemoradiation.

Analysis of covariance will be conducted to compare treatment groups with respect to the change in cognitive function. The outcome of this model is change in cognitive function. Treatment group assignment will be included in the model as a predictor, and the baseline measure of cognitive function will be included as a covariate. Additional analyses will explore the impact of BMX-001 on cognition within patient subgroups defined by WHO grade and histological subtype.

Measurement of cognition will continue after initiation of adjuvant temozolomide. Longitudinal analyses will compare treatment arms with respect to the pattern of cognitive change observed over time. Initially a repeated measures analysis will be conducted. However, a significant amount of the follow-up cognitive assessments may be missing due to health decline, disease progression, and death. The patterns of “drop-out” in the two treatment arms may also differ. To address these problems, additional analyses will be conducted. These analyses may focus on only assessments with relative complete data, or may consider alternative analytic methods such as pattern mixture models.

16.5.2.2 Secondary Objective #2 for Phase 2: Thrombocytopenia

The proportion of patients who experience grade 3 or 4 thrombocytopenia during concurrent temozolomide and radiation will be estimated within each treatment group. The proportion of patients who experience a platelet count less than 100K during concurrent temozolomide and radiation will also be estimated within each treatment group. The period of observation for these events will extend until 2 weeks after completion of radiation treatment. For both endpoints, a chi-square test will be conducted to compare the prevalence of such thrombocytopenia observed in patients with and without BMX-001.

In addition, the longitudinal patterns of platelet count over time will be explored in mixed linear models that account for within patient correlation. These longitudinal patterns will focus on the time period during BMX-001 treatment administration that continues for 2 weeks after radiation treatment is completed.

16.5.2.3 Secondary Objective #3 for Phase 2: Adverse Events

The phase 2 portion of this study has two adverse event endpoints: (1) The proportion of patients who experience any grade 3 or 4 adverse event during radiation and temozolomide treatment, and (2) The proportion of patients who experience a grade 3 or 4 adverse event that is definitely, possibly, or probably related to BMX-001 treatment during this same period. Adverse events occurring between baseline through 2 weeks after discontinuation of radiation will be the focus of these analyses.

Adverse Event Monitoring and Tabulations: Adverse events will be tabulated in a different manner for semi-annual reports to the DSMB-Plus / DSMB, the manuscript summarizing the results of this study, and the final report included within ClinicalTrials.gov.

DSMB-Plus / DSMB Monitoring: Two tabulations of adverse events occurring during concurrent chemoradiation will be generated for DSMB-Plus / DSMB review including one that includes all toxicities regardless of attribution, and another that includes only toxicities that are possibly, probably, and definitely related to BMX-001 treatment. For each of these tabulations, the maximum grade of each type of toxicity experienced by each patient will be summarized with frequency distributions within each treatment group. The period of observation that will be the focus of these tabulations will extend 2 weeks after termination of radiation treatment. Additional tabulations will summarize adverse events experienced during adjuvant treatment.

For the manuscript, similar summaries of adverse events will be generated.

For ClinicalTrials.gov, serious adverse events and other adverse events will be summarized separately. These tabulations will reflect the number of patients who experience each type of toxicity regardless of grade or attribution.

16.5.2.4 Secondary Objective #4 for Phase 2: Progression-Free Survival

Progression-Free Survival (PFS) is defined as the time between initiation of protocol treatment and disease progression or death. If the patient remains alive without disease progression, then PFS will be censored at last follow-up. Analyses similar to those described for OS will be generated for PFS.

16.5.2.5 Secondary Objective #5 for Phase 2: Radiographic Response

Radiographic response will be determined by brain MRI evaluations for assessment based on the RANO criteria. The radiographic response rate is defined as the proportion of patients of with a complete or partial response. The response rate within each arm will be calculated, and compared using an exact chi-square test. Additional analyses will describe the response rate within patient subgroups defined by arm, WHO grade and histological subtype. Exact binomial confidence intervals will be generated for each response rate estimate.

16.5.2.6 Secondary Objective #6 for Phase 2: Pharmacokinetics

Pharmacokinetics will be done as described in Appendix B (Section 19.2).

16.5.3 Exploratory Objectives for Phase 2

Three exploratory objectives will be pursued within this protocol. This includes an assessment of health-related quality of life, a description of hair loss, and an examination of changes in white matter integrity.

Many of the proposed exploratory analyses assume methods for normally distributed data are appropriate. If underlying assumptions for planned analyses are violated, plans will be modified. Alternative analytic methods may also be considered to assess the robustness of inferences.

Additional statistical analyses may be conducted besides those described in this protocol. These additional analyses may be motivated by evolving research outside of this protocol, or they may be suggested by statistical analyses pre-specified in this protocol.

16.5.3.1 Exploratory Objective #1 for Phase 2: Health-Related Quality of Life

Methods described in section 13.8.5 will be used to analyze HRQoL data. Given that numerous endpoints will be examined in these exploratory analyses, adjustments for multiple comparisons (e.g. false discovery rate methods or Bonferroni) may be considered.

16.5.3.2 Exploratory Objective #2 for Phase 2: Hair Loss

Qualitative assessments of hair loss depicted in longitudinal subject photographs will be conducted and summarized. Changes observed in Arm A will be compared to those observed in Arm B.

16.5.3.3 Exploratory Objective #3 for Phase 2: White Matter Integrity

The mean change between baseline and each follow-up assessment for white matter integrity, as measured by MRI diffusion tensor/susceptibility imaging will be computed. Assuming normality, analysis of variance or a repeated measures analysis may be used to compare change in Arms A and B. Additional analyses will be sub-divided in terms of WHO grade and histological subtype.

16.5.4 Interim Analysis for Phase 2

Interim analyses using an O'Brien-Fleming boundary will be conducted with the goal of terminating accrual early to accept or reject the null hypothesis of no difference in survival. Given the inflated type I error rate for this Phase 2 study, early termination will only be considered if it appears that the addition of BMX-001 treatment to radiation and temozolomide is decreasing survival. The interim analysis will be conducted to determine whether the addition of BMX-001 is possibly detrimental to the survival of patients.

Interim analyses for the primary endpoint (survival) will be conducted annually beginning June 2021. The decision rule for accrual suspension will be defined by the O'Brien-Fleming spending function and is dependent upon the number of deaths that will have been reported by the time of the interim analysis. If the boundary is crossed, accrual will be suspended while all other data (e.g. cognitive function data) is reviewed to determine whether accrual should be permanently terminated.

Though not part of the formal interim decision-making described above, additional interim analyses of secondary and exploratory outcomes will be conducted for multiple purposes, including assessment of data completeness and program planning. Currently, the planned time of these analyses is as follows:

Date	Planned Analysis
June 2020	Among the initial 80 patients treated, changes in platelet counts during radiation and temozolomide treatment and ending 2 weeks after termination of radiation will be examined. The consistency of these counts with AE reports will also be assessed.
December 2020	Among the initial 80 patients treated on the study, changes in neurocognition and quality of life PROs through week 24 will be examined. Patterns of data missingness will be assessed, including reasons for missed assessments.
June 2021	Among the initial 80 patients treated on the study, changes in neurocognition and quality of life PROs through week 52 will be examined. Patterns of data missingness will be assessed, including reasons for missed assessments. The

	conduct of this interim analysis may be dependent upon any issues occurring in the December 2020 analysis.

16.5.5 Sample Size Calculation for Phase 2

Though the primary outcome of this study as currently design is overall survival, an important secondary outcome is the change in cognition. Power calculations will be presented for both outcomes.

16.5.5.1 Power of Survival Comparisons

The primary goal of the Phase 2 portion of this study is to assess the effect of BMX-001 treatment in conjunction with standard of care treatment consisting of temozolomide and radiation on the survival of patients newly diagnosed with grade III or IV glioma. A permuted block randomization stratified by histologic grade (grade III / IV) and institution will be used to assign patients to treatment with or without BMX-001.

With standard treatment, the median survival of Grade IV patients is 14.6 months, and the median survival of Grade III is approximately 36 months. Given that we anticipate that approximately 10% of patients to be Grade III, we estimate that the overall median survival with standard treatment to be roughly 16.7 months.

Though this Phase 2 study is comparative, the goal is to determine whether BMX-001 is worthy of further investigation in combination with standard RT and TMZ treatment, and not to make definitive statements about the effect of BMX-001. Many aspects of the new treatment will be considered in the decision-making concerning further investigation, including relative toxicity and ease of treatment administration. As a Phase 2 study, there is a need to constrain the sample size requirements at the expense of either an increased false negative or false positive rate. A false-positive rate of 0.2 will be used to test the primary statistical hypothesis while maintaining reasonably high power [80-82].

The power of a statistical comparison of survival is a function of the number of deaths observed. In order that a 1-tailed logrank test conducted at the 0.2 level to have 90% power to detect a hazard ratio of 0.63, 84 deaths need to be observed among the 160 randomized patients. Under the assumption that survival is exponentially distributed, this hazard ratio represents an increase in median survival from 16.7 to 26.5 months.

With an accrual goal of 160 patients and an expected randomization rate of approximately 6 patients per month, we anticipate that accrual will be completed within approximately 27 months. It is estimated that approximately 10 months after accrual completion are needed to observe approximately 84 deaths among randomized patients.

16.5.5.2 Cognition as Outcome

Changes in cognition is an important issue with treatment of HGG patients with radiation. As such, an important secondary objective of this study is to determine whether treatment with BMX-001 in conjunction with standard RT and TMZ mitigates the reduction in cognitive function due to the radiation. The endpoint that is of interest is the change in cognitive function between baseline and week 24.

We focus on the week 24 assessment of cognitive function in this power calculation given that the study is a randomized study, even though we are interested in comparing groups with respect to change in cognitive function between baseline and week 24. Studies recently conducted at Duke

suggest that the standard deviation associated with a one-time assessment of executive function, a component of cognitive function, is approximately 30 units.

We anticipate that 120 of the 160 patients will have pre- and post-measurements of cognitive function. A two-tailed t-test conducted at the 0.05 level of significance (two-tailed) will have 80% power to detect a difference of 15.5.

16.5.5.3 Thrombocytopenia as Outcome

Platelet counts will be obtained during radiation and temozolomide treatment and ending 2 weeks after termination of radiation. The percentage of patients within each treatment group that experience grade 3 or 4 thrombocytopenia will be estimated. Gerber [1] reports that 15% of patients who receive standard radiation and temozolomide experience grade 3 or 4 thrombocytopenia. With 80 patients per arm, there is 80% power with a one-tailed chi-square test (α=0.05) to detect a reduction in grade 3 or 4 thrombocytopenia from 15% in Arm B (without BMX-001) to 4% in Arm A (with BMX-001).

17 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

17.1 Regulatory and Ethical Compliance

This protocol was designed and will be conducted and reported in accordance with the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

17.2 Institutional IRBs and Research Committees

It is expected that the protocol, informed consent form, advertising material (if applicable), and additional protocol-related documents must be submitted to the local clinical center Institutional Review Board's (IRB) and internal committees in addition to the external Central IRB (WIRB for this study) for review. The study will first be submitted to WIRB by the sponsor and then, after WIRB approval, to the local IRBs, if required. The study may be initiated only after the Principal Investigator has received written and dated approval from all required IRBs and Committees.

The Principal Investigator will have the responsibility to submit and obtain approval from the local IRB for all subsequent protocol amendments and changes to the informed consent form, continuing review, as well as informing any appropriate committee about protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/ exclusion criteria, agent administration, statistical analysis, etc.).

The sponsor will handle study level submissions to the Central IRB, and the site will be responsible for handling site-specific submissions to the Central IRB.

17.3 Informed Consent

The informed consent form must be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form must be approved by the IRB.

The Principal Investigator or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Potential subjects will have the opportunity to contact the Principal investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Principal Investigator must obtain written informed consent from the subject or a legally acceptable representative. The original informed consent form (including any updated consent (re-consent) documents) will be stored with the source documents, and a copy of the informed consent form will be provided to the subject. The Principal Investigator and the study team are responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

17.4 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a dedicated compliant database, which is housed in an encrypted and password-protected file on a secure network drive. Access to electronic databases (without edit rights) will be limited to the Principal Investigator, the study coordinator, and the statistical team. The only personnel with both access and edit rights to the electronic databases are the data management team, including the clinical trials manager.

For the diffusion tensor images, information that would directly identify the patient will be removed from the DICOM image datasets to produce a limited imaging dataset (for the purposes of this scan, a *limited dataset* has the 16 direct identifying information removed from the DICOM header, but may include information about the date/time of imaging and the imaging location). The limited imaging dataset will then be transferred to Duke University for review and analysis. The process of *cleaning* (removing patient identifiers from the images) and transferring the images is done by the clinical center staff. The sponsor has provided an Imaging Manual for specific instructions on this process.

The results of the BAC App tests conducted on the iPad and the audio recordings included with them will be stored on a secure cloud based server maintained by the sponsor's representative (VeraSci). The electronic files, including the audio files, will be retained for the period of time specified for all other study data or as required by law.

Upon completion of the study, research records will be archived and handled per institutional policy. Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

17.5 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the Data and Safety Monitoring Plan. For a more detailed description of the DSMP for this protocol, refer to refer to the Safety Monitoring and Reporting section and the Quality Control and Quality Assurance section.

17.6 Protocol Amendments

All protocol amendments must be initiated by the Sponsor and approved by the IRB prior to implementation. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigator must inform the Sponsor and the IRB and all other applicable regulatory agencies of such action immediately.

17.7 Records Retention

The Principal Investigator will maintain study-related records for the longer of a period of:

- at least two years after the date on which a New Drug Application is approved by the FDA, if an IND is involved
- at least two years after formal withdrawal of the IND associated with this protocol, if an IND is involved
- at least six years after study completion (Duke policy) or longer if required by another participating institution

17.8 Conflict of Interest

The Principal Investigator and Sub-Investigators must comply with applicable federal, state, and local regulations regarding reporting and disclosure of conflicts of interest. Conflicts of interest may arise from situations in which financial or other personal considerations have the potential to compromise or bias professional judgment and objectivity. Conflicts of interest include but are not limited to royalty or consulting fees, speaking honoraria, advisory board appointments, publicly-traded or privately-held equities, stock options, intellectual property, and gifts.

18 REFERENCES

1. Gerber DE, Grossman SA, Zeltzman M, Parisi MA, Kleinberg L. The impact of thrombocytopenia from temozolomide and radiation in newly diagnosed adults with high-grade gliomas. Neuro Oncol 2007 Jan; 9(1):47-52. PMID: 17108062, PMC1828105.
2. Ross, A.D., et al., Hemodynamic effects of metalloporphyrin catalytic antioxidants: structure-activity relationships and species specificity. Free Radic Biol Med, 2002. 33(12): p. 1657-69.
3. Ohgaki, H., Epidemiology of brain tumors. Methods Mol Biol, 2009. 472: p. 323-42.
4. Ross, G.M., Induction of cell death by radiotherapy. Endocr Relat Cancer, 1999. 6(1): p. 41-4.
5. Greene-Schloesser, D., et al., Radiation-induced brain injury: A review. Front Oncol, 2012. 2: p. 73.
6. Tofilon, P.J. and J.R. Fike, The radioresponse of the central nervous system: a dynamic process. Radiat Res, 2000. 153(4): p. 357-70.
7. Crossen, J.R., et al., Neurobehavioral sequelae of cranial irradiation in adults: a review of radiation-induced encephalopathy. J Clin Oncol, 1994. 12(3): p. 627-42.
8. Giovagnoli, A.R. and A. Boiardi, Cognitive impairment and quality of life in long-term survivors of malignant brain tumors. Ital J Neurol Sci, 1994. 15(9): p. 481-8.
9. Klein, M., et al., Effect of radiotherapy and other treatment-related factors on mid-term to long-term cognitive sequelae in low-grade gliomas: a comparative study. Lancet, 2002. 360(9343): p. 1361-8.
10. Meyers, C.A. and P.D. Brown, Role and relevance of neurocognitive assessment in clinical trials of patients with CNS tumors. J Clin Oncol, 2006. 24(8): p. 1305-9.
11. Osoba, D., et al., Effect of neurological dysfunction on health-related quality of life in patients with high-grade glioma. J Neurooncol, 1997. 34(3): p. 263-78.
12. Meyers, C.A., et al., Cognitive function as a predictor of survival in patients with recurrent malignant glioma. J Clin Oncol, 2000. 18(3): p. 646-50.
13. Klein, M., et al., Neurobehavioral status and health-related quality of life in newly diagnosed high-grade glioma patients. J Clin Oncol, 2001. 19(20): p. 4037-47.
14. Gilbert, M., T. Armstrong, and C. Meyers, Issues in assessing and interpreting quality of life in patients with malignant glioma. Semin Oncol, 2000. 27(3 Suppl 6): p. 20-6.
15. Liu, R., et al., Quality of life in adults with brain tumors: current knowledge and future directions. Neuro Oncol, 2009. 11(3): p. 330-9.
16. Budrukkar, A., et al., Prospective assessment of quality of life in adult patients with primary brain tumors in routine neurooncology practice. J Neurooncol, 2009. 95(3): p. 413-9.
17. Brown, P.D., et al., Prospective study of quality of life in adults with newly diagnosed high-grade gliomas. J Neurooncol, 2006. 76(3): p. 283-91.
18. Taylor, B.V., et al., Effects of radiation and chemotherapy on cognitive function in patients with high-grade glioma. J Clin Oncol, 1998. 16(6): p. 2195-201.
19. Gregor, A., et al., Neuropsychometric evaluation of long-term survivors of adult brain tumours: relationship with tumour and treatment parameters. Radiother Oncol, 1996. 41(1): p. 55-9.
20. Jones, L.W., et al., Assessment of physical functioning in recurrent glioma: preliminary comparison of performance status to functional capacity testing. J Neurooncol, 2009. 94(1): p. 79-85.
21. Peters, K., et al. Neurocognitive dysfunction in newly diagnosed high-grade glioma patients: utilization of standardized computerized neurocognitive testing. in Society for Neuro-Oncology. 2013. San Francisco, CA.
22. Gualtieri, C.T. and L.G. Johnson, Reliability and validity of a computerized neurocognitive test battery, CNS Vital Signs. Arch Clin Neuropsychol, 2006. 21(7): p. 623-43.
23. Peters, K.B., et al. Long-term survivorship in primary glioblastoma: a focus on quality of life. in Society for Neuro-Oncology. 2012. Washington, DC.
24. Keefe RS, Goldberg TE, Harvey PD, Gold JM, Poe MP, Coughenour L. The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. Schizophr Res. 2004;68(2-3):283-97. doi: 10.1016/j.schres.2003.09.011. PubMed PMID: 15099610.
25. Keefe RS, Harvey PD, Goldberg TE, Gold JM, Walker TM, Kennel C, et al. Norms and standardization of the Brief Assessment of Cognition in Schizophrenia (BACS). Schizophr Res. 2008;102(1-3):108-15. doi: 10.1016/j.schres.2008.03.024. PubMed PMID: 18495435.

26. Atkins AS, Tseng T, Vaughan A, Twamley EW, Harvey P, Patterson T, et al. Validation of the tablet-administered Brief Assessment of Cognition (BAC App). Schizophr Res. 2017;181:100-6. doi: 10.1016/j.schres.2016.10.010. PubMed PMID: 27771201.
27. Weitzner, M.A., et al., The Functional Assessment of Cancer Therapy (FACT) scale. Development of a brain subscale and revalidation of the general version (FACT-G) in patients with primary brain tumors. Cancer, 1995. 75(5): p. 1151-61.
28. Chawla, S., et al., Radiation Injury to the Normal Brain Measured by 3D-Echo-Planar Spectroscopic Imaging and Diffusion Tensor Imaging: Initial Experience. J Neuroimaging, 2013.
29. Haris, M., et al., Serial diffusion tensor imaging to characterize radiation-induced changes in normal-appearing white matter following radiotherapy in patients with adult low-grade gliomas. Radiat Med, 2008. 26(3): p. 140-50.
30. Xu, J.L., et al., Distinction between postoperative recurrent glioma and radiation injury using MR diffusion tensor imaging. Neuroradiology, 2010. 52(12): p. 1193-9.
31. Hope, T.R., et al., Serial diffusion tensor imaging for early detection of radiation-induced injuries to normal-appearing white matter in high-grade glioma patients. J Magn Reson Imaging, 2014.
32. Nagesh, V., et al., Radiation-induced changes in normal-appearing white matter in patients with cerebral tumors: a diffusion tensor imaging study. Int J Radiat Oncol Biol Phys, 2008. 70(4): p. 1002-10.
33. Ravn, S., et al., Differences in supratentorial white matter diffusion after radiotherapy--new biomarker of normal brain tissue damage? Acta Oncol, 2013. 52(7): p. 1314-9.
34. Chapman, C.H., et al., Diffusion tensor imaging of normal-appearing white matter as biomarker for radiation-induced late delayed cognitive decline. Int J Radiat Oncol Biol Phys, 2012. 82(5): p. 2033-40.
35. Butler, J.M., Jr., et al., A phase III, double-blind, placebo-controlled prospective randomized clinical trial of d-threo-methylphenidate HCl in brain tumor patients receiving radiation therapy. Int J Radiat Oncol Biol Phys, 2007. 69(5): p. 1496-501.
36. Kaleita, T.A., et al. Pilot study of modafinil for treatment of neurobehavioral dysfunction and fatigue in adult patients with brain tumors. in American Society of Clinical Oncology. 2006. Atlanta, GA: Journal of Clinical Oncology.
37. Shaw, E.G., et al., Phase II study of donepezil in irradiated brain tumor patients: effect on cognitive function, mood, and quality of life. J Clin Oncol, 2006. 24(9): p. 1415-20.
38. Zhao, W. and M.E. Robbins, Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. Curr Med Chem, 2009. 16(2): p. 130-43.
39. Navarro, J., et al., Blood glutathione as an index of radiation-induced oxidative stress in mice and humans. Free Radic Biol Med, 1997. 22(7): p. 1203-9.
40. Moeller, B.J., et al., Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. Cancer Cell, 2004. 5(5): p. 429-41.
41. Moulder, J.E. and E.P. Cohen, Future strategies for mitigation and treatment of chronic radiation-induced normal tissue injury. Semin Radiat Oncol, 2007. 17(2): p. 141-8.
42. Stone, H.B., et al., Models for evaluating agents intended for the prophylaxis, mitigation and treatment of radiation injuries. Report of an NCI Workshop, December 3-4, 2003. Radiat Res, 2004. 162(6): p. 711-28.
43. Takahashi, I., et al., Clinical study of the radioprotective effects of Amifostine (YM-08310, WR-2721) on chronic radiation injury. Int J Radiat Oncol Biol Phys, 1986. 12(6): p. 935-8.
44. Tannehill, S.P. and M.P. Mehta, Amifostine and radiation therapy: past, present, and future. Semin Oncol, 1996. 23(4 Suppl 8): p. 69-77.
45. Batinic-Haberle, I., et al., Diverse functions of cationic Mn(III) N-substituted pyridylporphyrins, recognized as SOD mimics. Free Radic Biol Med, 2011. 51(5): p. 1035-53.
46. Batinic-Haberle, I., et al., SOD therapeutics: latest insights into their structure-activity relationships and impact on the cellular redox-based signaling pathways. Antioxid Redox Signal, 2014. 20(15): p. 2372-415.
47. Batinic-Haberle, I., et al., Mechanistic considerations of the therapeutic effects of Mn porphyrins, commonly regarded as SOD mimics. Anticancer Therapy: Lessons from Brain and Lymphoma Studies. Supplement 2(0) 65.
48. Gridley, D.S., et al., Radiation and a metalloporphyrin radioprotectant in a mouse prostate tumor model. Anticancer Res, 2007. 27(5A): p. 3101-9.

49. Rabbani, Z.N., et al., Antiangiogenic action of redox-modulating Mn(III) meso-tetrakis(N-ethylpyridinium-2-yl)porphyrin, MnTE-2-PyP(5+), via suppression of oxidative stress in a mouse model of breast tumor. Free Radic Biol Med, 2009. 47(7): p. 992-1004.
50. Lee, M.Y., Y. Wang, and P.M. Vanhoutte, Senescence of cultured porcine coronary arterial endothelial cells is associated with accelerated oxidative stress and activation of NFkB. J Vasc Res, 2010. 47(4): p. 287-98.
51. Khan, I., I. Batinic-Haberle, and L.T. Benov, Effect of potent redox-modulating manganese porphyrin, MnTM-2-PyP, on the Na(+)/H(+) exchangers NHE-1 and NHE-3 in the diabetic rat. Redox Rep, 2009. 14(6): p. 236-42.
52. Batinic-Haberle, I., J.S. Reboucas, and I. Spasojevic, Superoxide dismutase mimics: chemistry, pharmacology, and therapeutic potential. Antioxid Redox Signal, 2010. 13(6): p. 877-918.
53. Sheng, H., et al., Effects of metalloporphyrin catalytic antioxidants in experimental brain ischemia. Free Radic Biol Med, 2002. 33(7): p. 947-61.
54. Bao, F. and D. Liu, Hydroxyl radicals generated in the rat spinal cord at the level produced by impact injury induce cell death by necrosis and apoptosis: protection by a metalloporphyrin. Neuroscience, 2004. 126(2): p. 285-95.
55. Ling, X. and D. Liu, Temporal and spatial profiles of cell loss after spinal cord injury: Reduction by a metalloporphyrin. J Neurosci Res, 2007. 85(10): p. 2175-85.
56. Liu, D., et al., Peroxynitrite generated at the level produced by spinal cord injury induces peroxidation of membrane phospholipids in normal rat cord: reduction by a metalloporphyrin. J Neurotrauma, 2005. 22(10): p. 1123-33.
57. Li, H., et al., Mn(III) meso-tetrakis-(N-ethylpyridinium-2-yl) porphyrin mitigates total body irradiation-induced long-term bone marrow suppression. Free Radic Biol Med, 2011. 51(1): p. 30-7.
58. Weitzel DH, Tovmasyan A, Ashcraft KA, Rajic Z, Weitner T, Liu C, Li W, Buckley AF, Prasad MR, Young KH, Rodriguiz RM, Wetsel WC, Peters KB, Spasojevic I, Herndon JE 2nd, Batinic-Haberle I, Dewhirst MW. Radioprotection of the brain white matter by Mn(III) N-butoxyethylpyridylporphyrin-based superoxide dismutase mimic, MnTnBuOE-2-PyP5+. Mol Cancer Ther. 2015 Jan; 14(1):70-9. PMID: 25319393, PMC4397941.
59. Garofalo, M.C., et al., A pilot study in rhesus macaques to assess the treatment efficacy of a small molecular weight catalytic metalloporphyrin antioxidant (AEOL 10150) in mitigating radiation-induced lung damage. Health Phys, 2014. 106(1): p. 73-83.
60. Moeller, B.J., et al., Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity. Cancer Cell, 2005. 8(2): p. 99-110.
61. Semenza, G.L., Oxygen sensing, homeostasis, and disease. N Engl J Med, 2011. 365(6): p. 537-47.
62. Schreck, R., K. Albermann, and P.A. Baeuerle, Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic Res Commun, 1992. 17(4): p. 221-37.
63. Koga, F., et al., ErbB2 and NFkappaB overexpression as predictors of chemoradiation resistance and putative targets to overcome resistance in muscle-invasive bladder cancer. PLoS One, 2011. 6(11): p. e27616.
64. Weitzel DH, Tovmasyan A, Ashcraft KA, Boico A, Birer SR, Roy Choudhury K, Herndon J 2nd, Rodriguiz RM, Wetsel WC, Peters KB, Spasojevic I, Batinic-Haberle I, Dewhirst MW. Neurobehavioral radiation mitigation to standard brain cancer therapy regimens by Mn(III) n-butoxyethylpyridylporphyrin-based redox modifier. Environ Mol Mutagen 2016 Jun; 57(5):372-81. PMID: 27224425.
65. Stupp, R., et al., Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med, 2005. 352(10): p. 987-96.
66. Ellingson BM, Wen PY, Cloughesy TF. Modified Criteria for Radiographic Response Assessment in Glioblastoma Clinical Trials. Neurotherapeutics. 2017;14(2):307-20. PMID: 28108885; PMC5398984.
67. Van Belle, S., et al., Comparison of proposed diagnostic criteria with FACT-F and VAS for cancer-related fatigue: proposal for use as a screening tool. Support Care Cancer, 2005. 13(4): p. 246-54.
68. Jacobs, S.R., et al., Evaluation of the functional assessment of cancer therapy cognitive scale with hematopoietic stem cell transplant patients. J Pain Symptom Manage, 2007. 33(1): p. 13-23.
69. Beck, A.T., et al., Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. J Pers Assess, 1996. 67(3): p. 588-97.
70. Godin, G. and R.J. Shephard, A simple method to assess exercise behavior in the community. Can J Appl Sport Sci, 1985. 10(3): p. 141-6.

71. Brandt, J. The Hopkins Verbal Learning Test: Development of a new memory test with six equivalent forms. Clin Neuropsychol, 1991. 5(2): p. 125–42.
72. Reitan, R. Validity of the Trail Making Test as an indicator of organic brain damage. Percept Mot Skills, 1958. 8: p. 271–6.
73. Ruff, R.M., et al. Benton Controlled Oral Word Association Test: Reliability and updated norms. Arch Clin Neuropsychol, 1996 11: p. 329-38.
74. Cheung, Y.K., Dose Finding by the Continual Reassessment Method. 2011.
75. Cheung, Y.K., Coherence principles in dose-finding studies. Biometrika, 2005. 92: p. 863-873.
76. Lee, S.M. and C. Ying Kuen, Model calibration in the continual reassessment method. Clin Trials, 2009. 6(3): p. 227-38.
77. Cheung, M., et al., A method for the consistent creation and quantitative testing of postoperative pelvic adhesions in a porcine model. J Invest Surg, 2009. 22(1): p. 56-62.
78. O'Quigley, J., M. Pepe, and L. Fisher, Continual reassessment method: a practical design for phase 1 clinical trials in cancer. Biometrics, 1990. 46(1): p. 33-48.
79. Wen, P.Y., et al., Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol, 2010. 28(11): p. 1963-72.
80. Ratain, M.J. and D.J. Sargent , Optimising the design of phase II oncology trials: the importance of randomisation. Eur J Cancer, 2009. 45(2): p. 275-80.
81. Rubinstein, L.V., et al., Design issues of randomized phase II trials and a proposal for phase II screening trials. J Clin Oncol, 2005. 23(28): p. 7199-206.
82. Rubinstein, L., et al., Randomized phase II designs. Clin Cancer Res, 2009. 15(6): p. 1883-90.

19 APPENDICES

19.1 Appendix A: RANO (Radiographic Assessment in Neuro-Oncology) Criteria for Newly Diagnosed Patients

RANO was published in April 2010 for the evaluation of HGG [79] and modified in 2017 [66].
For newly diagnosed HGG studies, the post radiation therapy MRI scan is required and is used as the baseline scan for which response will be determined. The post-operative MRI scan and pre-entry MRI scan are also desired and will vary based on the requirements of clinical protocols. In these criteria, particularly in regards to newly diagnosed subjects, the areas considered in determining comprehensive objective status in RANO criteria are target lesions (enhancing disease), new sites of measurable disease, neurological examination status, and corticosteroid usage and dose.

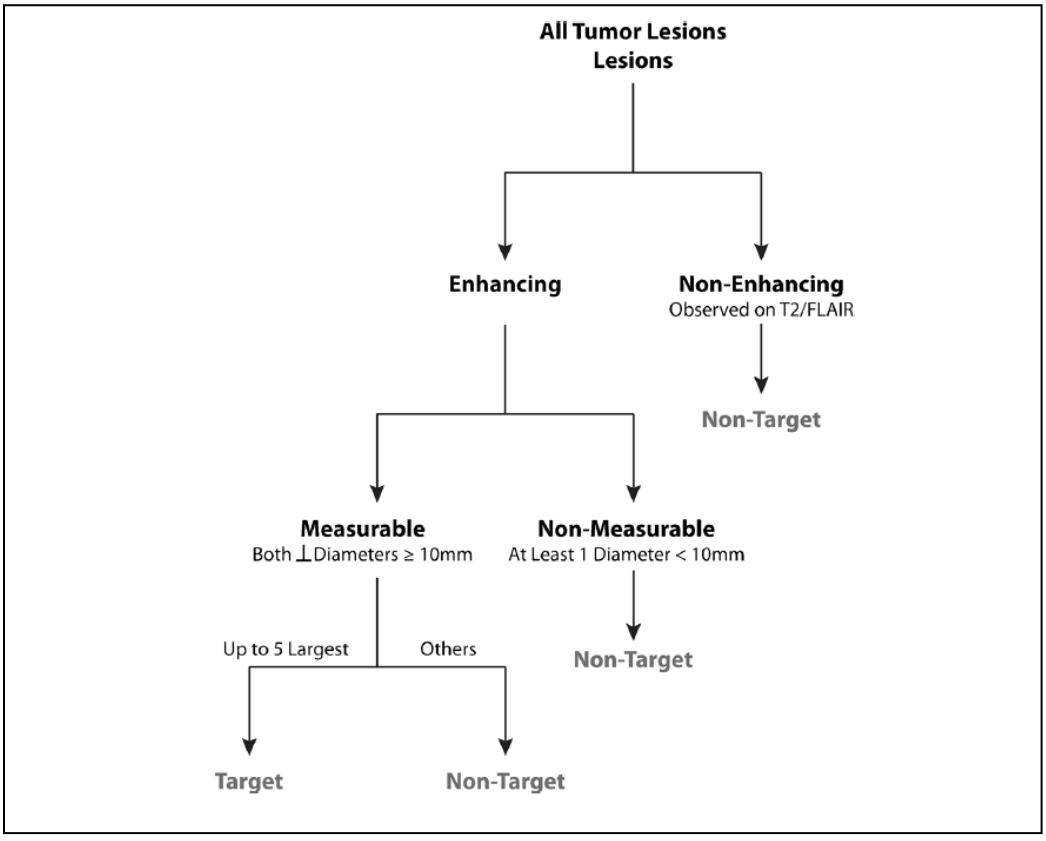
Definitions

Measurable lesions

- Contrast enhancing
- Two perpendicular diameters ≥10mm
- If slice + gap > 5mm, minimum is 2 x total
- Do not include cavity, cyst, or necrosis in the measurement

Non-measurable lesions

- Do not enhance (seen only on T2/FLAIR)
- Too small, e.g. 12 x 8 mm
- Poorly defined margin



Choosing a target lesion (2)

Target Lesions:

Take products of diameters and add them up → Sum of products of diameters (SPD)

A1 x B1 + A2 x B2 + … = SPD

Complete Response (CR): Requires all of the following:

- Disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. The first scan exhibiting disappearance of all enhancing measurable and non-measurable disease is considered "preliminary CR". If the second scan exhibits measurable enhancing disease with respect to the "preliminary CR" scan, then the response is not sustained, noted as "pseudoresponse", PsR, and is now considered "preliminary PD". If the second scan continues to exhibit disappearance of enhancing disease or emergence of non-measurable disease (less than 10mm bidimensional product), it is considered a durable CR and the patient should continue on therapy until confirmed PD is observed.
- Patients should be either off of corticosteroids or on physiologic replacement doses only.
- Stable or improved neurological examinations

Partial Response (PR): Requires all of the following

- Greater than or equal to a 50% reduction in the size (products of the largest perpendicular diameters) for all enhancing lesions compared with baseline, sustained for at least 4 weeks. The first scan exhibiting greater than or equal to a 50% decrease in the sum of the products of the perpendicular diameters of all measurable enhancing lesions compared with the baseline is considered "preliminary PR". If the second scan exhibits PD with respect to the "preliminary PR" scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered "preliminary PD" (note confirmed PD requires at least two sequential increases in tumor volume.) If the second scan exhibits SD, PR, or CR, it is considered a durable PR and the patient should continue on therapy until confirmed PD is observed.

- Patients should have either the same corticosteroid doses or on lower corticosteroid dose compared with baseline scan.

- Stable or improved neurological examinations

Stable Disease (SD): Evaluations that do not meet criteria for CR, PR or PD

Progressive Disease (PD): Defined as the following

- Greater than or equal to a 25% increase in the product of the largest perpendicular diameters of any enhancing lesion or any new enhancing tumor on MRI scans. The first scan exhibiting greater than or equal to a 25% increase in the sum of the products of perpendicular diameter of the enhancing lesions should be compared to the smallest tumor measurement obtained either at baseline (if no decrease) or best response (on stable or increasing corticosteroid dose) and is noted as "preliminary PD". If the second scan at least 4 weeks later exhibits a subsequent greater than or equal to 25% increase in the sum of products of perpendicular diameters of the enhancing lesions relative to the "preliminary PD" scan, it is considered "confirmed PD" and the patient should be discontinued from the study. If the second scan at least 4 weeks later exhibits SD or, PR/CR, this scan showing "preliminary PD" is noted as "pseudoprogression", PsP, and the subject should remain on study until a second increase in tumor size relative to the PsP scan is observed. Of note, any new measurable (> 10 mm X 10 mm) enhancing lesion

should not be immediately considered PD, but instead should be added to the sum of the bidimensional products representing the entire enhancing tumor burden.

- In the case where the baseline or best response demonstrates no measurable enhancing disease (visible or not visible), then any new measurable (>10mm x 10mm) enhancing lesions are considered PD after confirmed by a subsequent scan ≥4 weeks exhibiting greater than or equal to a 25% increase in sum of products of perpendicular diameters relative to the scan first illustrating new measurable disease. The first scan exhibiting new measurable disease is noted as "preliminary PD". If the second scan at least 4 weeks later exhibits a subsequent greater than or equal to a 25% increase in sum of products of perpendicular of enhancing lesions relative to the " preliminary PD" scan it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD, CR, PR, or becomes non-measurable, this scan showing "preliminary PD" is noted as "pseudoprogression", PsP, and the subject should continue on study until a second increase in tumor size relative to the "preliminary PD", or PsP, scan is observed. Note that any new measurable (>10mm x 10mm) enhancing lesions on the subsequent scan following the "preliminary PD" scan should not be immediately considered "confirmed PD", but instead should be added to the sum of bidimensional products representing the entire enhancing tumor burden.

- Unequivocal and significant worsening neurological examination not attributable to other causes.

- Of note, if there has been a major reduction in steroid dosage in the interval and the patient is felt to be clinically stable or improved, the proper assessment may be "indeterminate" and the therapy could be continued pending the next evaluation.

- Failure to return for evaluation as a result of death or deteriorating condition.

Not Evaluable (NE): Progression has not been documented and one or more sites have not been assessed.

Response Evaluation:

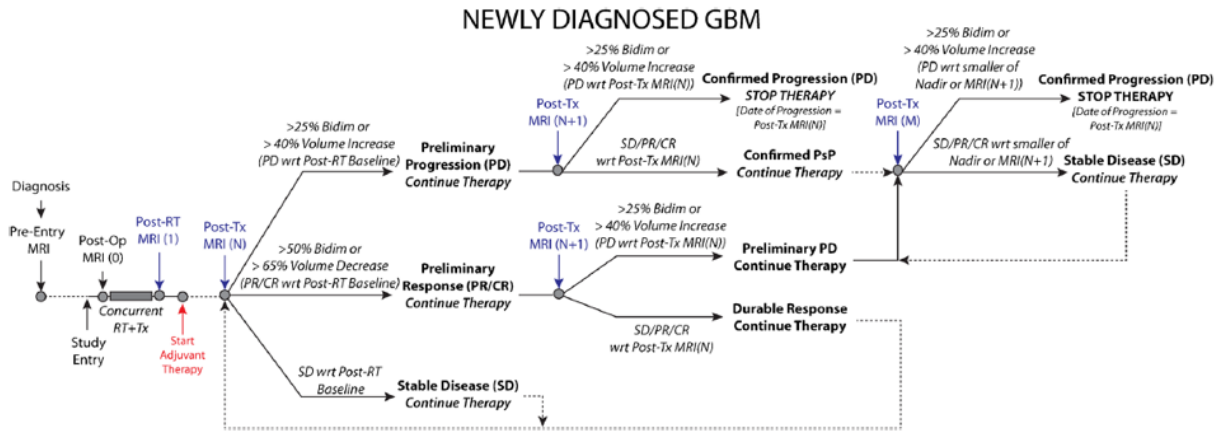
Table 3 Guidelines for determining comprehensive objective status

Target lesions (current scan)	Target lesions (previous scan)	New sites of measurable disease[a]	Neurological status	Steroid usage	Steroid dose	Overall objective status
CR	Not Evaluated	No	Stable/Better	No	N/A	Preliminary CR
PR	Not Evaluated	No	Stable/Better	Any	Stable/Decreasing	Preliminary PR
PD	Not Evaluated	Yes or No	Stable/Better	Any	Stable/Increasing	Preliminary PD
PD	Preliminary or Confirmed PR/CR	No	Stable/Better	Any	Stable/Increasing	Preliminary PD
SD	Preliminary or Confirmed CR/PR or SD/NE	No	Stable/Better	Any	N/A	SD
PR	Preliminary PR	Yes or No	Stable/Better	Any	Stable/Decreasing	Confirmed PR
SD	Preliminary PR	Yes or No	Stable/Better	Any	Stable/Decreasing	SD (Preliminary PR →Confirmed PR)
SD	Preliminary CR	Yes or No	Stable/Better	Any	Stable/Decreasing	SD (Preliminary CR →Confirmed CR)
CR	Preliminary CR	No	Stable/Better	No	N/A	Confirmed CR
SD	Preliminary PD	No	Stable/Better	Any	Stable/Decreasing	SD (Confirmed PsP)
CR/PR/SD PD/NE	CR/PR/SD/PD/NE	Yes or No	Worse	Any	Stable/Increasing	Confirmed PD
PD	Preliminary PD	Yes or No	Any	Yes	Stable/Increasing	Confirmed PD

[a] Note that new sites of measurable disease are added to the sum of bidimensional products or total lesion volume, or constitutes preliminary PD in the case of no measurable disease at baseline or best response

CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease, PsP=pseudoprogression, NE=not evaluable

Algorithm for Newly Diagnosed Glioblastoma (2)



MRI (0)= pre-entry MRI, MRI (N)=additional required MRI scan, MRI (N+1)=follow-up confirmatory MRI scan. Please note that while this figure references volumetric analysis, our standard operating procedure is to use bi-dimensional measures.

19.2 Appendix B: Pharmacokinetic Analysis

19.2.1 **Phase 1 Pharmacokinetic Analysis**

The major goal of the pharmacokinetic studies conducted in conjunction with this protocol will be to determine pharmacokinetics of BMX-001 in combination with RT and TMZ. These samples will be collected from patients during chemoradiation phase only.

Pharmacokinetic Assessment of BMX-001:. A series of blood samples will be taken for the measurement of the compound in order to define pharmacokinetics in these patients. Patients should have their dose administered on the same time of day. Instructions for the handling and shipping of plasma samples collected for pharmacokinetic analysis of BMX-001 are provided below. Blood sample volumes and schedule of samples are specified below.

Pharmacokinetic Blood Sample Collection and Handling: Blood samples (4 ml) will be collected into pre-cooled (ice bath) heparinized tubes and gently inverted 8-10 times and immediately placed in the ice bath. Within 30 minutes, plasma will be prepared by centrifugation (2000 x g, 4^0C, 10 min). Following centrifugation, 1 mL plasma will be transferred by polypropylene disposable pipette to a 2-mL polypropylene cryogenic vial containing 20 μL of 50% citric acid, inverted 8-10 times, and stored frozen at -80^0C until shipment and analysis.

Blood Sampling Time Schedule for BMX-001: Drug pharmacokinetics will be obtained on loading dose day, Day 8 or the next date on which drug is administered, and Day 36 or the next date on which drug is administered during the chemoradiation phase. Measures will be obtained at the following time-points: Pre Dose, 0.5 hour, 1 hour, 2 hours, 6 hours and 24 hours. Day 8 and Day 36 are intended to be the first maintenance dose of the second week and the first maintenance dose of the sixth week following initiation of radiation therapy.

PK Labelling: PK samples will labelled such that they are blinded to Dr. Ivan Spasojevic's labs. The cyro-vials provided to his lab will labelled with random numbers generated by statisticians and kept in a master file maintained by the study team. Samples will not be identified by patient, timepoint, dose level or cohort prior to being analysed by Dr. Spasojevic. Data generated by the samples will be evaluated after each enrolled cohort. Labelling and blinding procedures will be specified in the Blood Collection and Plasma Separation Storage SOP.

Analytical Method: Concentrations of BMX-001 will be determined using a high-pressure liquid chromatography – tandem-mass spectrometry (LC/MS/MS) by laboratory personnel designated by BioMimetix JV, LLC.

Samples will be taken/shipped to Dr. Ivan Spasojevic at the following address:

Ivan Spasojevic, PhD
Pharmacology Lab
Duke Hospital South
Orange Zone, Room 5317
200 Trent Drive
Durham, NC 27710
Tel: 919-684-8311
Cell: 919-323-5927
Fax: 919-684-8380

Pharmacokinetic Sample Collection for BMX-001 in Combination with Current Chemoradiation

Phase 1 Study Day	Timing	Range of time to be collected within
Loading Dose Day	Pre-dose	N/A
	30 minutes post-dose	+/- 2 minutes
	1 hour post-dose	+/- 5 minutes
	2 hours post-dose	+/- 5 minutes
	6 hours post-dose	+/- 5 minutes
	24 hours post-dose	+/- 10 minutes
Day 8 or the next day when drug is administered	Pre-dose	N/A
	30 minutes post-dose	+/- 2 minutes
	1 hour post-dose	+/- 5 minutes
	2 hours post-dose	+/- 5 minutes
	6 hours post-dose	+/- 5 minutes
	24 hours post-dose	+/- 10 minutes
Day 36 or the next day when drug is administered	Pre-dose	N/A
	30 minutes post-dose	+/- 2 minutes
	1 hour post-dose	+/- 5 minutes
	2 hours post-dose	+/- 5 minutes
	6 hours post-dose	+/- 5 minutes
	24 hours post-dose	+/- 10 minutes

19.2.2 **Phase 2 Pharmacokinetic Analysis**

In order to provide a wider population sample for pharmacokinetics, we will obtain sparse sampling from 6 patients in the Phase 2 Arm A portion of the study.

The major goal of the pharmacokinetic studies conducted in conjunction with this protocol will be to determine pharmacokinetics of BMX-001 in combination with RT and TMZ. These samples will be collected from patients during chemoradiation phase only.

Pharmacokinetic Assessment of BMX-001: A series of blood samples will be taken for the measurement of the compound in order to define pharmacokinetics in these patients. Patients should have their dose administered on the same time of day. Instructions for the handling and shipping of plasma samples collected for pharmacokinetic analysis of BMX-001 are provided below. Blood sample volumes and schedule of samples are specified below.

Pharmacokinetic Blood Sample Collection and Handling: Blood samples (4 ml) will be collected into pre-cooled (ice bath) heparinized tubes and gently inverted 8-10 times and immediately placed in the ice bath. Within 30 minutes, plasma will be prepared by centrifugation (2000 x g, 4^0C, 10 min). Following centrifugation, 1 mL plasma will be transferred by polypropylene disposable pipettes to create two 2-mL polypropylene cryogenic vial aliquots containing 20 μL of 50% citric acid, inverted 8-10 times, and stored frozen at -80^0C until shipment and analysis. One aliquot is acceptable if samples are not being shipped.

Blood Sampling Time Schedule for BMX-001: Drug pharmacokinetics will be obtained on Day 8 or the next day when drug is administered and Day 36 or the next day when drug is administered during the chemoradiation phase. Measures will be obtained at the following time-points: Pre Dose, 0.5 hour, 1 hour, 2 hours, 6 hours and 24 hours.

Analytical Method: Concentrations of BMX-001 will be determined using a high-pressure liquid chromatography – tandem-mass spectrometry (LC/MS/MS) by laboratory personnel designated by BioMimetix JV, LLC.

Samples will be taken/shipped to Dr. Ivan Spasojevic at the following address:

Ivan Spasojevic, PhD
Pharmacology Lab
Duke Hospital South
Orange Zone, Room 5317
200 Trent Drive
Durham, NC 27710
Tel: 919-684-8311
Cell: 919-323-5927
Fax: 919-684-8380

Pharmacokinetic Sample Collection for BMX-001 in Combination with Current Chemoradiation

Phase 2 Study Day	Timing	Range of time to be collected within
Day 8 or the next day when drug is administered	Pre-dose	N/A
	30 minutes post-dose	+/- 2 minutes
	1 hour post-dose	+/- 5 minutes
	2 hours post-dose	+/- 5 minutes
	6 hours post-dose	+/- 5 minutes
	24 hours post-dose	+/- 10 minutes
Day 36 or the next day when drug is administered	Pre-dose	N/A
	30 minutes post-dose	+/- 2 minutes
	1 hour post-dose	+/- 5 minutes
	2 hours post-dose	+/- 5 minutes
	6 hours post-dose	+/- 5 minutes
	24 hours post-dose	+/- 10 minutes

19.3 APPENDIX C: Statistical Tables

19.3.1 PROBABILITY OF MTD SELECTION WITH A LOGISTIC MODEL IN THE SECOND STAGE WITH VARIOUS TRUE UNDERLYING TOXICITY PROFILES AND COHORTS OF 2 AFTER FIRST STAGE WITH COHORTS OF 3

Initial Guess of Toxicity Probability=0.101 / 0.143 / 0.193 / 0.25 (delta=0.03; logistic)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0020	0.0060	0.0250	0.9670
21	0.0020	0.0060	0.0145	0.9775
24	0.0000	0.0035	0.0095	0.9870

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0045	0.0220	0.0815	0.8920
21	0.0030	0.0200	0.0830	0.8940
24	0.0010	0.0140	0.0790	0.9060

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0050	0.0290	0.1270	0.8390
21	0.0050	0.0305	0.1420	0.8225
24	0.0010	0.0210	0.1770	0.8010

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.2395	0.3665	0.2455	0.1485
21	0.2315	0.3970	0.2645	0.1070
24	0.2390	0.4285	0.2600	0.0725

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.3230	0.1915	0.2470	0.2385
21	0.3010	0.2430	0.2285	0.2275
24	0.2920	0.2590	0.2715	0.1775

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.4900	0.2455	0.1705	0.0940
21	0.4775	0.2945	0.1605	0.0675
24	0.5050	0.2935	0.1595	0.0420

Initial Guess of Toxicity Probability=0.044 / 0.089 / 0.158 / 0.25 (delta=0.05; logistic)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0030	0.0205	0.0465	0.9300
21	0.0015	0.0125	0.0475	0.9385
24	0.0015	0.0095	0.0280	0.9610

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0045	0.0305	0.1335	0.8315
21	0.0020	0.0255	0.1390	0.8335
24	0.0015	0.0180	0.1315	0.8490

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0030	0.0375	0.2020	0.7575
21	0.0015	0.0335	0.2160	0.7490
24	0.0015	0.0225	0.2215	0.7545

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.2040	0.4325	0.2820	0.0815
21	0.1855	0.4765	0.2810	0.0570
24	0.1840	0.5280	0.2490	0.0390

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.3200	0.2405	0.2740	0.1655
21	0.3050	0.2650	0.2900	0.1400
24	0.2935	0.2770	0.3040	0.1255

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.4685	0.3100	0.1735	0.0480
21	0.4635	0.3370	0.1680	0.0315
24	0.4555	0.3760	0.1410	0.0275

Initial Guess of Toxicity Probability=0.016 / 0.051 / 0.124 / 0.250 (delta=0.07; logistic)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0030	0.0410	0.0905	0.8655
21	0.0020	0.0250	0.1070	0.8660
24	0.0020	0.0160	0.0730	0.9090

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0030	0.0690	0.2005	0.7275
21	0.0035	0.0470	0.2170	0.7325
24	0.0025	0.0310	0.2010	0.7655

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0030	0.0530	0.2710	0.6730
21	0.0015	0.0330	0.3205	0.6450
24	0.0020	0.0245	0.2920	0.6815

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.1765	0.5295	0.2385	0.0555
21	0.1605	0.5580	0.2480	0.0335
24	0.1525	0.5875	0.2400	0.0200

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.3240	0.3060	0.2420	0.1280
21	0.3320	0.3135	0.2730	0.0815
24	0.2905	0.3320	0.2795	0.0980

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.4800	0.3505	0.1350	0.0345
21	0.4900	0.3540	0.1345	0.0215
24	0.4725	0.3790	0.1350	0.0135

19.3.2 PROBABILITY OF MTD SELECTION WITH AN EMPIRIC MODEL IN THE SECOND STAGE WITH VARIOUS TRUE UNDERLYING TOXICITY PROFILES AND COHORTS OF 2 AFTER FIRST STAGE WITH COHORTS OF 3

Initial Guess of Toxicity Probability=0.097 / 0.141 / 0.192 / 0.25 (delta=0.03; empiric)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0020	0.0060	0.0250	0.9670
21	0.0020	0.0060	0.0170	0.9750
24	0.0000	0.0035	0.0100	0.9865

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0045	0.0185	0.0835	0.8935
21	0.0035	0.0200	0.0820	0.8945
24	0.0010	0.0130	0.0705	0.9155

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0050	0.0265	0.1280	0.8405
21	0.0050	0.0305	0.1395	0.8250
24	0.0015	0.0210	0.1540	0.8235

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.2465	0.3540	0.2525	0.1470
21	0.2285	0.4025	0.2600	0.1090
24	0.2340	0.4285	0.2570	0.0805

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.3260	0.1860	0.2480	0.2400
21	0.3000	0.2420	0.2355	0.2225
24	0.2920	0.2555	0.2585	0.1940

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.4935	0.2360	0.1780	0.0925
21	0.4760	0.2960	0.1605	0.0675
24	0.5025	0.2955	0.1545	0.0475

Initial Guess of Toxicity Probability=0.036 / 0.084 / 0.157 / 0.25 (delta=0.05; empiric)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0050	0.0160	0.0430	0.9360
21	0.0020	0.0155	0.0430	0.9395
24	0.0010	0.0120	0.0330	0.9540

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0055	0.0260	0.1335	0.8350
21	0.0020	0.0255	0.1250	0.8475
24	0.0015	0.0190	0.1205	0.8590

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0045	0.0305	0.2050	0.7600
21	0.0020	0.0325	0.2090	0.7565
24	0.0010	0.0185	0.2275	0.7530

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.1755	0.4645	0.2600	0.1000
21	0.1835	0.4690	0.2955	0.0520
24	0.1430	0.5430	0.2700	0.0440

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.3030	0.2635	0.2635	0.1700
21	0.2920	0.2850	0.2750	0.1480
24	0.2805	0.2875	0.3020	0.1300

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.4520	0.3415	0.1520	0.0545
21	0.4705	0.3325	0.1680	0.0290
24	0.4415	0.3755	0.1560	0.0270

Initial Guess of Toxicity Probability=0.009 / 0.043 / 0.124 / 0.25 (delta=0.07; empiric)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0040	0.0400	0.0880	0.8680
21	0.0015	0.0285	0.1100	0.8600
24	0.0015	0.0255	0.0820	0.8910

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0045	0.0605	0.1770	0.7580
21	0.0020	0.0450	0.2385	0.7145
24	0.0025	0.0410	0.2030	0.7535

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.0045	0.0500	0.2560	0.6895
21	0.0015	0.0445	0.3085	0.6455
24	0.0015	0.0330	0.3060	0.6595

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.1845	0.5150	0.2400	0.0605
21	0.1655	0.5685	0.2305	0.0355
24	0.1460	0.6090	0.2215	0.0235

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.3185	0.3260	0.2375	0.1180
21	0.3185	0.3200	0.2720	0.0895
24	0.3010	0.3435	0.2830	0.0725

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
18	0.4655	0.3775	0.1200	0.0370
21	0.4770	0.3755	0.1260	0.0215
24	0.4635	0.4060	0.1160	0.0145

19.3.3 PROBABILITY OF MTD SELECTION WITH A LOGISTIC MODEL IN THE SECOND STAGE WITH VARIOUS TRUE UNDERLYING TOXICITY PROFILES AND COHORTS OF 3 AFTER FIRST STAGE WITH COHORTS OF 3

Initial Guess of Toxicity Probability=0.101 / 0.143 / 0.193 / 0.25 (delta=0.03; logistic)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.008	0.020	0.032	0.940
18	0.004	0.006	0.030	0.960
20	0.000	0.010	0.016	0.974

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.016	0.032	0.106	0.846
18	0.004	0.024	0.116	0.856
20	0.002	0.036	0.118	0.844

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.014	0.062	0.128	0.796
18	0.004	0.036	0.162	0.798
20	0.004	0.042	0.160	0.794

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.266	0.304	0.260	0.170
18	0.250	0.378	0.254	0.118
20	0.262	0.374	0.262	0.102

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.372	0.170	0.198	0.260
18	0.328	0.190	0.260	0.222
20	0.348	0.204	0.258	0.190

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.502	0.228	0.162	0.108
18	0.500	0.252	0.180	0.068
20	0.508	0.262	0.176	0.054

Initial Guess of Toxicity Probability=0.044 / 0.089 / 0.158 / 0.25 (delta=0.05; logistic)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.006	0.024	0.072	0.898
18	0.006	0.022	0.060	0.912
20	0.008	0.014	0.050	0.928

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.008	0.050	0.154	0.788
18	0.008	0.036	0.162	0.794
20	0.008	0.034	0.134	0.824

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.006	0.062	0.222	0.710
18	0.008	0.042	0.228	0.722
20	0.008	0.038	0.204	0.750

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.202	0.450	0.256	0.092
18	0.194	0.472	0.254	0.080
20	0.180	0.504	0.252	0.064

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.290	0.308	0.216	0.186
18	0.310	0.282	0.252	0.156
20	0.284	0.302	0.268	0.146

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.454	0.326	0.156	0.064
18	0.464	0.322	0.164	0.050
20	0.446	0.362	0.154	0.038

Initial Guess of Toxicity Probability=0.016 / 0.051 / 0.124 / 0.250 (delta=0.07; logistic)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.024	0.034	0.078	0.864
18	0.010	0.038	0.088	0.864
20	0.012	0.026	0.090	0.872

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.028	0.064	0.188	0.720
18	0.012	0.056	0.202	0.730
20	0.014	0.036	0.226	0.724

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.230	0.480	0.226	0.064
18	0.190	0.510	0.246	0.054
20	0.224	0.486	0.236	0.054

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.392	0.262	0.192	0.154
18	0.348	0.302	0.216	0.134
20	0.374	0.234	0.272	0.120

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.514	0.300	0.160	0.026
18	0.488	0.330	0.142	0.040
20	0.478	0.346	0.144	0.032

19.3.4 PROBABILITY OF MTD SELECTION WITH A EMPIRIC MODEL IN THE SECOND STAGE WITH VARIOUS TRUE UNDERLYING TOXICITY PROFILES AND COHORTS OF 3 AFTER FIRST STAGE WITH COHORTS OF 3

Initial Guess of Toxicity Probability=0.097 / 0.141 / 0.192 / 0.25 (delta=0.03; empiric)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.006	0.018	0.022	0.954
18	0.010	0.012	0.022	0.956
20	0.004	0.012	0.024	0.960

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.012	0.038	0.078	0.872
18	0.010	0.030	0.086	0.874
20	0.006	0.028	0.108	0.858

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.010	0.040	0.124	0.826
18	0.010	0.040	0.116	0.834
20	0.006	0.046	0.184	0.764

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.250	0.336	0.276	0.138
18	0.280	0.350	0.240	0.130
20	0.244	0.448	0.222	0.086

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.322	0.222	0.188	0.268
18	0.312	0.216	0.222	0.250
20	0.342	0.218	0.264	0.176

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.498	0.244	0.170	0.088
18	0.480	0.268	0.164	0.088
20	0.498	0.322	0.122	0.058

Initial Guess of Toxicity Probability=0.036 / 0.084 / 0.157 / 0.25 (delta=0.05; empiric)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.008	0.022	0.062	0.908
18	0.002	0.016	0.048	0.934
20	0.000	0.024	0.032	0.944

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.010	0.048	0.156	0.786
18	0.002	0.034	0.140	0.824
20	0.000	0.036	0.146	0.818

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.008	0.046	0.192	0.754
18	0.002	0.034	0.230	0.734
20	0.000	0.036	0.188	0.776

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.188	0.472	0.246	0.094
18	0.220	0.456	0.254	0.070
20	0.170	0.530	0.252	0.048

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.304	0.282	0.240	0.174
18	0.308	0.278	0.250	0.164
20	0.294	0.296	0.264	0.146

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.456	0.342	0.158	0.044
18	0.488	0.306	0.178	0.028
20	0.436	0.394	0.144	0.026

Initial Guess of Toxicity Probability=0.009 / 0.043 / 0.124 / 0.25 (delta=0.07; empiric)

True Probability of DLT=0.05 / 0.055 / 0.06 / 0.065

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.006	0.036	0.158	0.800
18	0.006	0.042	0.084	0.868
20	0.006	0.018	0.084	0.892

True Probability of DLT=0.05 / 0.07 / 0.11 / 0.2

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.006	0.076	0.262	0.656
18	0.010	0.064	0.234	0.692
20	0.006	0.046	0.218	0.730

True Probability of DLT=0.05 / 0.05 / 0.15 / 0.25

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.006	0.054	0.326	0.614
18	0.006	0.054	0.316	0.624
20	0.006	0.034	0.302	0.658

True Probability of DLT=0.1 / 0.25 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.218	0.488	0.224	0.070
18	0.198	0.522	0.224	0.056
20	0.180	0.544	0.238	0.038

True Probability of DLT=0.2 / 0.2 / 0.3 / 0.4

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.366	0.278	0.240	0.116
18	0.364	0.286	0.244	0.106
20	0.344	0.306	0.254	0.096

True Probability of DLT=0.2 / 0.3 / 0.4 / 0.5

n	Dose Level 1	Dose Level 2	Dose Level 3	Dose Level 4
16	0.510	0.318	0.134	0.038
18	0.508	0.334	0.126	0.032
20	0.472	0.374	0.138	0.016

19.4 Appendix D Cytochrome P450 Drug Interaction Table

Please see the following website for known cytochrome P450 inducers and strong inhibitors. The website is updated frequently as new information becomes available (Flockhart, 2007)

http://medicine.iupui.edu/clinpharm/ddis/main-table/

Dexamethasone is listed on this table, but is allowed in this protocol.

19.5 Appendix E Copy of BAC App screenshots

Brief Assessment of Cognition Application (BAC App)

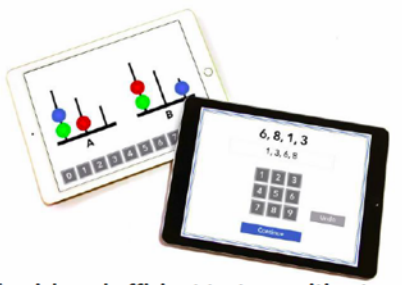
Task description and screen shots

NeuroCog Trials
3211 Shannon Road, Suite 300
Durham, NC 27707 (map)
Phone: +1 919 401-4642
Fax: +1 919 401-4644
Email: info@neurocogtrials.com

BAC App Description

- The **BAC App** was developed by NCT to allow tablet-based delivery and scoring of all subtests included in the original pen and paper BAC/BACS instrument
- The test battery can be:
  - Administered in full to yield subtest and total scores
  - Selected subtests can be chosen for administration
- By ensuring standardized administration of instructions and test stimuli, the BAC App :
  - Reduces error variance due to rater inconsistencies
  - Provides automated scoring
- Results can be:
  - Immediately reviewed on the tablet device (iPad®)
  - Transferred to a central data repository for later analysis

BAC App Overview



- Validated battery of **quick and efficient tests sensitive to cognitive impairment** in a variety of patient populations.
- Six-test battery is available for pen-and-paper and tablet:
  - **Verbal Memory**
  - **Digit Sequencing**
  - **Token Motor Task**
  - **Fluency**
  - **Symbol Coding**
  - **Tower of London**

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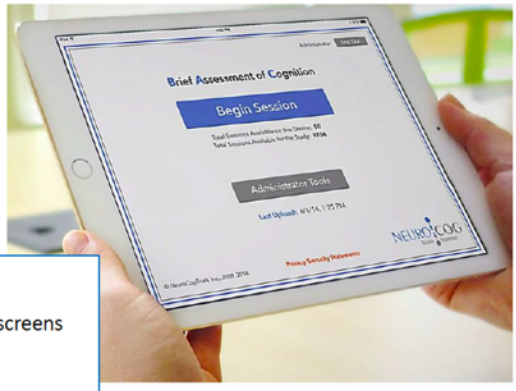
BAC App Cognitive Domains and Tests

Cognitive Domain	Test	Summary
Verbal Memory & Learning	Verbal Memory	Subjects are presented with 15 words and asked to recall as many as possible. This procedure is repeated 5 times.
Working Memory	Digit Sequencing	Subjects are presented with auditory clusters of numbers (e.g. 936) of increasing length and asked to tell the rater the numbers in order from lowest to highest.
Motor Function	Token Motor	Subjects are presented with tokens and asked to drag them to a center container as quickly as possible for 60 seconds.
Verbal Fluency	Semantic Fluency	Subjects are given 60 seconds to generate as many words as possible in a given category.
	Letter Fluency	In two separate trials, subjects are given 60 seconds to generate as many words as possible beginning with a given letter of the alphabet.
Speed of Processing	Symbol Coding	Subjects are provided a key and asked to fill in the corresponding numbers beneath a series of symbols as quickly as possible within 90 seconds.
Executive Function	Tower of London	Subjects are asked to give the minimum number of times the balls in one picture would need to be moved in order to make the arrangement of balls identical to that in the opposing picture.

Materials

- BAC Manual
- iPad®
- iPad® Stand
- Fluency Response Sheet
- Comments Sheet
- Subj. Recorded Statement Script



Prior to Administration

- Acquire stand for ***Administrator Facing*** screens
  - (Blue and grey border around screen)
- Ensure there is no glare for ***Subject Facing*** screens
  - (No border around screen)
- Sound turned on and up
- Quiet room, free of distractions

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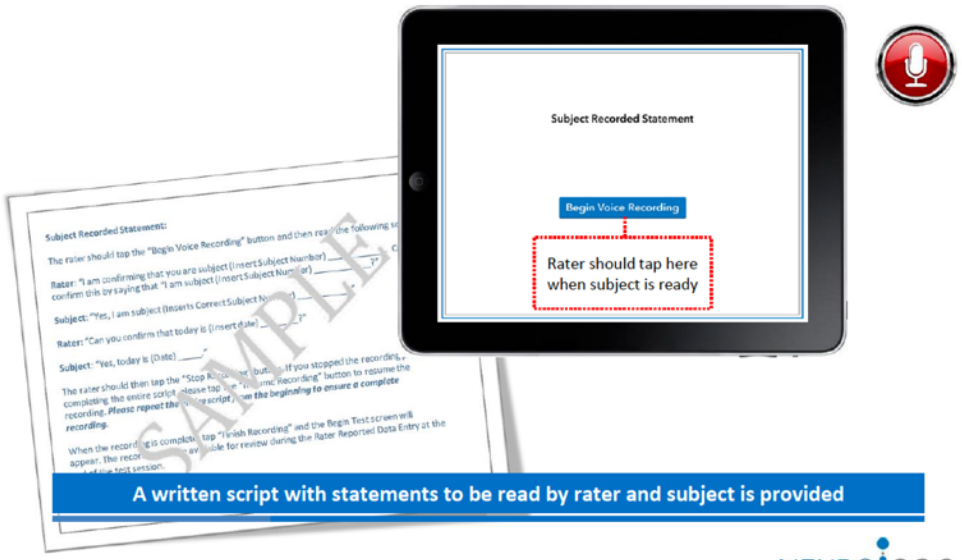
Subject Recorded Statement



A written script with statements to be read by rater and subject is provided

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Verbal Memory

Subjects are presented with 15 words and then asked to recall as many as possible.

Tablet Position	On stand facing administrator
Scored By	Application
Recording	Responses are audio recorded
# of Trials	5
# of Versions	7 (Version 2 omitted during normative BACS study)
Documentation	• Record all responses by tapping on the word. • Addition/Omission of simple suffixes receive credit. • Record intrusions by tapping "Intrusions".
Feedback	• You can tell the subject, if they ask, if they've already said a word. • If subject asks if a word was on the list, tell them that you cannot answer that, but ask if they would like it to be considered a response.

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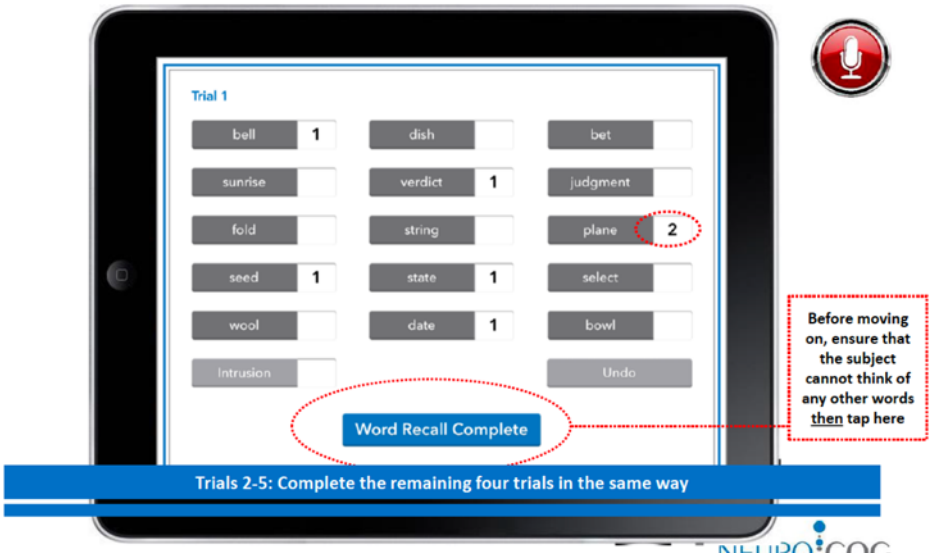
Verbal Memory



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Digit Sequencing

Subjects are presented with auditory clusters of numbers (e.g. 936) of increasing length. They are asked to tell the rater the numbers in order, from lowest to highest.

Tablet Position	On stand facing administrator
Scored By	Application
Recording	Responses are audio recorded
# of Trials	28
Discontinuation	Discontinues after 4 incorrect items in a sequence level
Feedback	Corrective feedback is provided (by the app) during 2 digit items
Prompts	Prompts are provided (by the app) for responses that are highest to lowest or a direct repetition of the presented stimulus (following an incorrect rule)

Before beginning the next test: Rater should move and sit next to the subject and place the iPad® flat on the table in front of the subject

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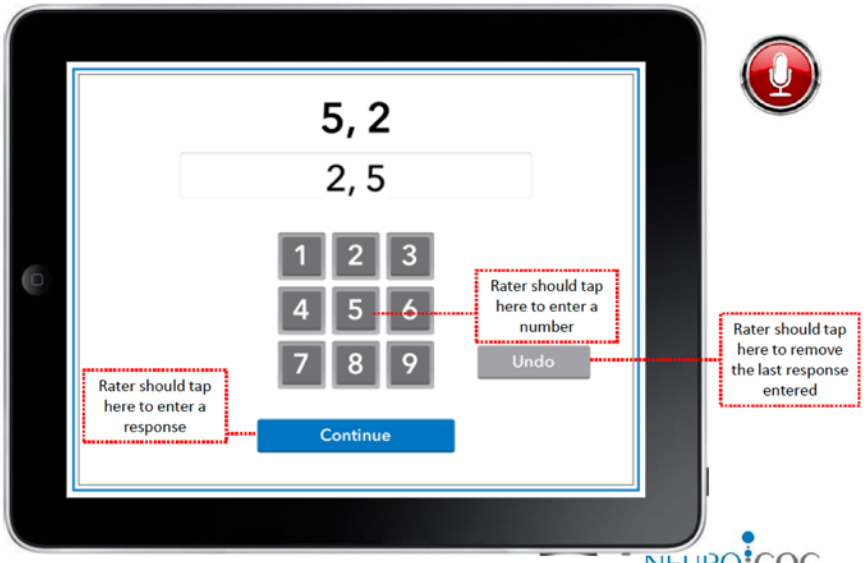
Digit Sequencing



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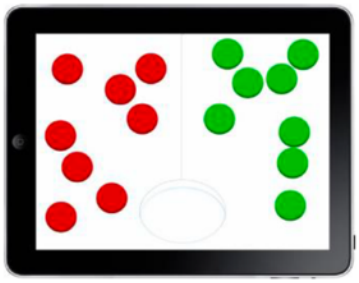
Token Motor

Subjects are given tokens and asked to drag them to a center container as quickly as possible for 60 seconds.

Tablet Position	Flat on table facing subject
Scored By	Application
# of Trials	1
Trial Duration	60 seconds
Discontinuation	Test discontinues if subject cannot complete practice accurately within ten tries.



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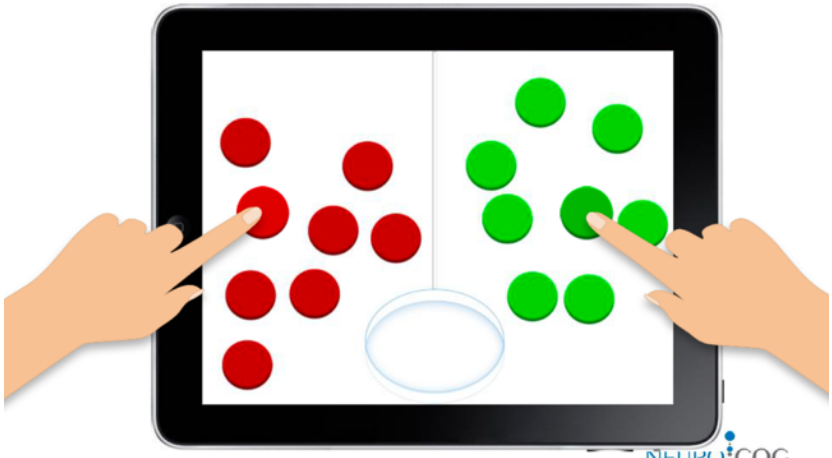
Token Motor



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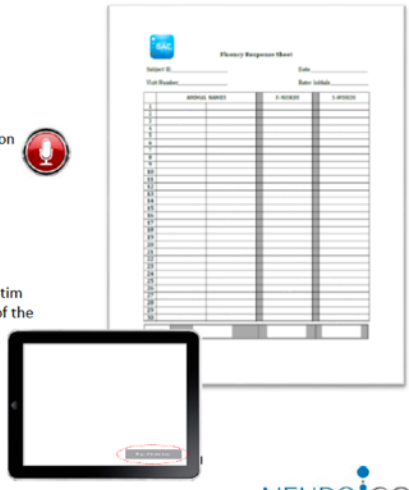
Semantic Fluency

Subjects are given 60 seconds to generate as many words as possible in a given category (Animals).

Tablet Position	Flat on table facing subject
Scored By	Rater
Recording	Responses are audio recorded **and** written on Fluency Response Sheet
# of Trials	1
Trial Duration	60 seconds
Documentation	Rater documents subject's responses verbatim (word-for-word) on paper then enters ALL of the responses at the end of the test



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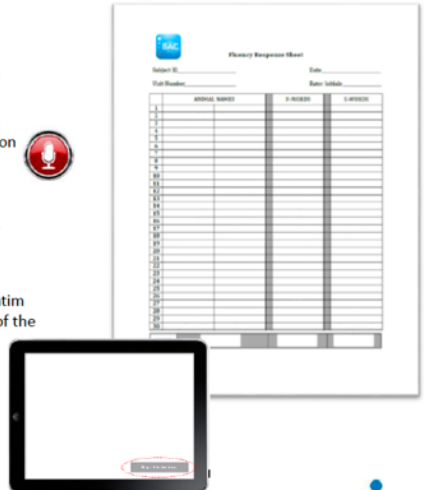
Letter Fluency

In two separate trials, subjects are given 60 seconds to generate as many words as possible beginning with a given letter of the alphabet.

Tablet Position	Flat on table facing subject
Scored By	Rater
Recording	Responses are audio recorded **and** written on Fluency Response Sheet
# of Trials	2 (F-words & S-words)
Trial Duration	60 seconds
Documentation	Rater documents subject's responses verbatim (word-for-word) on paper then enters ALL of the responses at the end of the test



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Symbol Coding

Subjects are given a key explaining how unique symbols correspond to the individual numerals 1-9. They are asked to fill in the corresponding number beneath a series of symbols as quickly as possible within a 90 second time limit.

Tablet Position	Flat on table facing subject
Scored By	Application
# of Trials	1
Trial Duration	90 seconds
# of Versions	8
Feedback	Feedback is provided for incorrect practice items (by the app)

Rater may repeat instructions for correcting a response if the subject asks

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Symbol Coding

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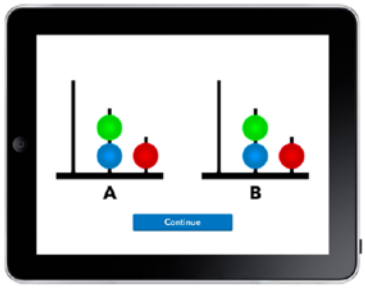
Tower of London

Subjects are instructed to look at two pictures simultaneously. Each picture shows three different colored balls arranged on three pegs, but the balls appear in a unique arrangement in each picture. Subjects are asked to give the minimum number of times the balls in one picture would need to be moved in order to make the arrangement of balls identical to that in the opposing picture.

Tablet Position	Flat on table facing subject
Scored By	Application
# of Trials	20 (2 additional trials if first 20 are correct)
Trial Duration	20 second time limit
Discontinuation	Discontinues after 5 incorrect responses in a row
# of Versions	8



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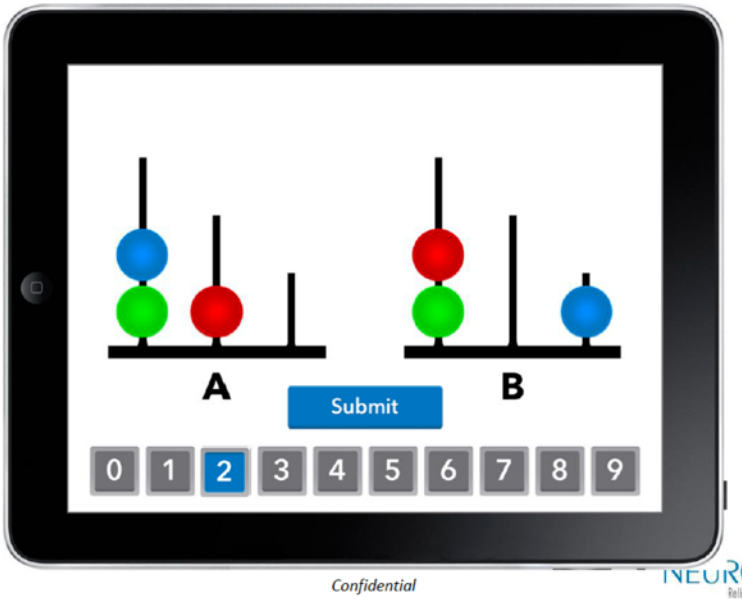
Tower of London



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19.6 Appendix F: Beck Depression Inventory- II (BDI-II)

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BDI-II

BDI-II

Please read each group of statements carefully, and then select the one statement in each group that best describes the way you have been feeling during the past 14 days, including today. Select the number beside the statement you have picked. If several statements in the group seem to apply equally well, select the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

Date form completed _____
(YYYY-MM-DD)

1. Sadness

- ◯ 0 - I do not feel sad.
- ◯ 1 - I feel sad much of the time.
- ◯ 2 - I am sad all the time.
- ◯ 3 - I am so sad or unhappy that I cont stand it.

2. Pessimism

- ◯ 0 - I am not discouraged about my future.
- ◯ 1 - I feel more discouraged about my future than I used to be.
- ◯ 2 - I do not expect things to work out for me.
- ◯ 3 - I feel my future is hopeless and will only get worse.

3. Past Failure

- ◯ 0 - I do not feel like a failure.
- ◯ 1 - I have failed more than I should have.
- ◯ 2 - As I look back, I see a lot of failures.
- ◯ 3 - I feel I am a total failure as a person.

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4. Loss of Pleasure

- ◯ 0 - I get as much pleasure as I ever did from the things I enjoy.
- ◯ 1 - I dont enjoy things as much as I used to.
- ◯ 2 - I get very little pleasure from the things I used to enjoy.
- ◯ 3 - I cant get any pleasure from things I used to enjoy.

5. Guilty Feelings

- ◯ 0 - I dont feel particularly guilty.
- ◯ 1 - I feel guilty over many things I have done or should have done.
- ◯ 2 - I feel quite guilty most of the time.
- ◯ 3 - I feel guilty all of the time.

6. Punishment Feelings

- ◯ 0 - I dont feel I am being punished.
- ◯ 1 - I feel I may be punished.
- ◯ 2 - I expect to be punished.
- ◯ 3 - I feel I am being punished.

7. Self-Dislike

- ◯ 0 - I feel the same about myself as ever.
- ◯ 1 - I have lost confidence in myself.
- ◯ 2 - I am disappointed in myself.
- ◯ 3 - I dislike myself.

8. Self-Criticism

- ◯ 0 - I dont criticize or blame myself more than usual.
- ◯ 1 - I am more critical of myself than I used to be.
- ◯ 2 - I criticize myself for all of my faults.
- ◯ 3 - I blame myself for everything bad that happens.

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9. Suicidal Thoughts or Wishes

- ◯ 0 - I dont have any thoughts of killing myself.
- ◯ 1 - I have thoughts of killing myself, but I would not carry them out.
- ◯ 2 - I would like to kill myself.
- ◯ 3 - I would kill myself if I had the chance.

10. Crying

- ◯ 0 - I dont cry anymore than I used to.
- ◯ 1 - I cry more than I used to.
- ◯ 2 - I cry over every little thing.
- ◯ 3 - I feel like crying, but I cant.

11. Agitation

- ◯ 0 - I am no more restless or wound up than usual.
- ◯ 1 - I feel more restless or wound up than usual.
- ◯ 2 - I am so restless or agitated that its hard to stay still.
- ◯ 3 - I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

- ◯ 0 - I have not lost interest in other people or activities.
- ◯ 1 - I am less interested in other people or things than before.
- ◯ 2 - I have lost most of my interest in other people or things.
- ◯ 3 - Its hard to get interested in anything.

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13. Indecisiveness

- ◯ 0 - I make decisions about as well as ever.
- ◯ 1 - I find it more difficult to make decisions than usual.
- ◯ 2 - I have much greater difficulty in making decisions than I used to.
- ◯ 3 - I have trouble making any decisions.

14. Worthlessness

- ◯ 0 - I do not feel I am worthless.
- ◯ 1 - I dont consider myself as worthwhile and useful as I used to.
- ◯ 2 - I feel more worthless as conpared to other people.
- ◯ 3 - I feel utterly worthless.

15. Loss of Energy

- ◯ 0 - I have as much energy as ever.
- ◯ 1 - I have less energy than I used to have.
- ◯ 2 - I dont have enough energy to do very much.
- ◯ 3 - I dont have enough energy to do anything.

16. Changes in Sleeping Pattern

- ◯ 0 - I have not experienced any change in my sleeping pattern.
- ◯ 1a - I sleep somewhat more than usual.
- ◯ 1b - I sleep somewhat less than usual.
- ◯ 2a - I sleep a lot more than usual.
- ◯ 2b - I sleep a lot less than usual.
- ◯ 3a - I sleep most of the day.
- ◯ 3b - I was up 1-2 hours early and cant get back to sleep.

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17. Irritability

- ◯ 0 - I am no more irritable than usual.
- ◯ 1 - I am more irritable than usual.
- ◯ 2 - I am much more irritable than usual.
- ◯ 3 - I am irritable all the time.

18. Changes in Appetite

- ◯ 0 - I have not experienced any change in my appetite.
- ◯ 1a - My appetite is somewhat less than usual.
- ◯ 1b - My appetite is somewhat greater than usual.
- ◯ 2a - My appetite is much less than before.
- ◯ 2b - My appetite is much greater than usual.
- ◯ 3a - I have no appetite at all.
- ◯ 3b - I crave food all the time.

19. Concentration Difficulty

- ◯ 0 - I can concentrate as well as ever.
- ◯ 1 - I cant concentrate as well as usual.
- ◯ 2 - Its hard to keep my mind on anything for very long.
- ◯ 3 - I find I cant concentrate on anything.

20. Tiredness or Fatigue

- ◯ 0 - I am no more tired or tatigued than usual.
- ◯ 1 - I get more tired or fatigued more easily than usual.
- ◯ 2 - I am too tired or fatigued to do a lot of the things I used to do.
- ◯ 3 - I am too tired or fatigued to do most of the things I used to do.

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21. Loss of Interest in Sex

- ◯ 0 - I have not noticed any recent change in my interest in sex.
- ◯ 1 - I am less interested in sex than I used to be.
- ◯ 2 - I am much less interested in sex now.
- ◯ 3 - I have lost interest in sex completely.

BDI-II Score _____

Comments _____

19.7 Appendix G: FACIT-Fatigue

FACIT Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

		Not at all	A little bit	Some-what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
An4	I have trouble finishing things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

19.8 Appendix H: Fact-BR

Title of measure:
Functional Assessment of Cancer Therapy-Brain (FACT-Br)

This summary was last revised 5 October 2010.

Brief overview:
The Functional Assessment of Cancer Therapy-Brain (FACT-Br) is a commonly used instrument measuring general quality of life (QOL) that reflects symptoms or problems associated with brain malignancies across 5 scales [1]. The measure yields information about total QOL, as well as information about the dimensions of physical well-being, social/family well-being, emotional well being, functional well-being, and disease-specific concerns. The FACT-Br is written at the 4th grade reading level, and patients can filled out it in 5-10 minutes. The self-report of quality of life can be completed by the patient or with the assistance of the examiner and does not require pre-certification.

Validated (Yes/No):
Yes [2]. The Functional Assessment of Cancer Therapy (FACT) scale has been developed following principles of test construction and evaluation and, recently, has undergone through psychometric testing for validity and reliability [1, 2]. The FACT-G (General version) was developed to provide information about health status that is specific to cancer patients. FACT-BR (brain) was developed as a new combined brain subscale questionnaire and checked for validation and reliability by Weitzner and coworkers [2] in 1995.

Psychometric properties and references:
FACT-Br subscale, brain tumor specific version, is a 23-item questionnaire that can be completed in 5 to 10 minutes with little or no assistance in patients who are not neurologically incapacitated. This brain subscale is usually used along with the core (general) questionnaire [2] that includes 27 items.
Patients rate all 5 items using a five-point Likert scale ranging from 0 "not at all" to 4 "very much." Overall, higher ratings suggest higher QOL. Items are totaled to produce the following subscales, along with an overall QOL score: physical well-being (7 items); social/family well-being (7 items); emotional well-being (6 items); functional well-being (7 items); and concerns relevant to patients with brain tumors (23 items).

Clinically significant changes:
Not specifically available for FACT-Br.

Website or how to register to use:
Go to www.facit.org and click on "Registration+Requests" to use one or more of the
FACT scales, which can be obtained by completing a User's Agreement and completing one Collaborator's Project Information Form per project. This information can be found under the "User's Agreement" link on the website. The permission information should be given to RTOG headquarters for each RTOG QOL study.

List any fees for usage:
Currently, there are no fees for use of any of the English versions of the FACT questionnaires.

Languages available:
The FACT has been translated into many languages, and translations are accessible at the FACIT web site, http://www.facit.org/translation/licensure.aspx.
Please check the website for the specific languages available for FACT-BR.

Instructions for CRAs and or credentialing for administration:
There is no credentialing needed for administration. Each protocol has instructions for the Clinical Research Associates. As well, a variety of information to assist in the administration of the FACT questionnaires is available from the website (under the administration and scoring guidelines link).

Quality assurance for administration (if needed):
None.

Scoring of instrument:
The website has a repository of information that assists in the scoring of the FACT questionnaires and in the interpretation of the results.

References:
1. Cella DF, Tulsky DS, Gray G, *et al*. The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. *J Clin Oncol* 1993;11:570-579.

2. Weitzner MA, Meyers CA, Gelke CK, et al. 1995. The Functional Assessment of Cancer Therapy (FACT) scale: Development of a brain subscale and revalidation of the general version (FACT-G) in patients with primary brain tumors. Cancer 75(5):1151-1161.

FACT-BR (Version 4)

Below is a list of statements that other people with your illness have said are important. By **circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please check this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
Br1	I am able to concentrate	0	1	2	3	4
Br2	I have had seizures (convulsions)	0	1	2	3	4
Br3	I can remember new things	0	1	2	3	4
Br4	I get frustrated that I cannot do things I used to	0	1	2	3	4
Br5	I am afraid of having a seizure (convulsion)	0	1	2	3	4
Br6	I have trouble with my eyesight	0	1	2	3	4
Br7	I feel independent	0	1	2	3	4
NTX 6	I have trouble hearing	0	1	2	3	4
Br8	I am able to find the right word(s) to say what I mean	0	1	2	3	4
Br9	I have difficulty expressing my thoughts	0	1	2	3	4
Br10	I am bothered by the change in my personality	0	1	2	3	4
Br11	I am able to make decisions and take responsibility	0	1	2	3	4
Br12	I am bothered by the drop in my contribution to the family	0	1	2	3	4
Br13	I am able to put my thoughts together	0	1	2	3	4
Br14	I need help in caring for myself (bathing, dressing, eating, etc.)	0	1	2	3	4
Br15	I am able to put my thoughts into action	0	1	2	3	4
Br16	I am able to read like I used to	0	1	2	3	4
Br17	I am able to write like I used to	0	1	2	3	4
Br18	I am able to drive a vehicle (my car, truck, etc.)	0	1	2	3	4
Br19	I have trouble feeling sensations in my arms, hands, or legs	0	1	2	3	4
Br20	I have weakness in my arms or legs	0	1	2	3	4
Br21	I have trouble with coordination	0	1	2	3	4
An 10	I get headaches	0	1	2	3	4

19.9 Appendix I: FACT-Cog

FACT-Cognitive Function (Version 3)

Below is a list of statements that other people with your condition have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PERCEIVED COGNITIVE IMPAIRMENTS	Never	About once a week	Two to three times a week	Nearly every day	Several times a day
CogA1	I have had trouble forming thoughts	0	1	2	3	4
CogA3	My thinking has been slow	0	1	2	3	4
CogC7	I have had trouble concentrating	0	1	2	3	4
CogM9	I have had trouble finding my way to a familiar place	0	1	2	3	4
CogM10	I have had trouble remembering where I put things, like my keys or my wallet	0	1	2	3	4
CogM12	I have had trouble remembering new information, like phone numbers or simple instructions	0	1	2	3	4
CogV13	I have had trouble recalling the name of an object while talking to someone	0	1	2	3	4
CogV15	I have had trouble finding the right word(s) to express myself	0	1	2	3	4
CogV16	I have used the wrong word when I referred to an object	0	1	2	3	4
CogV17b	I have had trouble saying what I mean in conversations with others	0	1	2	3	4
CogF19	I have walked into a room and forgotten what I meant to get or do there	0	1	2	3	4
CogF23	I have had to work really hard to pay attention or I would make a mistake	0	1	2	3	4
CogF24	I have forgotten names of people soon after being introduced	0	1	2	3	4

Page 1 of 3
Last Revised 10 February 2008

FACT-Cog (Version 3)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

		Never	About once a week	Two to three times a week	Nearly every day	Several times a day
CogF25	My reactions in everyday situations have been slow	0	1	2	3	4
CogC31	I have had to work harder than usual to keep track of what I was doing	0	1	2	3	4
CogC32	My thinking has been slower than usual	0	1	2	3	4
CogC33a	I have had to work harder than usual to express myself clearly	0	1	2	3	4
CogC33c	I have had to use written lists more often than usual so I would not forget things	0	1	2	3	4
CogMT1	I have trouble keeping track of what I am doing if I am interrupted	0	1	2	3	4
CogMT2	I have trouble shifting back and forth between different activities that require thinking	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	COMMENTS FROM OTHERS	Never	About once a week	Two to three times a week	Nearly every day	Several times a day
CogO1	Other people have told me I seemed to have trouble remembering information	0	1	2	3	4
CogO2	Other people have told me I seemed to have trouble speaking clearly	0	1	2	3	4
CogO3	Other people have told me I seemed to have trouble thinking clearly	0	1	2	3	4
CogO4	Other people have told me I seemed confused	0	1	2	3	4

FACT-Cog (Version 3)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	PERCEIVED COGNITIVE ABILITIES	Not at all	A little bit	Some-what	Quite a bit	Very much
Cog PC1	I have been able to concentrate	0	1	2	3	4
Cog PV1	I have been able to bring to mind words that I wanted to use while talking to someone	0	1	2	3	4
Cog PM1	I have been able to remember things, like where I left my keys or wallet	0	1	2	3	4
Cog PM2	I have been able to remember to do things, like take medicine or buy something I needed	0	1	2	3	4
Cog PF1	I am able to pay attention and keep track of what I am doing without extra effort	0	1	2	3	4
Cog PCH 1	My mind is as sharp as it has always been	0	1	2	3	4
Cog PCH 2	My memory is as good as it has always been	0	1	2	3	4
Cog PMT 1	I am able to shift back and forth between two activities that require thinking	0	1	2	3	4
Cog PMT 2	I am able to keep track of what I am doing, even if I am interrupted	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	IMPACT ON QUALITY OF LIFE	Not at all	A little bit	Some-what	Quite a bit	Very much
CogQ35	I have been upset about these problems	0	1	2	3	4
CogQ37	These problems have interfered with my ability to work	0	1	2	3	4
CogQ38	These problems have interfered with my ability to do things I enjoy	0	1	2	3	4
CogQ41	These problems have interfered with the quality of my life	0	1	2	3	4

19.10 Appendix J: Godin Leisure-Time Exercise Questionnaire

Godin Leisure-Time Exercise Questionnaire

INSTRUCTIONS

In this excerpt from the Godin Leisure-Time Exercise Questionnaire, the individual is asked to complete a self-explanatory, brief four-item query of usual leisure-time exercise habits.

CALCULATIONS

For the first question, weekly frequencies of strenuous, moderate, and light activities are multiplied by nine, five, and three, respectively. Total weekly leisure activity is calculated in arbitrary units by summing the products of the separate components, as shown in the following formula:

Weekly leisure activity score = (9 × Strenuous) + (5 × Moderate) + (3 × Light)

The second question is used to calculate the frequency of weekly leisure-time activities pursued "long enough to work up a sweat" (see questionnaire).

EXAMPLE

Strenuous = 3 times/wk

Moderate = 6 times/wk

Light = 14 times/wk

Total leisure activity score = (9 × 3) + (5 × 6) + (3 × 14) = 27 + 30 + 42 = 99

Godin, G., Shephard, R. J.. (1997) Godin Leisure-Time Exercise Questionnaire. Medicine and Science in Sports and Exercise. 29 June Supplement: S36-S38.

Godin Leisure-Time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

	Times Per Week
a) **STRENUOUS EXERCISE** **(HEART BEATS RAPIDLY)** (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	_____
b) **MODERATE EXERCISE** **(NOT EXHAUSTING)** (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____
c) **MILD EXERCISE** **(MINIMAL EFFORT)** (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)	_____

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

OFTEN	SOMETIMES	NEVER/RARELY
1. ☐	2. ☐	3. ☐

19.11 Appendix K: Trails Making Test (TMT) Parts A & B

Trail Making Test (TMT) Parts A & B

Instructions:

Both parts of the Trail Making Test consist of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1 – 25, and the patient should draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1 – 13) and letters (A – L); as in Part A, the patient draws lines to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The patient should be instructed to connect the circles as quickly as possible, without lifting the pen or pencil from the paper. Time the patient as he or she connects the "trail." If the patient makes an error, point it out immediately and allow the patient to correct it. Errors affect the patient's score only in that the correction of errors is included in the completion time for the task. It is unnecessary to continue the test if the patient has not completed both parts after five minutes have elapsed.

Step 1: Give the patient a copy of the Trail Making Test Part A worksheet and a pen or pencil.

Step 2: Demonstrate the test to the patient using the sample sheet (Trail Making Part A – *SAMPLE*).

Step 3: Time the patient as he or she follows the "trail" made by the numbers on the test.

Step 4: Record the time.

Step 5: Repeat the procedure for Trail Making Test Part B.

Scoring:

Results for both TMT A and B are reported as the number of seconds required to complete the task; therefore, higher scores reveal greater impairment.

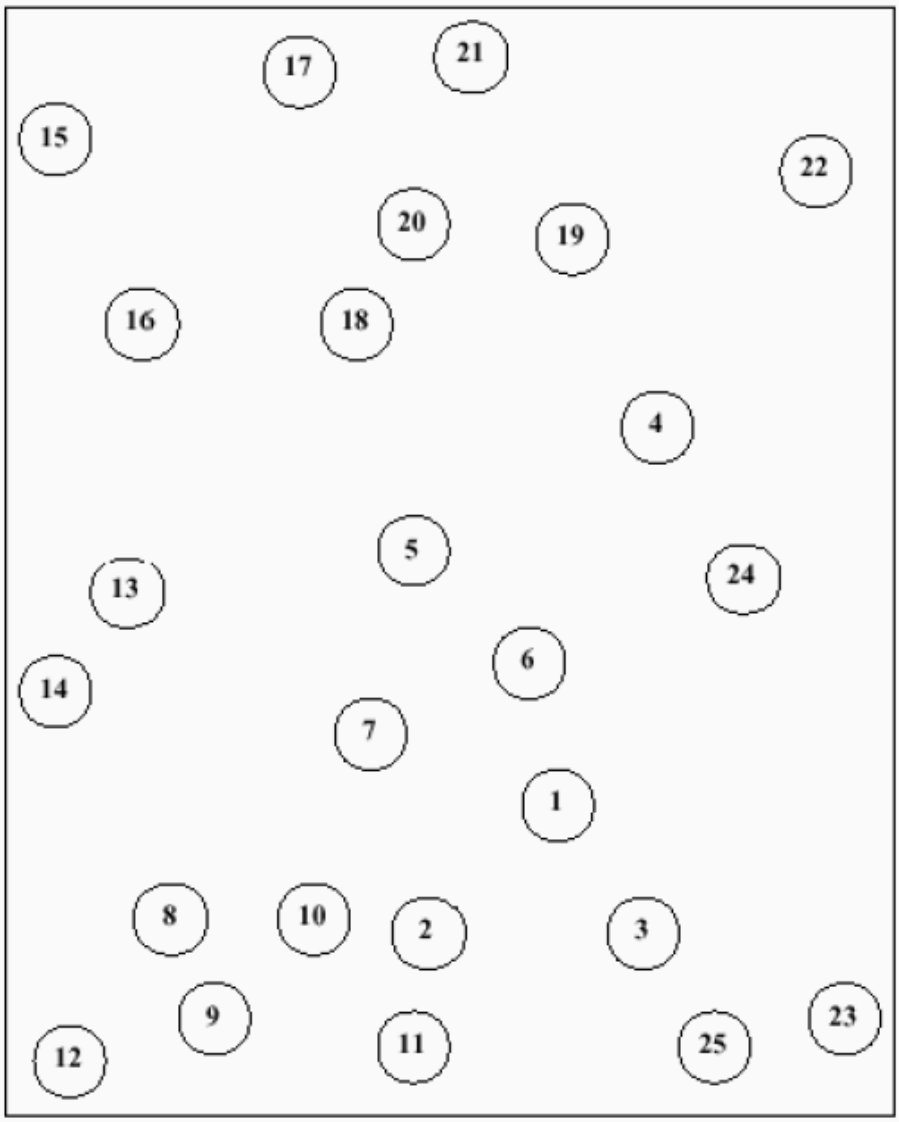
	Average	Deficient	Rule of Thumb
Trail A	29 seconds	> 78 seconds	Most in 90 seconds
Trail B	75 seconds	> 273 seconds	Most in 3 minutes

Sources:

- Corrigan JD, Hinkeldey MS. Relationships between parts A and B of the Trail Making Test. *J Clin Psychol*. 1987;43(4):402–409.
- Gaudino EA, Geisler MW, Squires NK. Construct validity in the Trail Making Test: what makes Part B harder? *J Clin Exp Neuropsychol*. 1995;17(4):529-535.
- Lezak MD, Howieson DB, Loring DW. *Neuropsychological Assessment*. 4th ed. New York: Oxford University Press; 2004.
- Reitan RM. Validity of the Trail Making test as an indicator of organic brain damage. *Percept Mot Skills*. 1958;8:271-276.

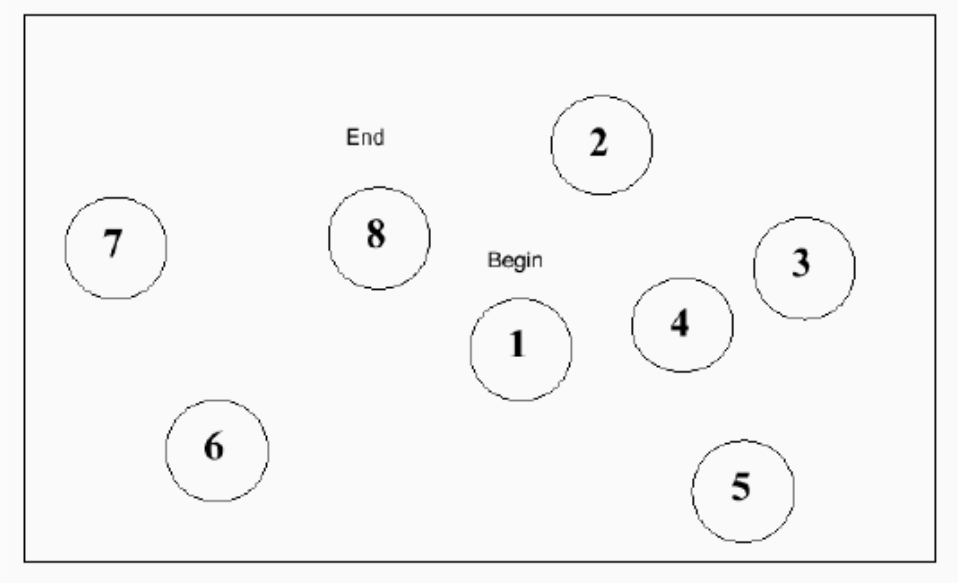
Trail Making Test Part A

Patient's Name: _____ Date: _____



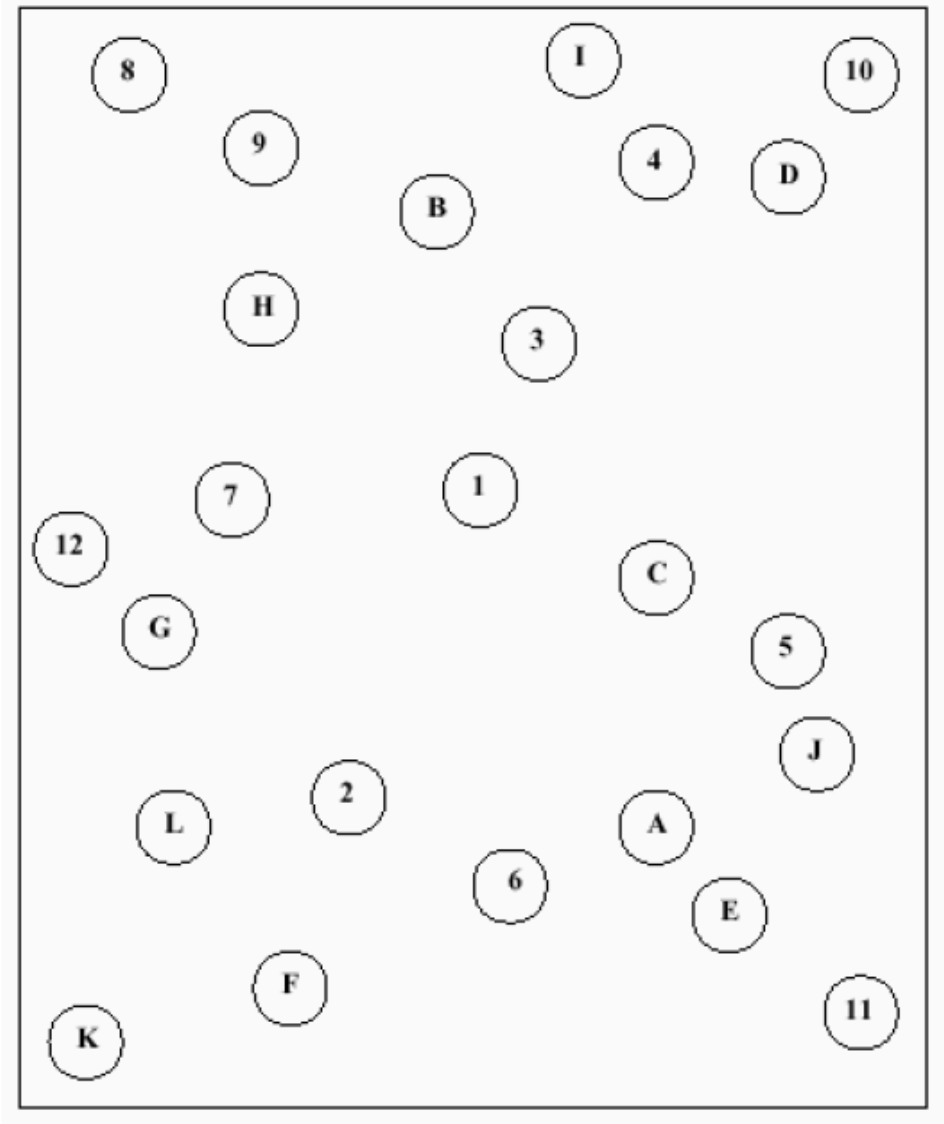
-Total time to complete in seconds: _____
- Number of errors: _____

Trail Making Test Part A – *SAMPLE*



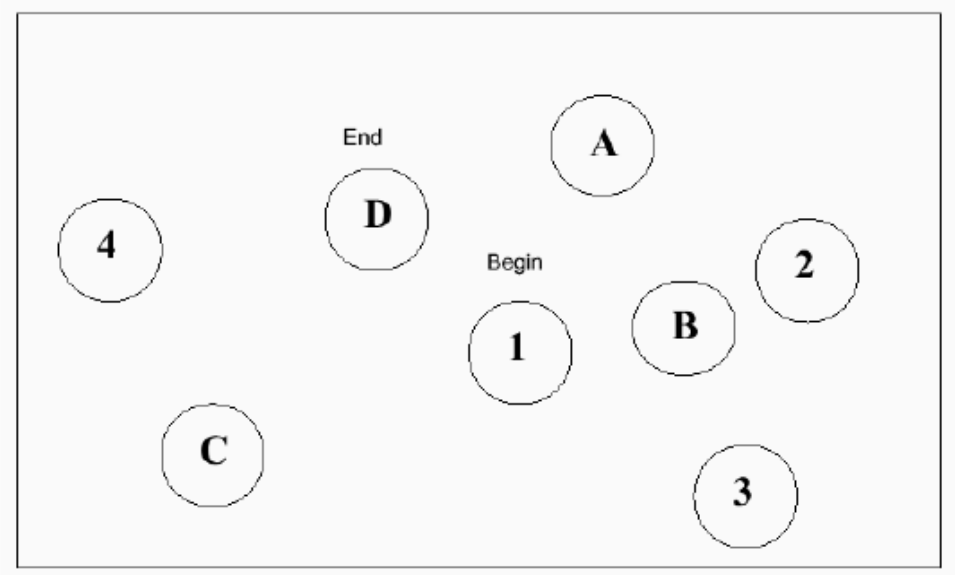
Trail Making Test Part B

Patient's Name: _____ Date: _____



-Total time to complete in seconds: _____
- Number of errors: _____

Trail Making Test Part B – *SAMPLE*



19.12 Appendix L: HVLT-R Forms 1, 2 and 3

HVLT-R™

Form 1

Test Booklet

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Learning Trial Instructions

Trial 1

Say the following:

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

- Repeat or paraphrase the instructions if necessary.
- Read the words at the rate of approximately one word every 2 seconds.
- If the individual does not spontaneously begin reporting words after the last word is read, say the following:

> **OK. Now tell me as many of those words as you can remember.**

Record the responses verbatim (including repetitions and intrusions) in the Trial 1 column. When the individual indicates no more words can be recalled, proceed to Trial 2.

Trial 2

Say the following:

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

Use the same procedure as in Trial 1 to record the responses in the column for Trial 2. Then proceed to Trial 3.

Trial 3

Say the following:

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

Record the responses in the column for Trial 3 using the same procedure as in the previous trials.

NOTE: *Do not tell the respondent that recall of the words will be tested later.*

Delayed Recall Trial Instructions

After the 20-25 minute delay, say the following:

> **Do you remember that list of words you tried to learn before?**

If the response is "No," remind the individual that you read the list three times and that he or she was asked to recall the words each time. Say the following:

> **Tell me as many of those words as you can remember.**

Form 1

Semantic Categories: Four-Legged Animals, Precious Stones, Human Dwellings

Name _____ Sex _____ Age _____ years _____ months

Examiner _____ Date ___/___/___

Word List	Learning Trials			Delayed Recall Trial (20-25 min.)
	Trial 1	Trial 2	Trial 3	Trial 4
LION				
EMERALD				
HORSE				
TENT				
SAPPHIRE				
HOTEL				
CAVE				
OPAL				
TIGER				
PEARL				
COW				
HUT				
Total correct responses =				

Completion Time Trial 3 _____ Start Time Trial 4 _____

→

3

Delayed Recognition Trial Instructions

The Delayed Recognition (Forced Choice) trial is administered immediately after the Delayed Recall trial. Say the following:

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

Read the words of the Delayed Recognition trial list in numerical order. Allow the individual as much time as needed to respond. You may use the prompt, **"Was horse on the list? Yes or no?"** The individual must give you a response for every word. If the individual is not sure, ask for a guess.

Delayed Recognition Trial (Forced Choice)			
1. HORSE Y N	7. house Y N	13. HUT Y N	19. TENT Y N
2. ruby Y N	8. OPAL Y N	14. EMERALD Y N	20. mountain Y N
3. CAVE Y N	9. TIGER Y N	15. SAPPHIRE Y N	21. cat Y N
4. balloon Y N	10. boat Y N	16. dog Y N	22. HOTEL Y N
5. coffee Y N	11. scarf Y N	17. apartment Y N	23. COW Y N
6. LION Y N	12. PEARL Y N	18. penny Y N	24. diamond Y N

Total number of true-positive responses ("hits"): _____ **/12** (no shading)

Semantically-related false-positive errors: _____ /6 (light shading)

Semantically-unrelated false-positive errors: _____ /6 (darker shading)

Total number of false-positive errors: _____ **/12**

	Raw score	*T* score
Total Recall (sum of total correct responses for Trials 1, 2, & 3)		
Delayed Recall (Trial 4)		
Retention (%) [(Trial 4 ÷ Higher score of Trials 2 and 3) x 100]		
Recognition Discrimination Index (Total no. of true-positives) – (Total no. of false-positives)		

Normative table (Appendix A): _____

HVLT-R™

Form 2

Test Booklet

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Learning Trial Instructions

Trial 1

Say the following:

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

- Repeat or paraphrase the instructions if necessary.
- Read the words at the rate of approximately one word every 2 seconds.
- If the individual does not spontaneously begin reporting words after the last word is read, say the following:

> **OK. Now tell me as many of those words as you can remember.**

Record the responses verbatim (including repetitions and intrusions) in the Trial 1 column. When the individual indicates no more words can be recalled, proceed to Trial 2.

Trial 2

Say the following:

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

Use the same procedure as in Trial 1 to record the responses in the column for Trial 2. Then proceed to Trial 3.

Trial 3

Say the following:

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

Record the responses in the column for Trial 3 using the same procedure as in the previous trials.

NOTE: *Do not tell the respondent that recall of the words will be tested later.*

Delayed Recall Instructions

After the 20-25 minute delay, say the following:

> **Do you remember that list of words you tried to learn before?**

If the response is "No," remind the individual that you read the list three times and that he or she was asked to recall the words each time. Say the following:

> **Tell me as many of those words as you can remember.**

Form 2

Semantic Categories: Kitchen Utensils, Alcoholic Beverages, Weapons

Name _____ Sex _____ Age _____ years _____ months

Examiner _____ Date ____/____/____

Word List
FORK
RUM
PAN
PISTOL
SWORD
SPATULA
BOURBON
VODKA
POT
BOMB
RIFLE
WINE

Learning Trials			Delayed Recall (20-25 min.)
Trial 1	Trial 2	Trial 3	Trial 4
Total correct responses =			

Completion Time Trial 3 _____ Start Time Trial 4 _____

→

3

Delayed Recognition Instructions

The Delayed Recognition (Forced Choice) trial is administered immediately after the Delayed Recall trial. Say the following:

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

Read the words of the Delayed Recognition trial list in numerical order. Allow the individual as much time as needed to respond. You may use the prompt, **"Was spoon on the list? Yes or no?"** The individual must give you a response for every word. If the individual is not sure, ask for a guess.

Delayed Recognition (Forced Choice)			
1. spoon Y N	7. harmonica Y N	13. knife Y N	19. WINE Y N
2. PISTOL Y N	8. can opener Y N	14. RUM Y N	20. lemon Y N
3. doll Y N	9. SWORD Y N	15. trout Y N	21. SPATULA Y N
4. whiskey Y N	10. pencil Y N	16. BOMB Y N	22. BOURBON Y N
5. FORK Y N	11. gun Y N	17. PAN Y N	23. beer Y N
6. POT Y N	12. VODKA Y N	18. gold Y N	24. RIFLE Y N

Total number of true-positive responses ("hits"): _____ **/12** (no shading)

Semantically-related false-positive errors: _____ /6 (light shading)

Semantically-unrelated false-positive errors: _____ /6 (darker shading)

Total number of false-positive errors: _____ **/12**

	Raw score	*T* score
Total Recall (sum of total correct responses for Trials 1, 2, & 3)		
Delayed Recall (Trial 4)		
Retention (%) [(Trial 4 ÷ Higher score of Trials 2 and 3) x 100]		
Recognition Discrimination Index (Total no. of true-positives) – (Total no. of false-positives)		

Normative table (Appendix A): _____

4

HVLT-R™

Form 3

Test Booklet

Jason Brandt, PhD
Ralph H. B. Benedict, PhD

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Learning Trial Instructions

Trial 1

Say the following:

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

- Repeat or paraphrase the instructions if necessary.
- Read the words at the rate of approximately one word every 2 seconds.
- If the individual does not spontaneously begin reporting words after the last word is read, say the following:

> **OK. Now tell me as many of those words as you can remember.**

Record the responses verbatim (including repetitions and intrusions) in the Trial 1 column. When the individual indicates no more words can be recalled, proceed to Trial 2.

Trial 2

Say the following:

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

Use the same procedure as in Trial 1 to record the responses in the column for Trial 2. Then proceed to Trial 3.

Trial 3

Say the following:

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

Record the responses in the column for Trial 3 using the same procedure as in the previous trials.

NOTE: *Do not tell the respondent that recall of the words will be tested later.*

Delayed Recall Instructions

After the 20-25 minute delay, say the following:

> **Do you remember that list of words you tried to learn before?**

If the response is "No," remind the individual that you read the list three times and that he or she was asked to recall the words each time. Say the following:

> **Tell me as many of those words as you can remember.**

Form 3

Semantic Categories: Musical Instruments, Fuels, Food Flavorings

Name _____ Sex _____ Age _____ years _____ months

Examiner _____ Date ___/___/___

Word List	Learning Trials			Delayed Recall (20-25 min.)
	Trial 1	Trial 2	Trial 3	Trial 4
SUGAR				
TRUMPET				
VIOLIN				
COAL				
GARLIC				
KEROSENE				
VANILLA				
WOOD				
CLARINET				
FLUTE				
CINNAMON				
GASOLINE				
Total correct responses =				

Completion Time Trial 3 _____ Start Time Trial 4 _____

3

Delayed Recognition Instructions

The Delayed Recognition (Forced Choice) trial is administered immediately after the Delayed Recall trial. Say the following:

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

Read the words of the Delayed Recognition trial list in numerical order. Allow the individual as much time as needed to respond. You may use the prompt, **"Was pepper on the list? Yes or no?"** The individual must give you a response for every word. If the individual is not sure, ask for a guess.

Delayed Recognition (Forced Choice)			
1. pepper Y N	7. ball Y N	13. TRUMPET Y N	19. KEROSENE Y N
2. GARLIC Y N	8. salt Y N	14. basement Y N	20. VANILLA Y N
3. WOOD Y N	9. priest Y N	15. CINNAMON Y N	21. GASOLINE Y N
4. drum Y N	10. chair Y N	16. FLUTE Y N	22. sand Y N
5. oil Y N	11. COAL Y N	17. electricity Y N	23. piano Y N
6. SUGAR Y N	12. CLARINET Y N	18. moon Y N	24. VIOLIN Y N

Total number of true-positive responses ("hits"): _____ **/12** (no shading)

Semantically-related false-positive errors: _____ /6 (light shading)

Semantically-unrelated false-positive errors: _____ /6 (darker shading)

Total number of false-positive errors: _____ **/12**

	Raw score	*T* score
Total Recall (sum of total correct responses for Trials 1, 2, & 3)		
Delayed Recall (Trial 4)		
Retention (%) [(Trial 4 ÷ Higher score of Trials 2 and 3) x 100]		
Recognition Discrimination Index (Total no. of true-positives) – (Total no. of false-positives)		

Normative table (Appendix A): _____

4

19.1 Appendix M: COWA

Multilingual Aphasia Examination III – Controlled Oral Word Association

Arthur Benton, Kerry deS. Hamsher, and Abigail Sivan

Record Sheet

Name _____ No. _____ Date ___/___/___ (month / day / year)

Age _____ Gender _____ Education (no. of years) _____ Handedness _____ Examiner _____

	First Letter (1 minute) C or P	Second Letter (1 minute) F or R	Third Letter (1 minute) L or W
	(Form A) (Form B)	(Form A) (Form B)	(Form A) (Form B)
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			
21.			
22.			
	No. of correct responses ☐ +	No. of correct responses ☐ +	No. of correct responses ☐ = ☐ Total Raw Score

Total Raw Score ☐ + Adjustment (see table) ☐ = ☐ Adjusted Score

Percentile Rank = ☐ (from manual)

Adjustment		Age (years)		
Education (years)		25-54	55-59	60-69
< 9	=	8	10	12
9-11	=	5	7	9
12-15	=	3	4	6
≥ 16	=	–	1	3

Remarks:

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