A phase I/II dose-escalation and dose-expansion study of disulfiram/copper with concurrent radiation therapy and temozolomide in patients with newly diagnosed glioblastoma

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Protocol Revision History

Initial Approval Version	**04/22/2016**
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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following ethical guidelines and regulations:

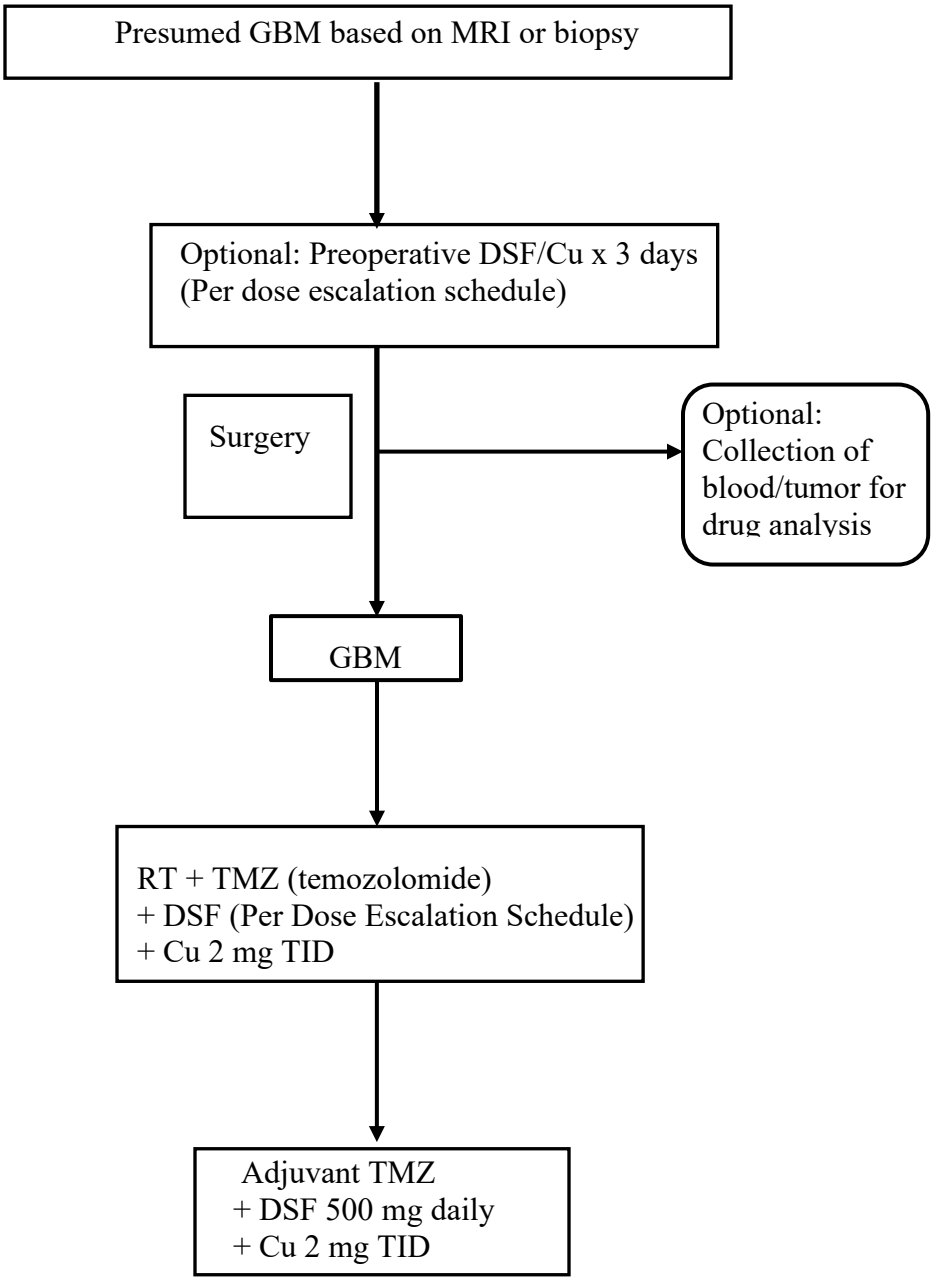
- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

A phase I/II dose-escalation and dose-expansion study of disulfiram/copper with concurrent radiation therapy and temozolomide in patients with newly diagnosed glioblastoma

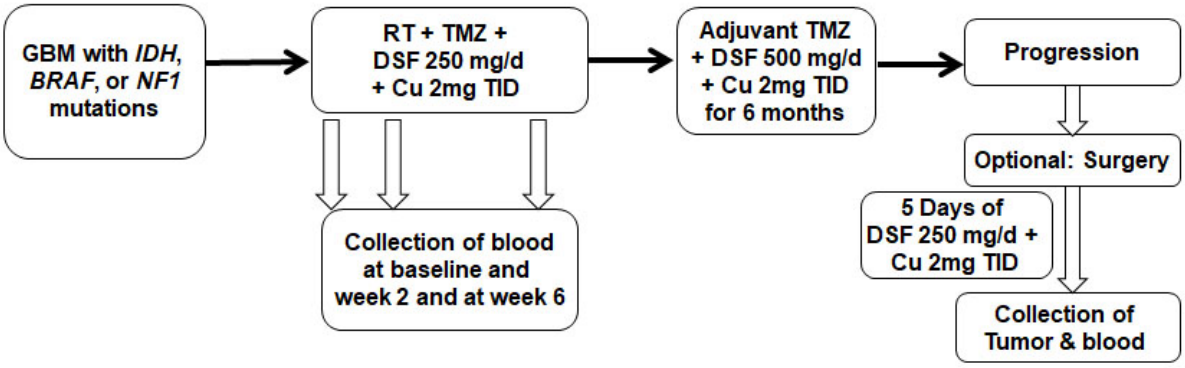
SCHEMA of the Dose-escalation Phase



Dose Escalation Schedule	
Dose Level	**Dose of Disulfiram**
Level 1	125 mg PO daily
Level 2	250 mg PO daily

Level 3	375 mg PO daily
Level 4	500 mg PO daily

SCHEMA of the Dose-expansion Phase



Glossary of Abbreviations

3DCRT	3-D conformal radiotherapy
AE	Adverse event
ALDH	Aldehyde dehydrogenase
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
ASCO	American Society of Clinical Oncologists
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BMP	Basic metabolic panel
CBC	Complete blood count
CFR	Code of Federal Regulations
CNS	Central nervous system
CR	Complete response
CRF	Case report form
CSC	Cancer stem cell
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTV	Clinical tumor volume
Cu	Copper supplement
DDTC	Diethyldithiocarbamate
DFS	Disease-free survival
DLTs	Dose Limiting Toxicities
DNA	Deoxyribonucleic acid

DSF	Disulfiram
DSM	Data and Safety Monitoring
DSMC	Data and Safety Monitoring Committee
FDA	Food and Drug Administration
GBM	Glioblastoma multiforme
GSC	GBM stem cell
GTV	Gross tumor volume
HRPO	Human Research Protection Office (IRB)
IMRT	Intensity modulated radiation therapy
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous (i.v.)
KPS	Karnofsky performance status
LFT	Liver function tests
LITT	Laser interstitial thermal therapy
MedDRA	Medical Dictionary for Regulatory Activity
MGMT	O6-methylguanine-DNA methyltransferase
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NED	No evidence of disease
NIH	National Institutes of Health
NYHA	New York Heart Association
OHRP	Office of Human Research Protections
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PI	Principal investigator
PO	Per os (by mouth)
PR	Partial response
PsP	Pseudoprogression
PTV	Planned tumor volume
QA	Quality assurance
QASMC	Quality Assurance and Safety Monitoring Committee
QD	Quaque die (daily)
RANO	Response Assessment in Neuro-Oncology criteria
RT	Radiation therapy
RTOG	Radiation Therapy Oncology Group
SAE	Serious adverse event
SCC	Siteman Cancer Center
SD	Stable disease

TITE-CRM	Time-to-Event Continual Reassessment Method
TMZ	Temozolomide
TTP	Time to progression
ULN	Upper limit of normal
UPN	Unique patient number
WBC	White blood cell (count)
WHO	World Health Organization
WUSM	Washington University School of Medicine

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1.0 BACKGROUND AND RATIONALE

1.1 Glioblastoma Multiforme

Glioblastoma multiforme (GBM, World Health Organization/WHO grade IV) is the most common malignant primary brain tumor and one of the most devastating cancers [1]. Between 2004 and 2007, there were 37,690 patients newly diagnosed with GBM, with an estimated incidence rate of 3 cases per 100,000 people in the United States [2]. The current standard of care for GBM includes maximal safe resection followed by radiotherapy (RT) and temozolomide (TMZ). The incorporation of TMZ in the treatment of these patients represented the first breakthrough against this fatal cancer in decades and was shown to significantly improve outcome in a randomized study. However, despite such multi-modality therapy, the median survival is approximately 14 months, with a five-year survival of less than 10% [3]. The combined RT and TMZ regimen was associated with relatively low rates of grade 3-4 toxicities: 4% neutropenia, 3% thrombocytopenia, 3% infection, 7% fatigue, 1% rash, <1% nausea/vomiting. Completion of therapy was also very high: 5% were unable to complete 90% of RT (4% due to disease progression, 1% due to incompliance), 13% did not complete concurrent TMZ (5% due to toxicity, 4% due to disease progression, and 4% due to other causes) [4].

Many institutions have attempted to increase the dose of RT to improve tumor control using various methods, including radiosurgery and brachytherapy, but each method has failed to improve outcome [5-7]. A recent retrospective study from our research group at Washington University showed that moderate RT dose-escalation with concurrent TMZ did not appear to improve outcome as compared to standard dose RT and TMZ [8]. Thus, novel therapeutic approaches are desperately needed for this devastating disease.

Recent evidence suggests that a cellular hierarchy exists in GBM [9,10]. A small fraction of GBM cells have the ability to regenerate the entire tumor and share many similar characteristics as neural stem cells, such as self-renewal, extensive proliferation, and differentiation [11-14]. Those cells have been referred to as cancer stem cells (CSCs) or tumor-initiating cells. Experiments have shown that CSCs are more resistant to RT and chemotherapy than the rest of the tumor cell population [15,16]. Therefore, CSCs may represent an important reason why GBM may initially respond but invariably recur after chemoradiotherapy. Importantly, recent laboratory study has suggested that CSC hierarchy may be reversible and that non-CSCs can convert into CSCs [17], which would imply that the optimal therapeutic approach to GBM would be to combine conventional therapy that eradicates non-CSCs with CSC-directed therapy. Other preclinical studies have also suggested that disulfiram (DSF), a FDA-approved, non-cancer therapeutic drug, may target the CSC-like subpopulation of GBM. Its mechanism is still unclear but may be due to aldehyde dehydrogenase (ALDH) inhibition, O6-methylguanine-DNA methyltransferase (MGMT) inhibition, NF-kB inhibition, proteasome inhibition, DNA repair inhibition, or increased intracellular reactive oxygen species (ROS) generation (Brar, et al. 2004; Klein, et al. 1994).

1.2 Disulfiram

1.2.1 Current Usage of Disulfiram and Proteasome Inhibition

Disulfiram (Antabuse, Teva Pharmaceuticals, North Wales, PA) is an FDA-approved medication that has been used for treating alcoholism since 1951. It inhibits ALDH, which leads to accumulation of acetaldehyde in the blood after ingestion of alcohol. The resulting acetaldehyde causes unpleasant symptoms such as sweating, flushing, nausea, and vomiting. The association of these aversive reactions with drinking thus discourages further consumption of alcohol. More recently, disulfiram (DSF) has also been used to treat cocaine addiction [18]. Upon absorption, DSF is immediately reduced to its active metabolite, diethyldithiocarbamate (DDTC). DDTC is a potent copper (Cu) chelator and can readily penetrate the blood brain barrier [19]. Increasing data has emerged that the Cu-DDTC complex appears to be a proteasome inhibitor [20,21].

A proteasome is a large multi-subunit complex in eukaryotes which controls the degradation of intracellular proteins [22]. As an important regulator of many cellular processes such as cell-cycle and apoptosis, proteasomes have emerged as a promising target for anticancer therapy [23]. Proteasomes contain at least three known catalytic activities: chymotrysin-like, trypsin-like, and caspase-like. Specifically, the inhibition of the proteasomal chymotrypsin-like activity has been shown to be associated with apoptosis of tumor cells [24,25]. One of the first proteasome inhibitors, bortezomib (Velcade, Millennium Pharmaceuticals, Cambridge, MA), is approved for the treatment of multiple myeloma. Bortezomib has also shown significant cytotoxic effect on GBM cells in vitro [26] but has limited penetration across the blood brain barrier [27,28]. It has been shown to be ineffective for recurrent GBM [29]. Although the results of the phase II study of bortezomib combined with vorinostat are disappointing, disease response may be complicated by bortezomib's poor intracranial penetration and its focus on heavily pretreated tumors.

1.2.2 In Vitro Studies of DSF on GBM Cells

DSF has shown selective anti-tumor activity against breast cancer, melanoma, and prostate cancer *in vitro* with minimal cytotoxicity against their normal tissue counterpart [20,30,31]. Recently, a few studies have also demonstrated promising activity against GBM models. Hothi *et al.* have performed a high-throughput screening study of 2000 compounds and identified DSF as a potent inhibitor of patient-derived GBM stem cells (GSCs), with its greatest effect at a concentration above 0.75 μM. Similar to the previous reports for other tumor models, DSF showed remarkable selectivity against GSCs as compared to normal neural stem cells (IC_{50} of 31 nM versus 283 nM, respectively). The study further identified that the effect is dependent on a complex formation with Cu and its inhibition of chymotrypsin-like proteasomal activity [32].

Liu *et al.* have also confirmed the Cu-dependent cytotoxicity of DSF against GBM cell lines and provided evidence that DSF preferentially targets the GSC subpopulation. They observed that the IC_{50} ranged from 119.7 to 464.9 nM for different GBM cell lines. Furthermore, after only 3 hour exposure to 0.5 μM of DSF with 1 μM of Cu, the neurosphere-forming ability of different GBM cell lines was completely abolished, which is a key characteristic of the GBM stem-like cells. They also showed that DSF-Cu complex could induce intracellular reactive oxygen species (ROS) to trigger intrinsic apoptosis of GBM cells through activation of JNK and p38 pathways [33].

In a third study, Triscott *et al.* showed that DSF is active against TMZ-resistant GBM cell lines and provided synergistic activity when combined with TMZ. They reported that the IC_{90} for the TMZ resistant cells was 100 nM. At such concentration, the neurosphere-forming ability of the GBM cells was abolished completely, but the normal astrocytes were not affected. Altogether, those in vitro studies support that DSF has selective activity against the GSC-like subpopulations and may also increase the efficacy of TMZ [34].

1.2.3 *In Vivo* Studies of DSF

Paranjpe *et al.* showed that DSF increased sensitivity of GBM cells to TMZ through inhibition of O^6-methylguanine-DNA methyltransferase (MGMT) and demonstrated that DSF preferentially inhibited tumor MGMT in GBM xenografts as compared to MGMT in the liver. MGMT is a DNA repair protein that removes the mutagenic O^6-akyl groups from guanines, which would negate the effect of TMZ on tumor cells. The inhibition of MGMT provided a mechanism explanation for the synergistic effect with TMZ [35]. Lun *et al.* have also recently reported that a combination of DSF, Cu gluconate, and TMZ could significantly improve survival of mice with orthotopic GBM tumors. They have also shown that DSF inhibited the 26S proteasome in a Cu-dependent manner and induced activation of both heat shock and unfolded protein responses [36]. Altogether, the *in vivo* studies have confirmed the findings of the *in vitro* experiments and support clinical trial to test the synergistic benefit of combining DSF and copper with RT and TMZ for treating GBM.

DSF has also been tested on breast and prostate cancer xenografts. Chen *et al.* injected 50 mg/kg of DSF intraperitoneally daily for 29 days to mice implanted with breast cancer xenografts. They found that DSF reduced tumor growth by 74% as compared to the control group. They also confirmed that the proteasome chymotrypsin-like activity was inhibited by 87% in the tumor tissues treated with DSF as compared to the control. Along with the proteasome inhibition, there was also increased apoptosis within the treated tumor, including increased caspase-3 activity and cleaved PARP [20]. When given to mice implanted with prostate cancer xenografts, daily treatment of 200 mg/kg/day DSF in olive oil for three weeks also reduced tumor growth by 40% as compared to olive oil alone [31].

These preclinical studies further support the promising role of DSF as a novel approach to treat cancer.

1.2.4 Safety and Toxicology

DSF has been used clinically for more than 50 years, so its safety profile is well known. The current FDA-recommended dose of DSF is 250-500 mg daily for alcohol abstinence. The primary pharmacological action of DSF is its irreversible inhibition of ALDH, leading to an accumulation of acetaldehyde with ingestion of alcohol. The acetaldehyde then produces unpleasant DSF-ethanol reaction, characterized by flushing, palpitation, headache, nausea, and vomiting [18]. In the early clinical practice, a much higher dose of 1000-3000 mg per day was used [37]. At high dosages, the DSF-ethanol reaction may be severe and even fatal, but such high dosage in the absence of alcohol is well tolerated. In the early 1950s, 4 patients (out of an estimated 11,000 patients prescribed high doses of DSF) died of sudden respiratory or cardiovascular causes likely related to the DSF-ethanol reaction [38]. At such high dosages, there were also case reports of psychosis in the absence of alcohol ingestion [39,40]. A previous phase I study combining a single dose of oral DSF and cisplatin every 3 weeks observed dose-limiting confusion at 3000 mg/m^2 (approximately 4800 mg) [41]. High doses of DSF may inhibit cerebrospinal dopamine B-hydroxylase [42], and people with very low activity of dopamine hydroxylase may be prone to transient psychosis with such inhibition [43].

Early toxicology studies done in mice, rats, dogs, and rabbits have shown that DSF has very low toxicity, with LD_{50} between 1.8-10g/kg when administered orally. At those extreme doses, demyelination of brain and spinal cord was observed on histopathology [44]. Interestingly, long-term administration of high doses of DSF to rats did not induce any laboratory or histological signs of liver damage [45]. High doses of DSF (up to 6 g daily) are relatively nontoxic in humans. Symptoms of overdose include vomiting, headache, apathy, ataxia, motor restlessness, irritability, hallucinations, psychosis, loss of consciousness and convulsions. Death occurs by respiratory arrest, preceded by ascending paralysis, and pathological lesions are seen in the liver, spleen, kidney and CNS, with congestion in the adrenal gland and edema in the heart muscle.

1.2.5 Pharmacology and Drug Metabolism

After oral ingestion, DSF is partially reduced to DDTC in the acidic stomach, which in turn forms a complex with Cu to form $Cu(DDTC)_2$. Both the parent DSF and $Cu(DDTC)_2$ are absorbed through the gastrointestinal tract. Generally, more than 80% of an oral dose is absorbed, and enteric formulation and oil may enhance improved absorption. After absorption, DSF is again reduced to DDTC and then $Cu(DDTC)_2$. Downstream metabolites also include diethylamine, carbon disulphide, diethyldithiomethylcarbamate (Me-DDTC), and glucuronic acid of DDTC. Me-DDTC is biotransformed into active inhibitors of ALDH [19]. Experiments with radiolabeled DSF have shown distribution in the blood, liver,

kidney, heart, adrenal gland, thyroid, pancreas, testes, spleen, marrow, muscles, and, most importantly, brain [46].

1.2.6 Clinical Experience

Clinical experience with DSF for the treatment of alcoholism has been extensive over the last 50 years. At the dose of 250-500 mg per day as recommended by the FDA, DSF is safe and well-tolerated [18,37]. A few clinical studies have been reported in the literature on its use in cancer patients. A phase II study randomized 64 non-metastatic breast cancer patients after mastectomy to chemotherapy either with DDTC (the main metabolite of DSF) or placebo. The group that received DDTC had significantly better disease-free survival (DFS) and overall survival (OS) as compared to the placebo group [47]. A phase I study treated non-metastatic recurrent prostate cancer patients with 250-500 mg of DSF (9 patients with 250 mg and 10 patients with 500 mg). Only 5 of the 19 patients completed at least 6 months of DSF, and the biochemical response appeared to be minimal. There were 3 patients with grade 3 toxicity for each dose level, including double vision, hearing loss, LFT abnormality, diarrhea, constipation, and ataxia. Due to lack of clear signal for treatment response and the toxicities reported, the authors did not recommend additional testing of DSF in the recurrent prostate cancer population [48]. A recent double-blinded, randomized phase II study compared chemotherapy with and without DSF for metastatic non-small cell lung cancer [49]. DSF was given at 40 mg three times daily (TID). Patients who received concurrent DSF had significantly better PFS and OS than who received chemotherapy alone (5.9 vs. 4.9 months, and 10.0 vs. 7.1 months, respectively). The authors recommended a larger phase III studies to test DSF with chemotherapy for metastatic non-small cell lung cancer.

We have recently conducted a phase I pharmacodynamic study combining 500-1000 mg of DSF with adjuvant temozolomide for newly diagnosed GBM patients after RT and concurrent TMZ, which has determined 500 mg of DSF per day as the maximum tolerated dose (MTD) in combination with adjuvant TMZ. Dose-limiting toxicity, which was defined as within the first 28 days of DSF administration, occurred at 1000 mg of disulfiram per day and included delirium and ataxia. At the MTD of 500 mg per day, 2 of 7 patients eventually stopped DSF after the first month due to possibly DSF-related toxicity. One patient developed grade 3 delirium after 55 days of DSF; another patient developed grade 3 motor neuropathy (lower extremity weakness and foot drop). Both toxicities resolved after discontinuing DSF. Grade 2-3 toxicities that were possibly or probably related to DSF are described in table below. All the toxicities were self-limiting or resolved within 30 days of cessation of DSF. A description of this clinical trial is available on http://clinicaltrials.gov/show/NCT01907165.

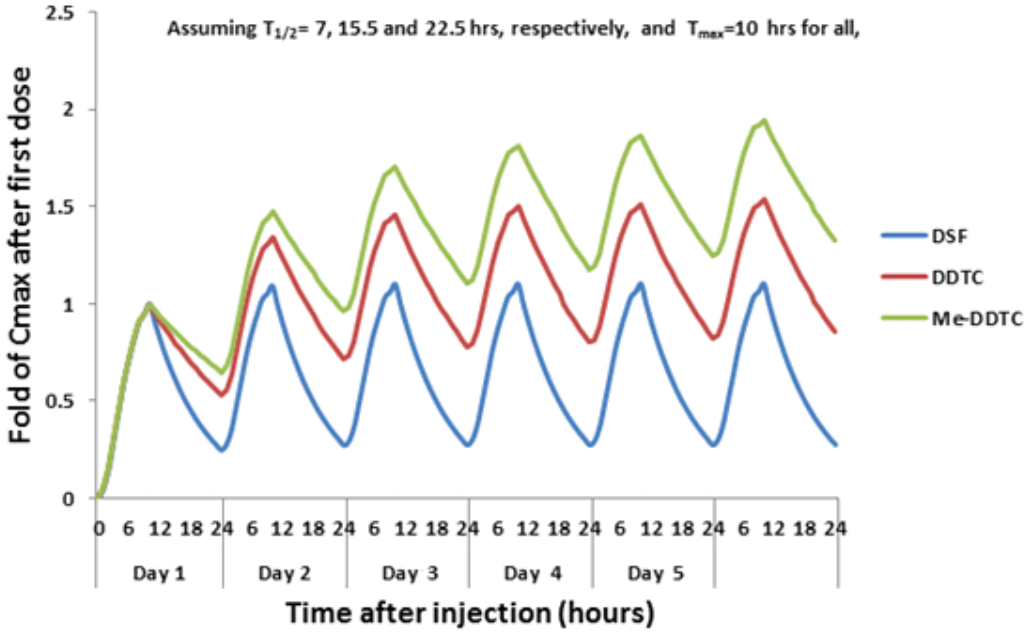
Toxicities*	DSF 500 mg (n=7)		DSF 1000 mg (n=5)	
	Grade 2	Grade 3	Grade 2	Grade 3
Ataxia	1 (14%)	0	2 (40%)	1 (20%)†
Delirium	0	1 (14%)†	2 (40%)	2 (40%)§
Dizziness	1 (14%)	0	1 (20%)	0
Fatigue	3 (43%)	0	1 (20%)	0
Peripheral motor neuropathy	0	1 (14%)†	1 (20%)	0
Peripheral sensory neuropathy	2 (29%)	0	1 (20%)	0

*Grade 2-3 adverse events according to the Nation Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0 that were possibly or probably related to disulfiram (DSF) and that were not present at baseline.
†Not dose-limiting toxicity (DLT) as occurred after the first month of DSF.
§Dose-limiting toxicity (DLT) as occurred within the first month of DSF.

1.2.7 Pharmacokinetics

Previous pharmacokinetic studies were done on alcoholic patients after either single dose or repeated doses for 12 consecutive days. Apparent half-lives of DSF, DDTC, and Me-DDTC were 7.3, 15.5, and 22.1 hours, respectively. Average time to reach maximal plasma concentration was 8-10 hours for DSF and its metabolites. The mean peak plasma concentrations of DSF and Me-DDTC were 1.3 nM and 4.7 nM, respectively. However, there was marked intersubject variability in the plasma concentrations [50,51]. Doses as low as 100 mg of DSF could produce detectable plasma concentrations of Me-DDTC and complete blockade of ALDH activity in erythrocytes [52]. As seen in Figure 1, three daily doses of DSF will allow for DSF and its metabolites to reach steady state.

Figure 1: Predicted pharmacokinetic profile of DSF and its metabolites (DDTC & Me-DDTC)



The metabolites of DSF are mainly excreted via kidneys, feces, and the lungs. Up to 20% of an oral dose may not be absorbed and thus excreted in the feces. About 65% is eliminated through the kidneys, mostly as the glucuronide of DDTC and inorganic sulfates. The metabolite carbon disulphide is mostly eliminated via the lungs [19].

In the blood, both DSF and Me-DDTC are mostly bound to albumin, with average binding percentages of 96 and 80% over the ranges 200-800 and 345-2756 nM, respectively. The average number of binding sites was approximately 1 for both substances, suggesting a single binding site for both. The average association constants were 7.1×10^4 and 6.1×10^3 M^{-1}, respectively. Therefore, patients with impaired protein synthesis and decreased albumin levels may have considerably different plasma concentration of DSF and its metabolites. Both DSF and Me-DDTC are also very lipophilic, with Log P (octanol-water partition coefficient) of 2.81 and 1.85, respectively [53], which support their ability to cross the BBB. However, specific measurement of DSF and its metabolites in gliomas have not been conducted to date.

1.2.8 Measurement of Intratumoral Drug Concentration using Resected Tumors

Although no studies have analyzed uptake of DSF and its metabolites in gliomas, a few prospective studies have investigated intracranial drug penetration by administering chemotherapy before planned resection of brain tumors. In a study measuring cisplatin level, high-grade gliomas appeared to have higher drug

concentration than low-grade gliomas but lower when compared to brain metastases, suggesting break-down of the blood-brain barrier plays an important factor in intratumoral drug concentration [54]. Previous studies have shown a sample size of 5 is typically sufficient to detect difference in drug concentration between dose levels [55]. Given the tumor specimen invariably contain blood, high plasma concentration may lead to over-estimation of the tissue drug concentration [56]. Thus, an accurate estimation of intratumoral drug concentration would require measurement of concurrent plasma level and would ideally be measured when the plasma level has peaked.

1.2.9 Co-administration of Copper (Cu)

Previous *in vitro* studies have consistently shown the effect of DSF is dependent on Cu, at a concentration of approximately 1 uM. Based on 130 autopsy cases of non-poisoned people, the Cu concentration of brain, blood, and stomach were approximately 49.0 uM (median) [range: 10.6-118.3], 13.1 uM [9.0-20.6], 15.7 uM [3.8-76.7], respectively [57]. Furthermore, cancer cells including gliomas cells appear to contain highly elevated levels of Cu as compared to surrounding normal tissues [58-60]. However, typical Western diets provide only 1 mg of Cu daily, less than the lower limit of 1.5 to 3.0 mg which is estimated as a safe and adequate daily dietary intake (ESADDI) of copper [61,62]. Therefore, dietary Cu supplementation may be required for DSF to be maximally effective as an antineoplastic drug. The upper level of recommended daily elemental Cu is 8 mg a day (Institute for Medicine 2002), administered as Cu gluconate, a substance generally regarded as safe (GRAS) (Code of Federal Regulation 2004). Large amounts of ingested Cu could result in hepatic failure and hemolysis similar to that seen with Wilson's disease, the rare genetic syndrome from hyperabsorption of Cu.

Prior reports have shown that 10 mg of elemental Cu can be administered for 2-3 months without any apparent side-effect nor any significant increase of serum copper level [63,64]. A previous phase I study for patients with advanced liver metastasis has shown 6 mg of elemental Cu in the form of Cu gluconate is well tolerated when given with 250 mg of DSF daily. In this study, Cu gluconate was given in the morning half hour before breakfast, and DSF was given with the evening meals [65].

1.2.10 Potential Risks and Benefits

The safety risks of DSF are well established based on over 60 years of clinical use [18,37,66]. From 1968 to 1991, 154 adverse drug reactions were reported to the Danish Committee on Adverse Drug Reactions: hepatic (34%), neurological (21%), cutaneous (15%), psychiatric (4%), and other (26%). However, some patients may have received much higher doses than the currently approved dosage and may have consumed alcohol while taking DSF. Rate of adverse event is estimated to be one per 200-2000 patients per year [67]. The more common and mild side effects include: drowsiness, usually of short duration (can be managed by taking

medication in the evening); headache; nausea and vomiting; metallic or garlic-like aftertaste; allergic dermatitis; impotence; DSF-ethanol reaction after ingestion of alcohol (flushing, headache, nausea, sweating, palpitation, dyspnea, tachycardia, hypotension, and rarely cardiovascular collapse). These symptoms are generally transient and typically resolve within first two weeks. DSF-ethanol reaction can be avoided by abstinence from drinking alcohol, and case reports of fatal DSF-ethanol reaction in the past occurred with much higher doses of DSF. Other rare but more severe toxicities include: hepatotoxicity or hepatitis, typically reversible if DSF is discontinued early (should be followed with liver function tests); peripheral neuropathy; optic neuritis; extrapyramidal symptoms; psychosis. Patients with hepatic dysfunction, active pregnancy, and idiopathic seizure disorder should not be given DSF.

As discussed in Section 1.2.6, our previous phase 1 study of DSF in combination with adjuvant TMZ established 500 mg of DSF per day as the MTD. At this dose, most patients tolerated DSF with adjuvant TMZ well, but 2 of 7 patients developed dose-limiting confusion and motor neuropathy after prolonged administration. Possible or probable DSF-related toxicities were all neurological and were mostly self-limiting or reversible. The investigators and patients should pay particular attention of any early signs of the neurological symptoms seen in the previous phase I study including delirium, ataxia, and neuropathy, but careful workup to rule out tumor progression or other causes should also be considered before attributing those symptoms to DSF.

Given its long track record of safety when used alone, especially below 1000 mg, DSF should pose minimal risk when given to presumed GBM patients before their surgical resection. Although patients without histological confirmation would qualify for this study, a misdiagnosis of GBM based on MRI finding is relatively uncommon. Furthermore, since it is not a cytotoxic chemotherapy, a short-course administration of pre-operative DSF for 3 days to a patient with a different diagnosis should not adversely affect his/her subsequent treatment. As for overlapping toxicity with TMZ or RT, DSF appears to have preferential inhibition of tumor MGMT versus liver MGMT in vitro, and our previous phase I study demonstrated safety when combining it with TMZ. Since DSF may be radiosensitizing due to its possible effect on DNA repair based on *in vitro* studies, its tolerability with RT is currently unknown. To monitor for severe toxicity, patients will be followed frequently during RT with weekly clinical evaluation and CBC. Liver function test will be checked before starting DSF, and every month while taking DSF.

1.2.11 Potential Adverse Events Related to DSF

MedDRA Term	**Frequency:** *Likely: greater than 10%* *Less Likely: 1-10%* *Rare: 1% or less*
Neoplasms	*Rare:* Tumor necrosis
Blood and lymphatic system disorders	*Rare*: Neutropenia, anemia, leukopenia, thrombocytopenia, lymphopenia (likely due to TMZ)
Immune system disorders	*Rare:* Hypersensitivity
Metabolism and nutrition disorders	*Likely:* Alcohol intolerance, metallic or garlic-like aftertaste
Psychiatric disorders	*Less Likely*: psychosis, delirium (need to rule out tumor progression)
Nervous system disorders	*Likely*: Drowsiness, headache, confusion *Less Likely:* ataxia, gait disturbance, peripheral neuropathy *Rare*: Extrapyramidal symptoms
Eye disorders	*Less Likely*: Optic neuritis
Cardiac disorders	*Less Likely*: Tachycardia, hypotension (need to rule out DSF-alcohol reaction)
Respiratory, thoracic, and mediastinal disorders	*Less Likely*: Dyspnea (need to rule out DSF-alcohol reaction)
Gastrointestinal disorders	*Likely*: Nausea, vomiting, diarrhea *Less Likely:* constipation (likely due to TMZ)
Hepatobiliary disorders	*Rare*: Hepatitis
Skin and subcutaneous tissue disorders	*Less Likely*: Allergic dermatitis
Musculoskeletal	*Rare*: Arthralgia, myalgia
Renal and urinary disorders	*Rare*: Dysuria, hematuria
Reproductive system	*Less likely*: impotence
General disorders	*Likely*: Fatigue

1.3 Study Rationale

Preclinical *in vitro* studies have identified DSF as a promising and selective drug against GBM cells. *In vivo* experiments have showed synergistic effect of DSF with RT and TMZ against orthotopic GBM models. Given its well-established safety profile, excellent penetration across the blood brain barrier, relatively low cost, and ease of administration with an oral formulation, the potential of DSF to enhance the efficacy of RT and TMZ for GBM warrants clinical trial investigation. We have previously demonstrated the feasibility

and safety of combining DSF with adjuvant TMZ after RT and concurrent TMZ. Adding DSF to RT and concurrent TMZ in the initial treatment GBM is a rational next step to test the potential of DSF to improve clinical outcome of GBM patients. Since Cu has been shown in the preclinical studies to be crucial for the activity of DSF, patients will also take Cu TID with meals to maximize the biological activity of DSF. Therefore, the purpose of this study is to identify the optimal dose of DSF to combine with RT and TMZ.

The proposed study will be a single arm, phase I/II study of DSF/Cu in combination with adjuvant standard RT and TMZ for patients with newly diagnosed GBM. There will be an optional phase 0 pharmacokinetic study where patients will take 3 days of DSF/Cu prior to their surgery to allow for collection of blood and tumor samples during surgery for analysis of drug uptake.

As for the rationale for the proposed expansion cohort, this is based on the promising finding of the dose-escalation phase of the study. After enrollment of the 18 patients for the dose-escalation phase, 8 patients were treated with DSF of 250 mg/day (dose level 2) and 10 patients with 375 mg/day (dose level 3). Three dose-limiting toxicities (DLTs) were observed: 1 with 250 mg/day (grade 2 urinary incontinence and ataxia), and 2 with 375 mg/day (both grade 3 elevated liver enzymes). The TITE-CRM model estimated the DLT probability of DSF to be 10% (95% CI: 3-29%) at 250 mg/day, and 21% (95% CI: 7-42%) at 375 mg/day. Thus, we have declared that the MTD of DSF in combination with RT/TMZ/Cu for GBM is 375 mg/day, and the recommended phase II dose is 250 mg/day. Furthermore, tumor mutations were evaluated with next-generation sequencing for all patients to explore possible biomarkers for response. Notably, GBM patients with *IDH* (n = 6), *BRAF* (n = 2), and *NF1* (n = 1) mutations exhibited much better PFS and OS than those without these mutations (n = 9): 1-year PFS of 100% vs 22%, respectively, p = 0.001; 1-year OS of 100% vs 42%, respectively, p = 0.006. Given that the historical PFS and OS of newly diagnosed GBM patients treated with RT and TMZ is approximately 27% and 60% at one year [4], respectively, the preliminary data of this molecular subset of GBM are thought-provoking and raise the possibility that these mutations make GBM tumors more sensitive to DSF/Cu in combination with chemoradiotherapy. Interestingly, all three mutations, which affect approximately 15-20% of GBM tumors, make tumor cells more dependent on glutamate for cellular metabolism [68-70]. As DSF metabolite can affect glutamate uptake, this may be the biological mechanism for the improved outcomes for GBM with these mutations. Thus, we have proposed to add an expansion cohort to this phase I/II study to treat additional GBM patients with *IDH*, *BRAF*, or *NF1* mutations to increase the statistical power of our preliminary finding.

2.0 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
Dose-escalation Phase: To determine the maximum tolerated dose (MTD) or the recommended phase 2 dose (RP2D) of disulfiram (DSF) when administered with concurrent radiation therapy (RT) and temozolomide (TMZ) in patients with newly diagnosed glioblastoma (GBM). **Dose-expansion Phase:** To evaluate the OS of the molecularly-defined cohort of newly diagnosed GBM with *IDH, BRAF,* or *NF1* mutations when treated with the combination of DSF/Cu plus concurrent RT and TMZ.	**Dose-escalation Phase:** The maximum tolerated dose (MTD) of DSF with concurrent RT+TMZ will be defined as the dose associated with a 20% probability of dose-limiting toxicity (DLT). Toxicity will be coded using CTCAE version 4.0, and DLT will be defined as toxicity that occurs within 18 weeks from the first day of RT and is possibly, probably or definitely related to DSF/Cu (or 12 weeks from the completion of RT if there is a delay/interruption of RT). **Dose-expansion Phase:** For the expansion cohort of GBM with *IDH, BRAF, and NF1* mutations, the overall survival (OS) of this molecularly-defined cohort will be measured from the first day of RT.
Secondary	
1. To describe the toxicities of DSF when given concurrently with RT and TMZ. 2. To evaluate the PFS of the molecularly-defined cohort of newly diagnosed GBM with IDH, BRAF, or NF1 mutations when treated with the combination of DSF/Cu plus concurrent RT and TMZ 3. To determine the active DSF metabolite concentration in plasma and tumor tissues.	1. The adverse events will be graded by NCI CTCAE version 4.0. 2. Progression-free survival (PFS) will be determined from the first day of RT to the time of tumor progression or death, whichever occurs first. Tumor progression will be determined using the RANO criteria. 3. Intratumor and plasma concentration of DSF metabolite (ditiocarb-copper complex) will be determined using mass spectrometer method.
Tertiary/Exploratory	
1. To estimate rate of pseudo-progression after chemoradiotherapy 2. To explore the pharmacodynamics effect of DFS on glutamate metabolism in plasma and tumor tissues	1. Pseudo-progression is defined as a transient increase of tumor after chemoradiotherapy that subsequently stabilizes without a change of therapy 2. Pharmacodynamics studies on glutamate metabolism will include measurement of glutamine, glutamate, aspartate, glucose, and lactate levels in blood and tumor tissues using mass spectrometry method.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Diagnosis of GBM or its histological variants (WHO grade IV). Patients who are participating in the optional pre-operative pharmacokinetic study may have presumed GBM based on clinical/radiological findings. However, patient must have histologically confirmed GBM before continuing to receive DSF with concurrent RT/TMZ.

2. Expansion Cohort: must have a diagnosis of GBM (or its histological variants) with *IDH, BRAF or NF1* mutations. Confirmation of these mutations may be either by immunohistochemistry or next-generation sequencing.

3. At least 18 years of age.

4. Karnofsky performance status (KPS) of at least 60% (see Appendix A).

5. For patients who will participate in the optional DSF pharmacokinetic study, they should be eligible for surgical resection for which at least 0.2 cubic cm or approximately 200 mg of tumor will be removed in additional to tumor specimen required for pathology evaluation. Patients enrolled after undergoing surgical resection or biopsy with histologically confirmed GBM are not required to meet this point of inclusion.

6. Eligible for and planning to receive standard fractionated RT with concurrent TMZ.

7. Willing to remain abstinent from consuming alcohol while on DSF.

8. Willing to defer definitive surgery for one week while taking DSF and Cu. Patients who declined the optional pre-operative pharmacokinetic study or enrolled after undergoing surgical resection or biopsy with histologically confirmed GBM are not required to meet this point of inclusion.

9. Meets the following laboratory criteria:
   a. Absolute neutrophil count ≥ 1,500/mcL
   b. Platelets ≥ 100,000/mcL
   c. Hemoglobin > 10.0 g/dL (transfusion and/or ESA allowed)
   d. Total bilirubin ≤ 2x institutional upper limit of normal (ULN)
   e. AST and ALT < 3 x ULN
   f. Serum creatinine < 1.5 x ULN or creatinine clearance > 50 mL/min (by Cockcroft-Gault)

10. Females of childbearing potential (defined as a female who is non-menopausal or surgically sterilized) must be willing to use an acceptable method of birth control (i.e., hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.

11. Able to take oral medication.

12. Able to understand and willing to sign an IRB-approved written informed consent document (legally authorized representative permitted).

3.2 Exclusion Criteria

1. Receipt of any other investigational agents within 14 days prior to study treatment.

2. Enrolled on another clinical trial testing a novel therapy or drug.

3. History of allergic reaction to DSF or Cu.

4. Treatment with the following medications are contraindicated with DSF when taken within 7 days prior to the first dose of DSF + Cu: metronidazole, isoniazid, dronabinol, carbocisteine, lopinavir, paraldehyde, ritonavir, sertraline, tindazole, tizanidine, atazanavir. (Note: the following medications are not contraindicated but should be cautioned if taking concurrently with DSF: warfarin, phenytoin, theophylline, chlorzoxazone, chlordiazepoxide, diazepam. If the patient is taking warfarin, INR should be monitored closely. If the patient has to remain on phenytoin, its serum concentration and response should be monitored closely.)

5. Active or severe hepatic, cardiovascular, or cerebrovascular disease, including myocardial infarction within 6 months prior to enrollment, New York Heart Association (NYHA) Class III or IV heart failure (Appendix B), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities.

6. History of idiopathic seizure disorder, psychosis, or schizophrenia.

7. History of Wilson's disease or family member with Wilson's disease.

8. History of hemochromatosis or family member with hemochromatosis.

9. Pregnant and breastfeeding women will be excluded because of the known teratogenic effect of RT and the unknown effect of TMZ and DSF on fetal development. Women of childbearing potential must have a negative pregnancy test within 14 days of initiation of treatment.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 PATIENT REGISTRATION

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of the patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

1. Confirm patient eligibility by collecting the information listed below: Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Pretreatment Evaluation

Prior to enrollment, patient must have a complete history, physical examination, evaluation of performance status (KPS), baseline laboratory studies (CBC, BMP, and LFTs), and MRI. For the expansion cohort, patients must have confirmed diagnosis of GBM with *IDH, BRAF,* or *NF1* mutations. Diagnosis will be confirmed through the pathology report as a part of standard of care. Although for the dose-escalation phase, there was an optional pre-operative disulfiram study before the initial surgery, this will no longer be applicable for the expansion cohort and has been removed.

5.2 Radiation Therapy

RT should start approximately 4-6 weeks after surgery (up to 8 weeks is permissible). Standard fractionated RT to 60 Gy in 30 daily fractions will be administered in this study. Gross tumor volume (GTV) is defined as T1 contrast enhancing abnormality and surgical cavity. Clinical tumor volume 1 (CTV1) is defined as GTV with a margin of 1.5 cm. Clinical tumor volume 2 (CTV2) is defined as GTV with a margin of 0.5-0.7 cm. CTVs may be modified to respect natural barriers such as bone or tentorium. CTV1 and CTV2 should be expanded uniformly by 0.3-0.5 cm into planning target volume 1 and 2 (PTV1 and PTV2), respectively to account for setup uncertainty. PTV1 should be treated to 46 Gy in 23 fractions of 2 Gy each, and PTV2 should be boosted to an additional 14 Gy in 7 fractions of 2 Gy each for a cumulative total dose of 60 Gy in 30 fractions of 2 Gy each. 3D conformal RT (3DCRT) and intensity modulated RT (IMRT) are all permitted for RT delivery.

5.3 Concurrent Temozolomide Treatment

Concurrent TMZ will be administered continuously (Monday through Sunday) from Day 1 of RT to the last day of RT at a daily oral dose of 75 mg/m^2 for a maximum of 49 days as per standard clinical care. TMZ should be taken before RT. The drug should typically be administered orally at least 1 hour before breakfast every morning. TMZ should not be taken within one hour of DSF and Cu administration. Patients will be instructed to bring all unused drug and the completed medication diary (Appendix C) to each study visit for assessment of compliance.

5.4 Disulfiram and Copper Administration during Chemoradiotherapy

DSF and Cu will be administered during chemoradiotherapy as per dose escalation instructions in Section 5.10. For patients enrolling in the expansion cohort, the dose of DSF will be 250 mg/day. During chemoradiotherapy, DSF should be taken in the morning with approximately 8 ounces of water approximately 1 hour after breakfast, and 2 mg of Cu gluconate should be taken TID with meals but not concurrently with DSF. The rationale of giving Cu in 3 doses is to maintain relatively constant plasma copper throughout the day while DSF is absorbed and metabolized. Concurrent DSF during chemoradiotherapy is administered in the morning to provide the maximum radiosensitizing effect. DSF and Cu should not be taken within one hour of TMZ administration. If the patient misses a dose, s/he should be instructed not to make up that dose but should instead resume dosing with the next scheduled dose. Patients will be instructed to bring all unused drug and the completed medication diary (Appendix D) to each study visit for assessment of compliance.

Medication Schedule	
TMZ	At least 1 hour before breakfast and before RT, but not within 1 hour of DSF + Cu
DSF	1 hour after breakfast
Cu	With breakfast, lunch, and dinner

5.5 Laboratory Studies and Tissue Collection during Chemoradiotherapy

Routine laboratory studies (CBC, BMP, and LFTs) will be obtained before the start of chemoradiotherapy and weekly (for CBC) and monthly (for BMP and LFTs) during chemoradiotherapy or more frequently as clinically indicated. If, during chemoradiotherapy treatment, the patient develops disease progression and undergoes surgical resection of the glioblastoma, a portion of the tissue removed during surgery and along with blood will be archived for future research purposes (please refer to Section 9.0).

5.6 Adjuvant Temozolomide and Disulfiram Treatment

Approximately 4-6 weeks after completion of RT with concurrent TMZ, adjuvant TMZ may be administered for 6 cycles. Dosage of each cycle is 150-200 mg/m^2 PO per day on Days 1-5 of every 28-day cycle. DSF will be administered at 500 mg PO daily continuously in combination with adjuvant TMZ for up to 6 cycles as established by our previous phase I study. The timing for DSF and Cu are same as in Section 5.7. Additional adjuvant TMZ beyond 6 cycles may be given at the discretion of the treating medical oncologist but not with DSF. Per standard clinical care, adjuvant TMZ is typically given in the evening before bedtime rather than in the morning. The timing of adjuvant TMZ will be established at the discretion of the treating medical oncologist. Tumor-treating fields or Optune device (Novocure) as per routine clinical care during adjuvant TMZ is permitted at the discretion of the treating physician.

During adjuvant TMZ, the patient should be assessed every 2 months as per routine clinical care; however, more frequent visits may be performed as per the treating physician. During adjuvant DSF with concurrent TMZ, if a patient develops disease progression and undergoes surgical resection of the GBM, a portion of the tissue removed during surgery and along with blood will be archived for future research purposes (please refer to Section 9.0).

5.7 Optional Preoperative Disulfiram before Salvage Surgery

After completion of adjuvant DSF, patients should be followed every 2-3 months as per routine clinical care. If a patient develops recurrent tumor during follow-up and plans to undergo another resection, he/she may opt for an optional preoperative DSF study prior to salvage surgery. Patients who would like to participate in this optional portion of the study will be treated with a 5-day (+/- 2 days) lead-in with oral DSF and Cu. DSF should be taken as 250 mg per day approximately 1 hour after dinner in the evening, and Cu should be taken as 2mg TID with each meals. Patient should not take DSF or Cu on the day of the surgery and should complete the drug diary (Appendix E).

5.8 Disulfiram Dose Escalation Criteria

The first patient will be treated at Dose Level 2 with 250 mg of DSF daily, and doses for subsequent patients will be provided at the time of registration based on the toxicity experience of the previous patients on study using the TITE-CRM method, as detailed in Section 13.3.

Dose Escalation Schedule	
Dose Level	**Dose of Disulfiram**
Level 1	125 mg PO daily
Level 2	250 mg PO daily
Level 3	375 mg PO daily
Level 4	500 mg PO daily

For patients enrolling in the expansion cohort, the dose of DSF during RT will be 250 mg/day.

5.9 Definition of MTD, DLT, Dose Escalation Criteria, and Toxicity, Response, and DLT Evaluations

5.9.1 Definition of Maximum Tolerated Dose (MTD)

The maximum tolerated dose (MTD) of DSF is defined as the dose level at which 20% of the cohort experience DLT within 18 weeks from start of RT (or 12 weeks from the end of RT if there is a delay in RT). MTD is assessed from the first dose of DSF in combination with TMZ and RT; patients will not be assessed for DLT during the pre-surgery period when they are receiving the lead-in doses of DSF.

5.9.2 Dose Limiting Toxicities (DLTs)

A DLT is defined as a clinically significant adverse event or abnormal laboratory value assessed as unrelated to disease progression, intercurrent illness, or concomitant medications/TMZ/adjuvant DSF which occurs within 18 weeks following the first dose of DSF with RT+TMZ (corresponding to approximately 6 weeks during RT and 12 weeks after RT) and meets any of the following criteria (all toxicity will be graded using CTCAE version 4.0):

- Any delay in RT treatment > 14 days that is possibly, probably, or definitely related to DSF will be considered a DLT.
- Any adverse event that requires dose reduction or discontinuation of DSF during RT that is possibly, probably, or definitely related to DSF will be considered a DLT.
- Grade 4 neutropenia or thrombocytopenia.

Note: These dose limiting hematological toxicities are most likely due to TMZ and may be attributed to TMZ. However, as a conservative assessment of the ability to combine DSF with RT and TMZ, these toxicities will be counted as a DLT as they would prompt discontinuation of TMZ.

- Serum creatinine > 2.0 x ULN
- Total bilirubin > 2.0 x ULN; AST or ALT > 3.0 x ULN
- Other grade 3 or grade 4 non-hematologic toxicity that is possibly, probably, or definitely related to DSF, with the following specific exceptions:
  - Grade 3 fatigue
  - Grade 3 arthralgias/myalgias
  - Grade 3 or 4 nausea, vomiting, or anorexia that does not require discontinuing RT or TMZ
  - Diarrhea that can be adequately managed with outpatient medication such as loperamide and does not require hospitalization

Headaches are frequent in the brain tumor population and are usually due to diffuse increased intracranial pressure or compression of pain-sensitive intracranial structures (example: dura). The oncologists participating in this study are experienced clinicians accustomed to evaluation of brain tumor patients with headaches. Each patient with a headache is approached individually with a systematic assessment as to the etiology of the pain. Appropriate tests may include vital signs, CT or MRI scans, or other investigations. If, in the opinion of the treating oncologist, the headache is due to DSF, then the toxicity will be graded as above, and if ≥ grade 3, it will be defined as a DLT.

Seizures are also a common complication associated with brain tumors. As noted in the preceding paragraph each patient will be approached individually and assessment for the cause of the seizure will be performed. If, in the opinion of the treating oncologist, the seizure is due to DSF, then the toxicity will be graded, and if ≥ 3, it will be defined as a DLT.

5.9.3 Toxicity, Response, and DLT Evaluations

All patients who receive at least one dose of DSF are evaluable for toxicity. However, patients have to receive the combination of DSF with RT+TMZ to be evaluable for DLT. Of note, DLT evaluation starts when patient starts DSF with RT and does not apply during the preoperative DSF administration. Patients who do not have at least one dose of DSF with RT+TMZ will not be evaluable for DLT and will be replaced.

5.10 General Concomitant Medication and Supportive Care Guidelines

The following medications and procedures are prohibited during the study:

- Any antineoplastic therapy other than RT, temozolomide, and DSF, except for the tumor-treating fields or Optune device when used in conjunction with adjuvant temozolomide and DSF
- Any investigational therapy other than DSF

All other medical conditions or manifestations of the patient's malignancy should be treated at the discretion of the investigator in accordance with local community standards of medical care.

Patients should not drive, operate dangerous tools or machinery, or engage in any other potentially hazardous activity that requires full alertness and coordination if they experience sedation while enrolled in this study.

Patients are to be instructed to abstain from alcohol while enrolled in this study.

5.10.1 Nausea and Vomiting

Prophylactic antiemetic therapy may be used in this study at the discretion of treating physician. Because of the potential of benzodiazepines to interact with DSF through the cytochrome P450 system, the use of benzodiazepines for antiemetic prophylaxis should be reserved for patients who cannot be satisfactorily managed otherwise.

5.10.2 Diarrhea

Antidiarrheal medications will not be used prophylactically; however, patients will be instructed to take loperamide, 4 mg total, at the occurrence of the first loose stool and then 2 mg every 2 hours until they are diarrhea-free for at least 12 hours. During the night, patients may take 4 mg of loperamide every 4 hours. Fluid intake should be maintained to avoid dehydration.

5.10.3 Central Nervous System Effects

In our previous experience with 500-1000 mg of DSF per day with adjuvant TMZ, significant neurological toxicities, including delirium/psychosis, ataxia, visual changes, peripheral neuropathy, were observed, especially at the 1000 mg dose. Early signs of those symptoms should be carefully monitored. Once other causes such as tumor progression are ruled out, dose reduction of DSF during adjuvant TMZ should be considered if the toxicity is grade 2 or greater. If symptoms are not improving with dose reduction, DSF should be discontinued. Patients whose symptoms are not considered immediately life-threatening should be carefully monitored. Each patient may be approached individually with a systematic

assessment to rule out other causes. Appropriate tests may include vital signs; CT or MRI scans or other investigations.

If the patient's level of consciousness is considered to be life-threatening, the patient should be hospitalized and necessary measures should be instituted to secure the airway, ventilation, and intravenous access.

5.10.4 Management of Disulfiram-Alcohol Reaction

Severe DSF reactions caused by the patient's ingestion of alcohol should lead to emergency evaluation and supportive measures to restore blood pressure. Other recommendations include: oxygen, carbogen (95% oxygen and 5% carbon dioxide), vitamin C intravenously in massive doses (1 g), and ephedrine sulfate. Antihistamines have also been used intravenously. Potassium levels should be monitored, particularly in patients on digitalis, since hypokalemia has been reported.

5.11 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, or women who have had a tubal ligation) are required to have a negative serum pregnancy test within 14 days prior to the first dose of the study treatment.

Female and male patients (along with their female partners) are required to use a method of acceptable contraception, including one barrier method, during participation in the study and for 4 months following the last dose of study treatment.

If a patient is suspected to be pregnant, study treatment should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume treatment.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 4 months after the last dose of study treatment, the investigator must be notified in order to facilitate outcome follow-up.

5.12 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, DSF treatment may continue until completion of 6 cycles of adjuvant TMZ or in the event of following:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious noncompliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

5.13 Duration of Follow-up

Patients are evaluated for adverse events for 30 days after the last dose of DSF or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Follow-up after the conclusion of adjuvant TMZ + DSF will be per routine clinical care. Two years after the patient comes off study, the chart will be reviewed to collect data on progression and survival.

6.0 DOSE MODIFICATIONS

6.1 Temozolomide Dose Modifications during Radiation Therapy

No dose reduction will be made, but delay or discontinuation of TMZ administration will be decided weekly according to hematologic and non-hematologic adverse events, as specified below. Additionally, TMZ can be held at the discretion of the treating physician. If the administration of TMZ has to be interrupted, RT will proceed normally. Missed doses of TMZ will not be made up at the end of RT. The total number of days and total dose of temozolomide will be recorded on the patient's medication diary (Appendix C).

If one or more of the following are observed:

- ANC $< 1.5 \times 10^9$/ L (Grade 2)
- Platelet count $< 75 \times 10^9$/L (Grade 2)
- Grade 3 non-hematologic AE (except alopecia, nausea and vomiting while on maximal antiemetic therapy, and fatigue)

Then treatment with concurrent TMZ will be withheld until all of the following conditions are met:

- ANC $\geq 1.5 \times 10^9$/L
- Platelet count $\geq 75 \times 10^9$/L
- Grade ≤ 1 non-hematologic AE (except alopecia, nausea and vomiting, and fatigue)

In case of hematologic AE as defined above, a complete blood count (CBC) should be performed at least weekly. In case of non-hematologic AE, the patient should be assessed at least weekly with relevant laboratory test(s). As soon as all of the above conditions are met, the administration of temozolomide will resume at the same dose as used initially.

If one or more of the following are observed:

- ANC $< 0.5 \times 10^9$/L (Grade 4)
- Platelet count $< 25 \times 10^9$/L (Grade 4)
- Grade 4 non-hematologic AE (except alopecia, nausea and vomiting unless the patient has failed maximal antiemetic therapy, and fatigue)

Then treatment with concurrent temozolomide should be discontinued.

AE	Value	Action
Grade 3 neutropenia	≥ 0.5 and $< 1.0 \times 10^9$/L	Hold TMZ until --ANC $\geq 1.5 \times 10^9$/L --Platelet $\geq 75 \times 10^9$/L --Grade ≤ 1 non-hematologic AE
Grade 2-3 thrombocytopenia	≥ 25 and $< 75 \times 10^9$/L	
Grade 3 non-hematologic (except alopecia, nausea/vomiting unless on maximal antiemetic therapy)	NA	
Grade 4 neutropenia	$< 0.5 \times 10^9$/L	Stop TMZ
Grade 4 thrombocytopenia	$< 25 \times 10^9$/L	
Grade 4 non-hematologica (except alopecia, nausea/vomiting)	NA	

If RT has to be temporarily interrupted for technical or medical reasons unrelated to TMZ, then treatment with daily TMZ should continue. If RT has to be permanently discontinued then TMZ should stop until it is re-initiated at the adjuvant phase of treatment.

6.2 DSF Dose Modifications

During radiotherapy, if a patient experiences any adverse event that requires dose reduction in the opinion of the investigator and that is considered by the investigator to be possibly, probably, or definitely related to DSF, DSF/Cu should be discontinued and the event will be considered a DLT. If RT has to be temporarily interrupted for technical or medical reasons unrelated to TMZ or DSF/Cu, then treatment with daily TMZ and DSF/Cu should continue. If RT has to be permanently discontinued then TMZ and DSF/Cu should stop until re-initiated at the adjuvant phase of treatment. If TMZ is held due to toxicity unrelated to DSF/Cu, DSF/Cu should continue as tolerated.

During adjuvant TMZ, the dose of DSF may be reduced from 500 mg per day to 250 mg per day due to toxicity or intolerance at the discretion of the treating physician. Expansion patients who discontinued DSF during RT and concurrent TMZ due to toxicity may be considered to resume DSF with adjuvant TMZ but at 250 mg/day as long as the toxicity has resolved prior to the start of cycle 1 of adjuvant TMZ and after discussion between the treating physician and the PI. Special attention should be paid to neurological symptoms such as delirium/psychosis, gait disturbance/ataxia, and peripheral neuropathy. A grade 2 toxicity of the above neurological symptoms should prompt a consideration for further work-up and consideration of dose reduction if thought to be related to DSF. No second dose reduction (below 250 mg/day) is allowed—if a second dose reduction is required, DSF and Cu will be permanently discontinued. Of note, hematologic toxicity is uncommon for DSF and is likely related to TMZ.

6.2.1 Administration of DSF to Patients with Abnormal Hepatic Function

DSF/Cu should only be administered if hepatic function is within the parameters established in the eligibility criteria. Hepatic toxicity from DSF/Cu is uncommon but may occur. Therefore, hepatic dysfunction that occurs while the patient is on study should prompt an evaluation to determine the cause, including the possibility of hepatotoxicity from concurrent medications including TMZ.

6.2.2 Hypersensitivity Reactions

Hypersensitivity reactions rarely occur. If they do occur, minor symptoms such as flushing, skin reactions, dyspnea, lower back pain, hypotension, or tachycardia may require no intervention; however, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema, or generalized urticaria require immediate discontinuation of study drug administration and aggressive symptomatic therapy. Patients who experience a severe hypersensitivity reaction to DSF should discontinue DSF immediately and should not be re-challenged.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 7.2.

7.1 Definitions

7.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

7.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

7.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

7.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

7.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research

procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

7.2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

7.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

7.4 Timeframe for Reporting Required Events

Adverse events will be tracked until the last dose of DSF. However, DLTs will only be evaluated for 18 weeks from the first day of DSF with RT+TMZ or at least 12 weeks following the completion of RT in the event there is a delay/interruption of RT. All clinically or laboratory adverse events that are > grade 1 (excluding lymphopenia) will be collected.

8.0 PHARMACEUTICAL INFORMATION

8.1 Disulfiram

8.1.1 DSF Description

DSF is an alcohol antagonist drug approved by the FDA for the treatment of alcoholism. Its powder is white, odorless, and almost tasteless. It is soluble in water to the extent of about 20mg in 100mL, and in alcohol to the extent of about 3.8 g in 100 mL.
Molecular formula: $C_{10}H_{20}N_2S_4$
Chemical name: bis(diethylthiocarbamoyl) disulfide.
Molecular weight: 296.54.

8.1.2 Clinical Pharmacology

DSF is mostly known as an irreversible inhibitor of aldehyde dehydrogenase, which affects alcohol metabolism and causes accumulation of acetaldehyde. However, increasing preclinical studies have shown that DSF is also a proteasome inhibitor, specifically the chymotrypsin-like activity. GBM cells and its stem-like subpopulation (also referred to as CSC) may be more susceptible to the effect of

proteasomal inhibition than normal brain cells. DSF is very lipophilic and readily crosses the blood-brain barrier.

8.1.3 Supplier

DSF (trade name Antabuse) is commercially available and will be provided to participants by the study.

8.1.4 Dosage Form

DSF is supplied as white, round tablets of 250 mg. Each tablet also contains colloidal silicon dioxide, anhydrous lactose, magnesium stearate, microcrystalline cellulose, sodium starch glycolate, and stearic acid. The tablet may be cut in half to obtain 125 mg dose.

8.1.5 Storage and Stability

DSF is dispensed in a tight, light-resistant container. It should be stored at controlled room temperature (20° to 25°C or 68° to 77°F) in its original container to protect from bright light.

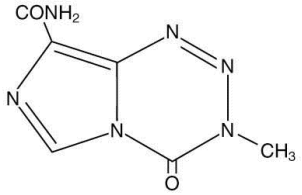
8.1.6 DSF Administration

DSF is taken by mouth daily. It should be taken with meal to improve absorption. It should not be taken within one hour of temozolomide. Patient should not have consumed any alcohol at least 12 hours prior to the first dose. In the rare event of a severe hypersensitivity reaction, discontinue DSF immediately.

8.2 Temozolomide (Temodar)

8.2.1 Temozolomide Description

Chemical name: 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-*as*-tetrazine-8-carboxamide.
Structural formula:



8.2.2 Clinical Pharmacology

Temozolomide is not directly active but undergoes rapid nonenzymatic conversion at physiologic pH to the reactive compound 5-(3-methyltraizen-1-yl)-imaidazole-4-carboxamide (MTIC). The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O^6 and N^7 positions of guanine.

8.2.3 Pharmacokinetics and Drug Metabolism

Temozolomide is rapidly and completely absorbed after oral administration with a peak plasma concentration (C_{max}) achieved in a median T_{max} of 1 hour. Food reduces the rate and extent of temozolomide absorption.

Temozolomide is spontaneously hydrolyzed at physiologic pH to the active species, MTIC and to temozolomide acid metabolite. MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis, and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P540 enzymes play only a minor role in the metabolism of temozolomide and MTIC.

About 38% of the administered temozolomide total radioactive dose is recovered over 7 days: 37.7% in urine and 0.8% in feces. Temozolomide is rapidly eliminated, with a mean elimination half-life of 1.8 hours.

8.2.4 Supplier

Temozolomide is commercially available.

8.2.5 Dosage Form and Preparation

Temozolomide capsules are supplied in child-resistant sachets containing the following capsule strengths: 5 mg, 20 mg, 100 mg, 140 mg, 180 mg, and 250 mg.

8.2.6 Storage and Stability

Store temozolomide capsules at 35°C (77°F); excursions permitted to 15-30°C (59-86°F).

8.2.7 Administration

Please refer to Sections 5.6 and 5.9.

8.2.8 Special Handling Instructions

The use of gloves is recommended.

9.0 CORRELATIVE STUDIES

The correlative studies to measure drug concentration will be performed in all enrolled patients that have agreed to take part in collection of blood and tumor samples. Drug concentration of DSF metabolites (such as ditiocarb-copper complex) from the serum and tumor samples will be measured by mass spectrometry by our collaborator Dr. Martin Mistrik at Palacky University

Olomouc in Czech Republic as previously described [71]. **Of note, the tumor, plasma, cell pellet or lysate may be stored at -80 ºC for up to 2 years before batch analysis.**

9.1 Collection of Specimens

If a patient is participating in the optional tumor collection at time of recurrence, a tumor specimen of at least 0.2 cubic cm or approximately 200 mg will be collected. At the time of salvage surgery, approximately 20 mL of peripheral blood (10 mL into each of 2 green top tube containing sodium heparin) will also be collected. Tumor and blood samples will be submitted at ambient temperature to Tissue Procurement Core (TPC) for processing and storage.

10.0 STUDY CALENDAR

10.1 Dose-escalation phase:

Baseline evaluations are to be conducted up to 14 days prior to start of pre-operative DSF. Labs and MRIs must be done no more than 30 days prior to the start of the protocol therapy.

	B/l	3 days pre-surg[18]	Surg[12]	Pre-RT[13]	Wks 1-6[14]	Adjuvant TMZ[5] Cycles						F/U[16]
						1	2	3	4	5	6	
Informed consent	X											
Physical exam	X			X	X[1]	X	X	X	X	X	X	
KPS	X			X								
CBC	X		X	X	X[1]	X	X	X	X	X	X	
BMP	X		X	X	X[20]	X	X	X	X	X	X	
LFTs	X		X	X	X[20]	X	X	X	X	X	X	
β-hCG[2]	X			X								
MRI w/ and w/o contrast	X			X[19]			X		X		X	
Path. conf. of GBM diagnosis				X								
RT					X[15]							
Temozolomide					X[3]	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]	
DSF		X[10]			X[11]	X[6]	X[6]	X[6]	X[6]	X[6]	X[6]	
Cu[7]		X[10]			X	X	X	X	X	X	X	
Review of medication diary			X		X[8]							
Adverse events		X		X	X[8]	X[8]	X[8]	X[9]	X[9]	X[9]	X[9]	X[17]
Collection of tumor[17]			X									
Collection of blood			X									

1. Weekly during RT + TMZ
2. Women of childbearing potential only
3. Daily during RT
4. Days 1-5 of every 28-day cycle
5. Adjuvant TMZ will start 4-6 weeks after the end of RT and will be given for 6 28-day cycles
6. 500 mg daily with adjuvant TMZ
7. 2 mg TID with meals while taking DSF
8. Reviewed weekly by the treating radiation oncologist or the institutional PI during concurrent RT + TMZ, and reviewed every 8 weeks during adjuvant TMZ.
9. Only if patient is still receiving DSF
10. DSF/Cu are taken for 3 days prior to surgery, and preoperative DSF should be taken in the evening approximately 1 hour before or 1 hour after dinner. The dose of DSF is as per dose-escalation criteria.
11. DSF is taken in the morning approximately1 hour before or 1 hour after breakfast. The dose of DSF is as per dose-escalation criteria
12. Must be resection with at least 200 mg of tissue available beyond what is required for pathology. This is only required for patients that have opted into the preoperative DSF + Cu phase of treatment.
13. For patients consented for the optional preoperative DSF, eligibility must be reconfirmed after surgery and before the start of RT. For patients consented after surgery, they need to meet eligibility prior to RT.
14. RT and TMZ will start 4-6 weeks after resection (up to 8 weeks is permissible)
15. 30 fractions of RT given over 6 weeks
16. The patient will be followed for 30 days after the last dose of disulfiram for adverse events; 2 years after coming off study, a review of the patient's medical records will be conducted to collect data on progression and survival
17. If the tumor recurs during the 6-month treatment period and the patient has surgery, we will request a portion of the tissue removed during surgery for future correlative studies

18. OPTIONAL; patients whose disease recurs and who will undergo another resection may opt to take p 5 days (+/- 2 days) of preoperative DSF (250 mg per day in the morning) and Cu (2mg PO TID with meals) until the day before the planned surgery. Patient should not take DSF/Cu on the day of surgery.
19. Simulation MRI can be used as pre-RT MRI if conducted with and without contrast
20. Monthly during RT + TMZ.

10.2 Dose-expansion phase:

Baseline evaluations, labs and MRIs must be done no more than 30 days prior to the start of the protocol therapy.

	B/l	Wks 1-6[1]	Adjuvant TMZ[5] Cycles						F/U[3]	Optional: Tissue collection at recurrence[4]
			1	2	3	4	5	6		
Informed consent	X									X
Physical exam	X	X[5]	X	X	X	X	X	X		
KPS	X									
CBC	X	X[5]	X	X	X	X	X	X		
BMP	X	X[6]	X	X	X	X	X	X		
LFTs	X	X[6]	X	X	X	X	X	X		
β-hCG[7]	X									
MRI w/ and w/o contrast	X[8]			X		X		X		
Path. diagnosis of GBM with IDH, BRAF, or NF1 mutations	X									
RT		X[9]								
Temozolomide		X[10]	X[11]	X[11]	X[11]	X[11]	X[11]	X[11]		
DSF		X[12]	X[13]	X[13]	X[13]	X[13]	X[13]	X[13]		X[16]
Cu[14]		X	X	X	X	X	X	X		X[16]
Review of medication diary		X[15]								X
Adverse events		X[15]	X[15]	X[15]	X[15]	X[15]	X[15]	X[15]	X	X
Collection of tumor										X[17]
Collection of blood										X[17]

1. RT and TMZ will start 4-6 weeks after resection (up to 8 weeks is permissible)
2. Adjuvant TMZ will start 4-6 weeks after the end of RT and will be given for 6 28-day cycles
3. The patient will be followed for 30 days after the last dose of disulfiram for adverse events; 2 years after coming off study, a review of the patient's medical records will be conducted to collect data on progression and survival
4. If a patient develops recurrence and is eligible for salvage surgery, he/she will be eligible for optional tissue collection at time of salvage surgery. If he/she is not on DSF/Cu any more, he/she may opt to take 5 days (+/- 2 days) of preoperative DSF and Cu before the planned surgery.
5. Weekly during RT + TMZ
6. Monthly during RT + TMZ.
7. Women of childbearing potential only
8. Simulation MRI can be used as pre-RT MRI if conducted with and without contrast
9. 60 Gy in 30 fractions
10. 75 mg/m^2 daily during RT
11. Days 1-5 of every 28-day cycle
12. DSF is taken in the morning approximately1 hour after breakfast. The dose of DSF in the expansion cohort is 250 mg once daily during RT.
13. DSF dose during adjuvant TMZ will be 500 mg once daily
14. 2 mg TID with meals while taking DSF

15. Reviewed weekly by the treating radiation oncologist or the institutional PI during concurrent RT + TMZ, and reviewed every 8 weeks during adjuvant TMZ.
16. If patient is still receiving DSF/Cu on study, they should continue until the day before the planned surgery. If they are off DSF/Cu at time of recurrence, they may opt to take 5 days (+/- 2 days) of preoperative DSF (250 mg per day in the evening) and Cu (2mg PO TID with meals) until the day before the planned surgery. Patient should not take DSF/Cu on the day of surgery.
17. A portion of resected tumor tissue and blood will be collected for future correlative studies.

11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	**Submission Schedule**
Original Consent Form	Prior to registration
On-Study Form	Prior to starting treatment
Surgery Form	Time of surgery
Treatment Form	At completion of RT End of every cycle during adjuvant TMZ
Correlatives Form	Time of surgery Time of progression
Toxicity Form	Continuous
Treatment Summary Form	Completion of treatment
Follow Up Form	2 years after last dose of disulfiram
MedWatch Form	See Section 7.0 for reporting requirements

12.0 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 9 +/- 1 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 9 weeks (not less than 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology (RANO) working group guideline [78].

Criteria for Determining First Progression Depending on Time from Initial Chemoradiotherapy

First Progression	Definition
Progressive disease < 12 weeks after completion of chemoradiotherapy	Progression can only be defined using diagnostic imaging if there is new enhancement outside of the radiation field (beyond the high-dose region or 80% isodose line) or if there is unequivocal evidence of viable tumor on histopathologic sampling (eg, solid tumor areas [ie, > 70% tumor cell nuclei in areas], high or progressive increase in MIB-1 proliferation index compared with prior biopsy, or evidence for histologic progression or increased anaplasia in tumor). Note: Given the difficulty of differentiating true progression from pseudoprogression, clinical decline alone, in the absence of radiographic or histologic confirmation of progression, will not be sufficient for definition of progressive disease in the first 12 weeks after completion of concurrent chemoradiotherapy.
Progressive disease ≥ 12 weeks after chemoradiotherapy completion	1. New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids. 2. Increase by ≥ 25% in the sum of the products of perpendicular diameters between the first postradiotherapy scan, or a subsequent scan with smaller tumor size, and the scan at 12 weeks or later on stable or increasing doses of corticosteroids. 3. Clinical deterioration not attributable to concurrent medication or comorbid conditions is sufficient to declare progression on current treatment but not for entry onto a clinical trial for recurrence. 4. For patients receiving antiangiogenic therapy, significant increase in T2/FLAIR nonenhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not be a result of comorbid events (eg, effects of radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).

Criteria for Response Assessment Incorporating MRI and Clinical Factors (Adapted from JCO 2010)

Response	Criteria
Complete response	• Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks. • No new lesions; stable or improved nonenhancing (T2/FLAIR) lesions. • Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: • ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. • No progression of nonmeasurable disease.

Response	Criteria
	• Stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan. • Stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: • Does not qualify for complete response, partial response, or progression. • Stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: • ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*. The absolute increase in any dimension must be at least 5mm when calculating the products. • Significant increase in T2/FLAIR nonenhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). • Any new measureable lesion. • Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose. • Failure to return for evaluation as a result of death or deteriorating condition; or clear progression of nonmeasurable disease.

- NOTE. All measurable and nonmeasurable lesions must be assessed using the same techniques as at baseline.
- Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.
- Stable doses of corticosteroids include patients not on corticosteroids.

12.2 Disease Parameters

Measurable disease: Bi-dimensionally measurable lesions with clearly defined margins by MRI scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable or evaluable disease: Uni-dimensionally measurable lesions or lesions with margins not clearly defined such as areas of T2/FLAIR signal abnormality or poorly defined enhancing abnormality.

Note: For cystic lesions, the only measurable part is any enhancement area around the cyst that is clearly defined and bi-dimensionally measurable. The cyst itself should not be considered measurable or non-measureable disease.

Target lesions: All measurable lesions that are residual of the lesion treated with MLA should be identified as target lesions and recorded and measured. Target lesions should be selected on the basis of their size (lesions with the longest diameter), but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion, which can be measured reproducibly should be selected. When there are too many measurable lesions, choose the largest 3 lesions as target lesions to follow. The other measurable lesions should be considered evaluable for the purpose of objective status determination.

Non-target lesions: All non-measurable lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 2 weeks before the beginning of the treatment.

Clinical lesions: Clinical lesions will only be considered measurable on brain MRI when they are ≥ 5 mm diameter as assessed using a ruler.

Histology: This technique can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases when biopsy or surgical resection of a measureable lesion is clinically indicated.

Perfusion/CBV: This advanced brain MRI technique can be used as an adjunct test to determine treatment response or disease status. However, it should not be used as the primary or sole method to determine response or disease status.

Brain FDG-PET coupled with head CT or brain MRI: This advanced metabolic imaging technique can be used as an adjunct test to determine response or disease status. However it should be used as the primary or sole method of determining response or disease status.

12.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions.

Partial Response (PR): ≥ 50% decrease compared with baseline in the sum of

products of perpendicular diameters of all target lesions sustained for at least 4 weeks.

Progressive Disease (PD): At least a 25% increase in the sum of products of perpendicular diameters of at least 1 target lesion, taking as reference the smallest sum of products of perpendicular diameters on study (this includes the baseline sum if that is the smallest on study). The absolute increase in any dimension must be at least 5mm when calculating the products of perpendicular diameters.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of products of perpendicular diameters while on study.

12.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

12.3.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Summary of the RANO Response Criteria (Adapted from JCO 2010)

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.

* Progression occurs when this criterion is present.

† Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

NOTE: Patients may continue on treatment and remain under close observation and evaluation at 4-8 week intervals if there is uncertainty regarding whether pseudoprogression may be present as determined by the investigators. If subsequent radiographic or clinical assessments suggest that the patient is in fact experiencing progression, then the date of progression should be the time point at which this issue was first raised. Similarly, stable disease may be assigned in cases of presumed "pseudoprogression" associated with decreased steroid use.

12.3.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

12.3.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

During the phase I dose escalation, the Principal Investigator will review all patient data at least monthly (or before each dose-escalation if occurring sooner than monthly), and provide a semi-annual report to the Quality Assurance and Safety Monitoring Committee (QASMC). During the phase II, the Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASM Committee according to institutional guidelines.

14.0 STATISTICAL CONSIDERATIONS

14.1 Definition of Primary Endpoint

The maximum tolerated dose (MTD) of DSF with concurrent RT+TMZ will be defined as the dose associated with a 20% probability of dose-limiting toxicity (DLT). Toxicity will be coded using CTCAE version 4.0, and DLT will be defined as toxicity that occurs within 18 weeks from the first day of RT and is possibly, probably or definitely related to DSF/Cu (or 12 weeks from the completion of RT if there is a delay/interruption of RT).

For the expansion cohort of GBM with *IDH, BRAF, and NF1* mutations, the overall survival (OS) of this molecularly-defined cohort will be measured from the first day of RT.

14.2 Definition of Secondary and Exploratory Endpoints

1. The adverse events will be graded by NCI CTCAE version 4.0.
2. Progression-free survival (PFS) will be determined from the first day of RT to the time of tumor progression or death, whichever occurs first. Tumor progression will be determined using the RANO criteria.
3. Intratumor and plasma concentration of DSF metabolite (ditiocarb-copper complex) will be determined using mass spectrometer method.
4. Pharmacodynamics studies on glutamate metabolism will include measurement of glutamine, glutamate, aspartate, glucose, and lactate levels in blood and tumor tissues using mass spectrometry method.
5. Pseudo-progression is defined as a transient increase of tumor after chemoradiotherapy that subsequently stabilizes without a change of therapy.

14.3 Analytic Plan for Primary Endpoint

14.3.1 Background of Time-to-Event Continual Reassessment Method (TITE-CRM) and Trial Rules

The standard phase I 3+3 design is poorly suited for trials of RT, for which toxicities may occur up to several months after treatment. In this case, the 3+3 design, or any design that requires all patients to have completed observation for toxicity, is subject to openings and closings as patients present after a dose level potentially has filled, but before sufficient time has elapsed to be certain that treatment has not produced DLT.

As such, dose escalation for this trial will be guided by the Time-to-Event Continual Reassessment Method (TITE-CRM). TITE-CRM seeks to determine the target dose, defined as the dose most closely identified with the target rate, which is the largest acceptable probability of toxicity, determined a priori by the investigators based on the relative costs and benefits of the treatment (typically between 5% and 25%). As the trial progresses and patients do or do not experience toxicities at different doses, the estimates of probability of toxicity are recalculated using a

Bayesian expectation, and subsequent patients are assigned to doses under the principle to always treat at the target dose. In a Monte Carlo simulation of 60,000 phase I trials comparing 3+3 design to TITE-CRM in studies with delayed toxicity, the TITE-CRM trials were significantly shorter when toxicity observation times are long, treated more patients at or above the MTD, identified the MTD more accurately, but did not expose patients to significant additional risk [79]. Additionally, given that TITE-CRM concentrates most of the accrual around the MTD, early estimates of efficacy are possible for a phase I trial.

The Time-to-Event Continual Reassessment Method (TITE-CRM) proposed by Cheung and Chappell is an adaptive Phase I design [80]. In a TITE-CRM clinical trial, patients enroll as they become available to be studied. Each participant is assigned to a dose level from a set of dose levels pre-defined by investigators and is monitored for DLTs over time. The design is adaptive in that the dose level assigned to a newly enrolled patient depends on the dose level assignments and DLT outcomes of the patients already in the study. A patient's observation period ends at the occurrence of a DLT, or if a DLT does not occur, after a fixed time T of follow-up. The trial ends when a fixed number of patients, n, have been observed. Once the final patient has been observed, the MTD can be estimated using the available data. The TITE-CRM differs from the traditional CRM in that the estimation process is weighted to account for the proportion of the observation period that each currently enrolled patient has been observed. By not requiring complete observation before the enrollment of the next patient, new participants can be assigned a dose and begin evaluation as they become available, subsequently shortening the overall duration of the study.

The Biostatistics Unit at the University of Michigan Comprehensive Cancer Center has developed a program titecrm.sas for SAS 9 (SAS Institute, Inc.; Cary, NC) that makes all of the calculations necessary to determine dose allocation of a trial in real-time. They have also developed simulation programs that allow the user to determine the efficacy of the trial based on *a priori* estimates of the probabilities of toxicity and necessary sample sizes.

In order to properly utilize this type of trial, a few assumptions and many rules must be determined before the start of the trial. The main assumption that must be determined is how many different dose levels will be included in the trial and *a priori* estimates of the probability of a DLT at each of those dose levels. For this trial, we have 4 dose levels (d_1,…, d_4) ranging from 125mg PO daily to 500 mg PO daily, with initial toxicity probability estimates (p_1,…, p_4) ranging from 0.05 to 0.30, respectively. The following table shows the dose design with associated estimated probabilities of toxicity.

Dose Escalation Schedule		
Dose Level	**Dose of DSF**	**Initial Toxicity Estimate**
Level 1	125 mg PO daily	0.05
Level 2	250 mg PO daily	0.10
Level 3	375 mg PO daily	0.20
Level 4	500 mg PO daily	0.30

14.3.2 TITE-CRM Design for the Dose-escalation Phase

The starting dose can have a large impact on the properties of the design. After the trial is completed, most information about the MTD will be obtained if many patients are treated at or near the MTD, thus the sooner the choice of dose can converge on the MTD the more effective the trial will be. However, with concerns about safety we will start at the one dose level lower than the expected MTD. Therefore, our starting dose for this trial will be 250 mg PO daily.

The next thing to be considered is the dose escalation rules. For this trial, we will only allow a single dose level escalation per new patient enrolled on the trial. Therefore, if the previous patient was treated at dose level, d_k, then a new patient can only be treated at dose levels d_{k+1} or lower. In order to determine at which level to treat the new patient, we will use a non-weighted, cumulative time of exposure approach. The total length of follow-up for DLT for any patient is 18 weeks. In order to efficiently and effectively determine the MTD, we allow for a dose change when the cumulative time of exposure is 18 weeks. Also, since previously published data shows that toxic events occur evenly throughout the time of the study we will not implement a weighting function. The dose for the next patient is selected so that the estimated probability at that dose is close to the target level. A feature of the dose escalation part of the TITE-CRM program is the flexibility in specifying the margin for the dose selection. This allows the selection of the dose level for the next patient that may have an estimated probability of toxicity larger than the target level but less than the target level plus the margin. It finds the dose closest to the target probability even if it has a higher estimated probability. For this trial we set the admissible margin to be 5%, therefore, the MTD will be chosen as the dose that yields a posterior toxicity estimate closest to 20% while being between 15% and 25%. The admissible margin is also used to help determine stopping rules for the trial. In order to account for the possibility of poor initial outcomes we will employ a run-in of 6 patients, meaning that the minimum number of treated patients will be 6. If after these 6 patients, or any other time during the trial, the lowest dose level has a posterior probability higher than 25% then the trial will stop.

14.3.3 Simulation and Sample Size Calculations

The TITE-CRM manual details methods for utilizing trial simulation in order to obtain estimated posterior probabilities on toxicity estimates. We utilize the

simulation program to determine the efficacy of our trial and obtain estimates for needed sample size. While there is no exact power calculation for this type of trial, the effectiveness of the trial's sample size can be determined by looking at the stability of the posterior probability under different prior probabilities. We ran 1000 simulations under various increasing and decreasing prior probability estimates utilizing the previously described rules and assumptions for this trial and found that we will be able to determine the MTD with 18 patients. Simulations showed that approximately 98% of the time the trial will come to completion after 18 patients and the MTD will fall between the 2nd and 3rd dose level more than 95% of the time. Therefore, the sample size of this adaptive phase I/II study will be 18 patients.

14.3.4 Workflow

The first patient will be enrolled on the trial and treated at the 250 mg PO daily dose level, which is one dose level below the hypothesized MTD. That patient will be entered into the titecrm.sas program and follow-up will begin from start of treatment (RT+TMZ+DSF+Cu). Although the entire study duration is 34 weeks or at the completion of adjuvant DSF, for the purposes of DLT evaluation, the patient will be followed from start of treatment until the patient completes 18 weeks of follow-up, has a DLT, or is lost to follow-up. If any of these endpoints occur, the statistician will be notified and the date corresponding to the event will be entered into the program. While there is a maximum follow-up of 18 weeks for observation of possible DLTs, sometimes patients have a delay in their treatment resulting in the need for a prolonged observation period (at least 12 weeks after completion of RT if there is delay/interruption of RT). This delay will be accounted for as needed for this study to ensure completed 18 week follow-up. When a new patient is enrolled on the trial the statistician will be notified of the date of enrollment. At this time the statistician will update the follow-up for all patients currently on the trial and determine the dose that will be administered for the new patient. We will utilize the cumulative time of exposure method which will allow the contribution of patients with no DLT at this time to be partially weighted consistent with their time observed. At this point, the program provides a summary of the number of patients treated at each dose so far, along with the number of DLTs that have occurred. The posterior probability and 95% credible intervals of dose limiting toxicity for each dose level is calculated. At this point, the model determines the dose level that has an estimated toxicity rate closet to 20% within the 5% admissible margin. The newly enrolled patient will be enrolled in the corresponding dose level if that dose level is within one dose jump from the dose level the most current patient is enrolled on. If the suggested dose level is more than one dose level from the current dose level, the next patient will be enrolled on the dose level that is closest to the current level in the direction of the dose level determined from the estimated posterior probability. This continues until the trial has accrued all 18 patients or, if after 6 patients are enrolled, the estimated posterior probability for the lowest dose level is higher than 25%.

14.3.5 Sample Size for the Dose-expansion Phase

To further explore the effect of the addition of DSF/Cu to standard chemoradiotherpay for the subset of GBM with *IDH/BRFA/NF1* mutations, we propose to add an expansion cohort to further confirm that the addition of DSF/Cu to chemoradiotherapy may be a promising strategy to treat GBM with these mutations. Given historical data for a cohort of GBM is not readily available, some assumptions are used to derive a historical control benchmark. GBM with *BRAF* or *NF1* mutations are not known to have different prognosis than typical *IDH*-wildtype GBM [81,82], with historical OS of approximately 30% at 2 years and 10% at 5 years [3,83,84]. Both *BRAF* and *NF1* mutations affect the RAS/MAPK pathway and can also occur in the same patient, thus we will combine both mutations into one molecular cohort. However, *IDH*-mutant GBM is known to have better OS than *IDH*-wildtype GBM, with 2 year OS of approximately 50% and 5 year OS of approximately 20% [85], so we will define the second molecular cohort by the presence of *IDH* mutation. Therefore, a cohort with equal proportion of *IDH*-mutant GBM and *BRAF/NF1*-mutant GBM should theoretically have 2 year OS of 40% and 5-year OS of 15%. Based on such assumptions, a sample size of 24 patients (including 12 with *IDH* mutations and 12 with *BRAF/NF1* mutations) would provide 80% power with one-sided alpha of 0.10 to detect an improvement of 2-year OS from historical 40% to 60% (or a hazard ratio of 0.55). Since we have already enrolled 9 patients with these mutations (6 with *IDH* mutation, 2 with *BRAF* mutation, and 1 with *NF1* mutation), only additional 15 patients will be needed: 6 with *IDH* mutation, 9 patients with *BRAF* and/or NF1 mutations. For the dose-expansion phase, patients are considered evaluable if a patient has received at least 4 weeks of DSF with RT, at either the MTD (375mg/day) or the RP2D (250mg/day). Inevaluable patients will be replaced.

14.4 Analytic Plan for Secondary and Exploratory Endpoints

Adverse events will be tabulated by type and grade.

Descriptive statistics will be used to summarize and compare concentration of ditiocarb-copper complex and glutamate metabolites in plasma and tumor tissue, including median and range.

The cox proportional hazard models will be used to assess for associations between intratumor drug concentration with tumor control and survival.

The Kaplan-Meier product-limit method will be used to estimate tumor control and survival probabilities.

15.0 REFERENCES

REFERENCES

1. Johnson DR, O'Neill BP. Glioblastoma survival in the united states before and during the temozolomide era. *Journal of neuro-oncology* 2012;107:359-364.
2. Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, Dowling J, Wolinsky Y, Kruchko C, Barnholtz-Sloan J. Cbtrus statistical report: Primary brain and central nervous system tumors diagnosed in the united states in 2007-2011. *Neuro-oncology* 2014;16 Suppl 4:iv1-63.
3. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase iii study: 5-year analysis of the eortc-ncic trial. *The Lancet Oncology* 2009;10:459-466.
4. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *The New England journal of medicine* 2005;352:987-996.
5. Laperriere NJ, Leung PM, McKenzie S, Milosevic M, Wong S, Glen J, Pintilie M, Bernstein M. Randomized study of brachytherapy in the initial management of patients with malignant astrocytoma. *International journal of radiation oncology, biology, physics* 1998;41:1005-1011.
6. Nelson DF, Diener-West M, Horton J, Chang CH, Schoenfeld D, Nelson JS. Combined modality approach to treatment of malignant gliomas--re-evaluation of rtog 7401/ecog 1374 with long-term follow-up: A joint study of the radiation therapy oncology group and the eastern cooperative oncology group. *NCI Monogr* 1988:279-284.
7. Souhami L, Seiferheld W, Brachman D, Podgorsak EB, Werner-Wasik M, Lustig R, Schultz CJ, Sause W, Okunieff P, Buckner J, Zamorano L, Mehta MP, Curran WJ, Jr. Randomized comparison of stereotactic radiosurgery followed by conventional radiotherapy with carmustine to conventional radiotherapy with carmustine for patients with glioblastoma multiforme: Report of radiation therapy oncology group 93-05 protocol. *International journal of radiation oncology, biology, physics* 2004;60:853-860.
8. Badiyan SN, Markovina S, Simpson JR, Robinson CG, DeWees T, Tran DD, Linette G, Jalalizadeh R, Dacey R, Rich KM, Chicoine MR, Dowling JL, Leuthardt EC, Zipfel GJ, Kim AH, Huang J. Radiation therapy dose escalation for glioblastoma multiforme in the era of temozolomide. *International journal of radiation oncology, biology, physics* 2014;90:877-885.
9. Tabatabai G, Weller M. Glioblastoma stem cells. *Cell Tissue Res* 2011;343:459-465.
10. Venere M, Fine HA, Dirks PB, Rich JN. Cancer stem cells in gliomas: Identifying and understanding the apex cell in cancer's hierarchy. *Glia* 2011;59:1148-1154.
11. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F, Vescovi A. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer research* 2004;64:7011-7021.

12. Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI. Cancerous stem cells can arise from pediatric brain tumors. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100:15178-15183.
13. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. Identification of a cancer stem cell in human brain tumors. *Cancer research* 2003;63:5821-5828.
14. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. *Nature* 2004;432:396-401.
15. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;444:756-760.
16. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS. Analysis of gene expression and chemoresistance of cd133+ cancer stem cells in glioblastoma. *Mol Cancer* 2006;5:67.
17. Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyak K, Arendt LM, Kuperwasser C, Bierie B, Weinberg RA. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proceedings of the National Academy of Sciences of the United States of America* 2011;108:7950-7955.
18. Suh JJ, Pettinati HM, Kampman KM, O'Brien CP. The status of disulfiram: A half of a century later. *J Clin Psychopharmacol* 2006;26:290-302.
19. Johansson B. A review of the pharmacokinetics and pharmacodynamics of disulfiram and its metabolites. *Acta Psychiatr Scand Suppl* 1992;369:15-26.
20. Chen D, Cui QC, Yang H, Dou QP. Disulfiram, a clinically used anti-alcoholism drug and copper-binding agent, induces apoptotic cell death in breast cancer cultures and xenografts via inhibition of the proteasome activity. *Cancer research* 2006;66:10425-10433.
21. Cvek B, Milacic V, Taraba J, Dou QP. Ni(ii), cu(ii), and zn(ii) diethyldithiocarbamate complexes show various activities against the proteasome in breast cancer cells. *J Med Chem* 2008;51:6256-6258.
22. Ciechanover A. The ubiquitin-proteasome proteolytic pathway. *Cell* 1994;79:13-21.
23. Mani A, Gelmann EP. The ubiquitin-proteasome pathway and its role in cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005;23:4776-4789.
24. Daniel KG, Gupta P, Harbach RH, Guida WC, Dou QP. Organic copper complexes as a new class of proteasome inhibitors and apoptosis inducers in human cancer cells. *Biochemical pharmacology* 2004;67:1139-1151.
25. Lopes UG, Erhardt P, Yao R, Cooper GM. P53-dependent induction of apoptosis by proteasome inhibitors. *The Journal of biological chemistry* 1997;272:12893-12896.
26. Yin D, Zhou H, Kumagai T, Liu G, Ong JM, Black KL, Koeffler HP. Proteasome inhibitor ps-341 causes cell growth arrest and apoptosis in human glioblastoma multiforme (gbm). *Oncogene* 2005;24:344-354.
27. Hemeryck A, Geerts R, Monbaliu J, Hassler S, Verhaeghe T, Diels L, Verluyten W, van Beijsterveldt L, Mamidi RN, Janssen C, De Coster R. Tissue distribution and depletion kinetics of bortezomib and bortezomib-related radioactivity in male rats after single and

repeated intravenous injection of 14 c-bortezomib. *Cancer chemotherapy and pharmacology* 2007;60:777-787.
28. Mele G, Pinna S, Alloro E, Brocca MC, Coppi MR, Quarta G. Inefficacy of bortezomib therapy for cns involvement of refractory multiple myeloma. *Leuk Res* 2007;31:721-723.
29. Friday BB, Anderson SK, Buckner J, Yu C, Giannini C, Geoffroy F, Schwerkoske J, Mazurczak M, Gross H, Pajon E, Jaeckle K, Galanis E. Phase ii trial of vorinostat in combination with bortezomib in recurrent glioblastoma: A north central cancer treatment group study. *Neuro-oncology* 2012;14:215-221.
30. Cen D, Brayton D, Shahandeh B, Meyskens FL, Jr., Farmer PJ. Disulfiram facilitates intracellular cu uptake and induces apoptosis in human melanoma cells. *J Med Chem* 2004;47:6914-6920.
31. Iljin K, Ketola K, Vainio P, Halonen P, Kohonen P, Fey V, Grafstrom RC, Perala M, Kallioniemi O. High-throughput cell-based screening of 4910 known drugs and drug-like small molecules identifies disulfiram as an inhibitor of prostate cancer cell growth. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009;15:6070-6078.
32. Hothi P, Martins TJ, Chen L, Deleyrolle L, Yoon JG, Reynolds B, Foltz G. High-throughput chemical screens identify disulfiram as an inhibitor of human glioblastoma stem cells. *Oncotarget* 2012;3:1124-1136.
33. Liu P, Brown S, Goktug T, Channathodiyil P, Kannappan V, Hugnot JP, Guichet PO, Bian X, Armesilla AL, Darling JL, Wang W. Cytotoxic effect of disulfiram/copper on human glioblastoma cell lines and aldh-positive cancer-stem-like cells. *British journal of cancer* 2012;107:1488-1497.
34. Triscott J, Lee C, Hu K, Fotovati A, Berns R, Pambid M, Luk M, Kast RE, Kong E, Toyota E, Yip S, Toyota B, Dunn SE. Disulfiram, a drug widely used to control alcoholism, suppresses the self-renewal of glioblastoma and over-rides resistance to temozolomide. *Oncotarget* 2012;3:1112-1123.
35. Paranjpe A, Zhang R, Ali-Osman F, Bobustuc GC, Srivenugopal KS. Disulfiram is a direct and potent inhibitor of human o6-methylguanine-DNA methyltransferase (mgmt) in brain tumor cells and mouse brain and markedly increases the alkylating DNA damage. *Carcinogenesis* 2014;35:692-702.
36. Lun X, Wells JC, Hao X, Zhang J, Grinshtein N, Kaplan D, Luchman A, Weiss S, Cairncross JG, Senger DS, Robbins S. Disulfiram when combined with copper is an effective adjuvant therapy with tmz for treatment of human gliomblastoma. *Neuro-oncology* 2014;15:iii1-iii269. [Abstract: ET-064].
37. Fuller RK, Gordis E. Does disulfiram have a role in alcoholism treatment today? *Addiction* 2004;99:21-24.
38. Jacobsen E. Deaths of alcoholic patients treated with disulfiram (tetraethylthiuram disulfide) in denmark. *Q J Stud Alcohol* 1952;13:16-26.
39. Guild J, Epstein NB. Psychosis during the treatment of alcoholism with tetraethylthiuram disulfide. *Q J Stud Alcohol* 1951;12:360-366.
40. Martensen-Larsen O. Psychotic phenomena provoked by tetraethylthiuram disulfide. *Q J Stud Alcohol* 1951;12:206-216.
41. Stewart DJ, Verma S, Maroun JA. Phase i study of the combination of disulfiram with cisplatin. *American journal of clinical oncology* 1987;10:517-519.

42. Nilsson GE, Tottmar O, Wahlstrom G. Effects of aldehyde dehydrogenase inhibitors on hexobarbital sensitivity and neuroamine metabolism in rat brain. *Brain Res* 1987;409:265-274.
43. Major LF, Lerner P, Ballenger JC, Brown GL, Goodwin FK, Lovenberg W. Dopamine-beta-hydroxylase in the cerebrospinal fluid: Relationship to disulfiram-induced psychosis. *Biol Psychiatry* 1979;14:337-344.
44. Child GP, Crump M. The toxicity of tetraethylthiuram disulphide (antabuse) to mouse, rat, rabbit and dog. *Acta Pharmacol Toxicol (Copenh)* 1952;8:305-314.
45. Milandri M, Poulsen HE, Ranek L, Andreasen PB. Effect of long-term disulfiram administration on rat liver. *Pharmacology* 1980;21:76-80.
46. Faiman MD, Dodd DE, Hanzlik RE. Distribution of s35 disulfiram and metabolites in mice, and metabolism of s35 disulfiram in the dog. *Res Commun Chem Pathol Pharmacol* 1978;21:543-567.
47. Dufour P, Lang JM, Giron C, Duclos B, Haehnel P, Jaeck D, Jung JM, Oberling F. Sodium dithiocarb as adjuvant immunotherapy for high risk breast cancer: A randomized study. *Biotherapy* 1993;6:9-12.
48. Schweizer MT, Lin J, Blackford A, Bardia A, King S, Armstrong AJ, Rudek MA, Yegnasubramanian S, Carducci MA. Pharmacodynamic study of disulfiram in men with non-metastatic recurrent prostate cancer. *Prostate cancer and prostatic diseases* 2013;16:357-361.
49. Nechushtan H, Hamamreh Y, Nidal S, Gotfried M, Baron A, Shalev YI, Nisman B, Peretz T, Peylan-Ramu N. A phase iib trial assessing the addition of disulfiram to chemotherapy for the treatment of metastatic non-small cell lung cancer. *The oncologist* 2015;20:366-367.
50. Faiman MD, Jensen JC, Lacoursiere RB. Elimination kinetics of disulfiram in alcoholics after single and repeated doses. *Clin Pharmacol Ther* 1984;36:520-526.
51. Jensen JC, Faiman MD, Hurwitz A. Elimination characteristics of disulfiram over time in five alcoholic volunteers: A preliminary study. *Am J Psychiatry* 1982;139:1596-1598.
52. Johansson B, Angelo HR, Christensen JK, Moller IW, Ronsted P. Dose-effect relationship of disulfiram in human volunteers. Ii: A study of the relation between the disulfiram-alcohol reaction and plasma concentrations of acetaldehyde, diethyldithiocarbamic acid methyl ester, and erythrocyte aldehyde dehydrogenase activity. *Pharmacol Toxicol* 1991;68:166-170.
53. Johansson B. Plasma protein binding of disulfiram and its metabolite diethylthiocarbamic acid methyl ester. *J Pharm Pharmacol* 1990;42:806-807.
54. Stewart DJ, Molepo JM, Green RM, Montpetit VA, Hugenholtz H, Lamothe A, Mikhael NZ, Redmond MD, Gadia M, Goel R. Factors affecting platinum concentrations in human surgical tumour specimens after cisplatin. *British journal of cancer* 1995;71:598-604.
55. Sigmond J, Honeywell RJ, Postma TJ, Dirven CM, de Lange SM, van der Born K, Laan AC, Baayen JC, Van Groeningen CJ, Bergman AM, Giaccone G, Peters GJ. Gemcitabine uptake in glioblastoma multiforme: Potential as a radiosensitizer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2009;20:182-187.
56. van Tellingen O, Boogerd W, Nooijen WJ, Beijnen JH. The vascular compartment hampers accurate determination of teniposide penetration into brain tumor tissue. *Cancer chemotherapy and pharmacology* 1997;40:330-334.

57. Lech T, Sadlik JK. Copper concentration in body tissues and fluids in normal subjects of southern poland. *Biological trace element research* 2007;118:10-15.
58. Habib FK, Dembinski TC, Stitch SR. The zinc and copper content of blood leucocytes and plasma from patients with benign and malignant prostates. *Clinica chimica acta; international journal of clinical chemistry* 1980;104:329-335.
59. Rizk SL, Sky-Peck HH. Comparison between concentrations of trace elements in normal and neoplastic human breast tissue. *Cancer research* 1984;44:5390-5394.
60. Turecky L, Kalina P, Uhlikova E, Namerova S, Krizko J. Serum ceruloplasmin and copper levels in patients with primary brain tumors. *Klinische Wochenschrift* 1984;62:187-189.
61. Klevay LM. Lack of a recommended dietary allowance for copper may be hazardous to your health. *Journal of the American College of Nutrition* 1998;17:322-326.
62. Ma J, Betts NM. Zinc and copper intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individuals (csfii). *The Journal of nutrition* 2000;130:2838-2843.
63. Araya M, Olivares M, Pizarro F, Mendez MA, Gonzalez M, Uauy R. Supplementing copper at the upper level of the adult dietary recommended intake induces detectable but transient changes in healthy adults. *The Journal of nutrition* 2005;135:2367-2371.
64. Pratt WB, Omdahl JL, Sorenson JR. Lack of effects of copper gluconate supplementation. *The American journal of clinical nutrition* 1985;42:681-682.
65. Grossmann KF, Blankenship MB, Akerley W, Terrazas MC, Kosak KM, Boucher KM, Buys SS, Jones K, Werner TL, Agarwal N, Weis J, Sharma S, Ward J, Shami PJ. A phase i clinical study investigating disulfiram and copper gluconate in patients with advanced treatment-refractory solid tumors involving the liver. *Cancer research* 2011;71:[Abstract] nr 1308.
66. Chick J. Safety issues concerning the use of disulfiram in treating alcohol dependence. *Drug Saf* 1999;20:427-435.
67. Enghusen Poulsen H, Loft S, Andersen JR, Andersen M. Disulfiram therapy--adverse drug reactions and interactions. *Acta Psychiatr Scand Suppl* 1992;369:59-65; discussion 65-56.
68. Baenke F, Chaneton B, Smith M, Van Den Broek N, Hogan K, Tang H, Viros A, Martin M, Galbraith L, Girotti MR, Dhomen N, Gottlieb E, Marais R. Resistance to braf inhibitors induces glutamine dependency in melanoma cells. *Mol Oncol* 2016;10:73-84.
69. Seltzer MJ, Bennett BD, Joshi AD, Gao P, Thomas AG, Ferraris DV, Tsukamoto T, Rojas CJ, Slusher BS, Rabinowitz JD, Dang CV, Riggins GJ. Inhibition of glutaminase preferentially slows growth of glioma cells with mutant idh1. *Cancer research* 2010;70:8981-8987.
70. Sheikh TN, Patwardhan PP, Cremers S, Schwartz GK. Targeted inhibition of glutaminase as a potential new approach for the treatment of nf1 associated soft tissue malignancies. *Oncotarget* 2017;8:94054-94068.
71. Skrott Z, Mistrik M, Andersen KK, Friis S, Majera D, Gursky J, Ozdian T, Bartkova J, Turi Z, Moudry P, Kraus M, Michalova M, Vaclavkova J, Dzubak P, Vrobel I, Pouckova P, Sedlacek J, Miklovicova A, Kutt A, Li J, Mattova J, Driessen C, Dou QP, Olsen J, Hajduch M, Cvek B, Deshaies RJ, Bartek J. Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor npl4. *Nature* 2017;552:194-199.
72. Hochreiter J, McCance-Katz EF, Lapham J, Ma Q, Morse GD. Disulfiram metabolite s-methyl-n,n-diethylthiocarbamate quantitation in human plasma with reverse phase ultra

performance liquid chromatography and mass spectrometry. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* 2012;897:80-84.
73. Wilmotte R, Burkhardt K, Kindler V, Belkouch MC, Dussex G, Tribolet N, Walker PR, Dietrich PY. B7-homolog 1 expression by human glioma: A new mechanism of immune evasion. *Neuroreport* 2005;16:1081-1085.
74. Zhang L, Jiang Y, Jing G, Tang Y, Chen X, Yang D, Zhang Y, Tang X. A novel uplc-esi-ms/ms method for the quantitation of disulfiram, its role in stabilized plasma and its application. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* 2013;937:54-59.
75. Zhou Q, Xing A, Zhao K. Simultaneous determination of nickel, cobalt and mercury ions in water samples by solid phase extraction using multiwalled carbon nanotubes as adsorbent after chelating with sodium diethyldithiocarbamate prior to high performance liquid chromatography. *Journal of chromatography A* 2014;1360:76-81.
76. Morikawa A, Peereboom DM, Thorsheim HR, Samala R, Balyan R, Murphy CG, Lockman PR, Simmons A, Weil RJ, Tabar V, Steeg PS, Smith QR, Seidman AD. Capecitabine and lapatinib uptake in surgically resected brain metastases from metastatic breast cancer patients: A prospective study. *Neuro-oncology* 2015;17:289-295.
77. Elliott PJ, Soucy TA, Pien CS, Adams J, Lightcap ES. Assays for proteasome inhibition. *Methods Mol Med* 2003;85:163-172.
78. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, Degroot J, Wick W, Gilbert MR, Lassman AB, Tsien C, Mikkelsen T, Wong ET, Chamberlain MC, Stupp R, Lamborn KR, Vogelbaum MA, van den Bent MJ, Chang SM. Updated response assessment criteria for high-grade gliomas: Response assessment in neuro-oncology working group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010;28:1963-1972.
79. Normolle D, Lawrence T. Designing dose-escalation trials with late-onset toxicities using the time-to-event continual reassessment method. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2006;24:4426-4433.
80. Cheung YK, Chappell R. Sequential designs for phase i clinical trials with late-onset toxicities. *Biometrics* 2000;56:1177-1182.
81. Korshunov A, Chavez L, Sharma T, Ryzhova M, Schrimpf D, Stichel D, Capper D, Sturm D, Kool M, Habel A, Kleinschmidt-DeMasters BK, Rosenblum M, Absalyamova O, Golanov A, Lichter P, Pfister SM, Jones DTW, Perry A, von Deimling A. Epithelioid glioblastomas stratify into established diagnostic subsets upon integrated molecular analysis. *Brain pathology* 2018;28:656-662.
82. Vizcaino MA, Shah S, Eberhart CG, Rodriguez FJ. Clinicopathologic implications of nf1 gene alterations in diffuse gliomas. *Hum Pathol* 2015;46:1323-1330.
83. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, Carpentier AF, Hoang-Xuan K, Kavan P, Cernea D, Brandes AA, Hilton M, Abrey L, Cloughesy T. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *The New England journal of medicine* 2014;370:709-722.
84. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, Colman H, Chakravarti A, Pugh S, Won M, Jeraj R, Brown PD, Jaeckle KA, Schiff D, Stieber VW, Brachman DG, Werner-Wasik M, Tremont-Lukats IW, Sulman EP, Aldape KD, Curran WJ, Jr., Mehta MP. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *The New England journal of medicine* 2014;370:699-708.

85. Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Sicotte H, Pekmezci M, Rice T, Kosel ML, Smirnov IV, Sarkar G, Caron AA, Kollmeyer TM, Praska CE, Chada AR, Halder C, Hansen HM, McCoy LS, Bracci PM, Marshall R, Zheng S, Reis GF, Pico AR, O'Neill BP, Buckner JC, Giannini C, Huse JT, Perry A, Tihan T, Berger MS, Chang SM, Prados MD, Wiemels J, Wiencke JK, Wrensch MR, Jenkins RB. Glioma groups based on 1p/19q, idh, and tert promoter mutations in tumors. *The New England journal of medicine* 2015;372:2499-2508.

APPENDIX A: Karnofsky Performance Status Scale

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

APPENDIX B: NYHA Classification of Cardiac Disease

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

APPENDIX C: Concurrent Temozolomide Medication Diary

Today's Date: _____ Agent: Temozolomide Month: with RT

Patient Name: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form during radiation therapy. Take _____mg (___capsules) of temozolomide daily approximately 1 hr before breakfast. **Temozolomide should not be taken within one hour of your disulfiram or copper supplement.**
2. Record the date, the number of capsules taken, and when you took them.
3. If you forget to take temozolomide before 6:00PM, then do not take a dose that day. Restart it the next day.
4. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
5. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.
6. Avoid consuming alcohol before and throughout the entire study.

Day	Date	What time was dose taken?	# of tablets taken	Comments
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APPENDIX D: Concurrent Disulfiram and Copper Medication Diary

Today's Date: _____ Agent: Disulfiram/Copper Month: With RT

Patient Name: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take _____mg (___tablets) of disulfiram daily at approximately the same time each day (you should take with 8 ounces of water approximately 1 hour after BREAKFAST in the MORNING). Please also take 2 mg of copper gluconate supplement three time a day with each meal. Do **not** take disulfiram or copper within one hour of your dose of temozolomide.
2. Record the date, what time you took disulfiram on that date and how many times you took copper supplement on that date.
3. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
4. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done.
5. Avoid consuming alcohol before and throughout the entire study.

Day	Date	What time was disulfiram taken?	How many times was the copper supplement taken?	Comments
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APPENDIX E: Optional Pre-Surgery Disulfiram and Copper Medication Diary

Today's Date: _____ Agent: Disulfiram/copper Month: Pre-surgery

Patient Name: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form for the pre-surgery doses of disulfiram (3-7 doses). Take 250mg (1 tablet) of disulfiram daily at approximately the same time each day (you should take with 8 ounces of water approximately **1 hour after DINNER in the EVENING**). Please also take 2 mg of copper gluconate supplement three times a day with each meal. **You should not take disulfiram nor copper on the day of surgery.**
2. Record the date, what time you took disulfiram on that date, and how many times you took copper supplement on that date.
3. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
4. Please return the forms to your physician or your study coordinator on the day of surgery.
5. Avoid consuming alcohol before and throughout the entire study.

Day	Date	What time was Disulfiram taken?	How many times was the copper supplement taken	Comments
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APPENDIX F: Adjuvant Temozolomide Medication Diary

Today's Date: _____ Agent: Temozolomide Month: _____

Patient Name: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

7. Complete one form for each month. Take _____mg (___capsules) of temozolomide daily at approximately the same time each day as instructed by your oncologist. It is recommended that you take it at night before bedtime.
8. Record the date, the number of capsules taken, and when you took them.
9. If you forget to take temozolomide before midnight, then do not take a dose that day. Restart it the next day.
10. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
11. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done. **Please also inform your study coordinator of any new medications before starting.**
12. Avoid consuming alcohol before and throughout the entire study.

Day	Date	What time was dose taken?	# of tablets taken	Comments
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6		N/A	N/A	
7		N/A	N/A	
8		N/A	N/A	
9		N/A	N/A	
10		N/A	N/A	
11		N/A	N/A	
12		N/A	N/A	
13		N/A	N/A	
14		N/A	N/A	
15		N/A	N/A	
16		N/A	N/A	
17		N/A	N/A	
18		N/A	N/A	
19		N/A	N/A	
20		N/A	N/A	
21		N/A	N/A	
22		N/A	N/A	
23		N/A	N/A	
24		N/A	N/A	
25		N/A	N/A	
26		N/A	N/A	
27		N/A	N/A	
28		N/A	N/A	

APPENDIX G: Adjuvant Disulfiram Medication Diary

Today's Date: _____ Agent: Disulfiram Month: _____

Patient Name: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take ____ mg (___ tablets) of disulfiram daily at approximately the same time each day (1 hour after breakfast). Do **not** take within one hour of your dose of temozolomide.
2. Record the date, the number of capsules taken, and when you took them.
3. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
4. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done. **Please also inform your study coordinator of any new medications before starting.**
5. Avoid consuming alcohol before and throughout the entire study.

Day	Date	What time was dose taken?	# of tablets taken	Comments
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APPENDIX H: Adjuvant Copper Gluconate Diary

Today's Date: _____ Agent: Copper Gluconate Month: _____

Patient Name: _____ Study ID#: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take 2 mg of copper daily 3 times a day (with a meal is recommended).
2. Record the date, the number of pills taken, and when you took them.
3. If you have any questions or notice any side effects, please record them in the comments section. Record the time if you should vomit.
4. Please return the forms to your physician or your study coordinator when you go to your next appointment. Please bring your unused study medications and/or empty bottles with you to each clinic visit so that a pill count can be done. **Please also inform your study coordinator of any new medications before starting.**
5. Avoid consuming alcohol before and throughout the entire study.

Day	Date	What time was dose taken?			# of tablets taken	Comments
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