A Phase II Study of Disulfiram and Copper to Re-sensitize Temozolomide-Resistant Glioblastoma to Temozolomide

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Principal Investigator: **Jiayi Huang, M.D.**
Washington University School of Medicine
Department of Radiation Oncology
4921 Parkview Place // Box 8224
St. Louis, MO 63110
Telephone: 314-362-8516 // Fax: 314-747-9557
Email: jhuang@radonc.wustl.edu

Sub-Investigators	Institution	Department
Jian Campian M.D., Ph.D.	Washington University	Medical Oncology (Med Onc Co-chair)
Albert Kim, M.D., Ph.D.	Washington University	Neurosurgery (Neurosurgery Co-Chair)
George Ansstas, M.D.	Washington University	Medical Oncology
Amit Gujar, Ph.D.	Washington University	Neurosurgery
Christina Tsien, M.D.	Washington University	Radiation Oncology
Craig Lockhart	Washington University	Medical Oncology
Todd DeWees, Ph.D.	Washington University	Biostatistics/Radiation Oncology

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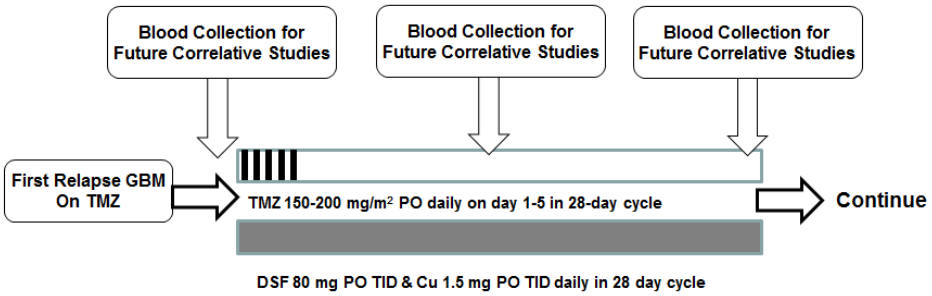
Table of Contents (Stephanie, this needs to be fixed)

Glossary of Abbreviations
Stephanie, can you insert this section:
FDA
GBM
RT
TMZ
ORR
WHO
BVZ
CCNU
OS
MGMT
OBG
PFS
ORR
PFS6
DSF
Cu
ALDH
GSC
DDTC
Me-DDTC
MRI
RANO
AST
ALT

ULN
FLAIR
CR
PR

SCHEMA

Schema:



Abbreviations: GBM = glioblastoma multiforme, TMZ = temozolomide, DSF = disulfiram, Cu = copper gluconate, PO = by mouth, TID = three time a day.

1.0 BACKGROUND AND RATIONALE

1.1 Recurrent Glioblastoma

Glioblastoma (GBM, World Health Organization/WHO grade IV) is the most common malignant primary brain tumor and one of the most devastating cancers (Johnson and O'Neill 2012). 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 (Ostrom, et al. 2014). The current standard of care for GBM includes maximal safe resection followed by radiotherapy (RT) and temozolomide (TMZ). However, despite optimal multi-modality therapy, median progression-free survival (PFS) is less than 7 months, and median overall survival (OS) is less than 15 months (Stupp, et al. 2005). Effective treatment for recurrent GBM is even more dismal. A meta-analysis of 8 phase II studies of different cytotoxic drugs or cytostatic agents on recurrent GBM showed objective response rate (ORR, including both complete response and partial response) of 6%, median progression-free survival (PFS) of 9 weeks, and PFS at 6 months (PFS6) of 15%. Based on remarkable radiological response from two single-arm, non-comparative phase II studies, bevacizumab (BVZ), an anti-angiogenesis antibody, was approved by the Food and Drug Administration (FDA) for the treatment of recurrent GBM (Friedman, et al. 2009; Kreisl, et al. 2009). However, the duration of response is relatively short with median PFS of 3-4 months and OS of 7-9 month (Piccioni, et al. 2014). A recent randomized study (EORTC 26101) showed that the addition of BVZ to lomustine (CCNU) improved PFS but failed to improve OS as compared to CCNU alone (Wilk, et al. 2015). Previously, two large randomized studies also showed that the addition of BVZ to standard RT and TMZ failed to improve OS (Chinot, et al. 2014; Gilbert, et al. 2014). Altogether, these data confirm that the radiological response from BVZ does not accurately reflect its anti-tumor effect and challenges the current prevalent approach of testing BVZ-based combination therapies in clinical studies for recurrent GBM. Therefore, a novel agent is desperately needed for recurrent GBM and should be ideally tested without BVZ in future trials.

1.2 Re-sensitizing Glioblastoma to Temozolomide

Currently, TMZ represents the only systemic therapy that has been shown to improve OS for GBM (Stupp, et al. 2005). If GBM recurs after at least a 2 month interval from discontinuing TMZ, re-challenging with TMZ has demonstrated promising results (Perry, et al. 2010). Another phase II study attempted to re-sensitize recurrent TMZ-resistant GBM (those who would recur while receiving TMZ or within 2 months of the last treatment of TMZ) using an O^6-methylguanine-DNA methyltransferase (MGMT) inhibitor called O^6-benzylguanine (OBG) (Quinn, et al. 2009). Since MGMT is an enzyme that repairs DNA damage by alkylating agents such as TMZ, a MGMT inhibitor is thought to be a rational agent to re-sensitize GBM to TMZ. Unfortunately, combination of OBG and TMZ did not show any evidence of significant re-sensitization: ORR of 3% (95% CI: 0-15%), PFS6 of 9% (95% CI: 2-21%), and median PFS of 7.5 weeks (95% CI: 4.2-7.9 weeks) (Quinn, et al. 2009).

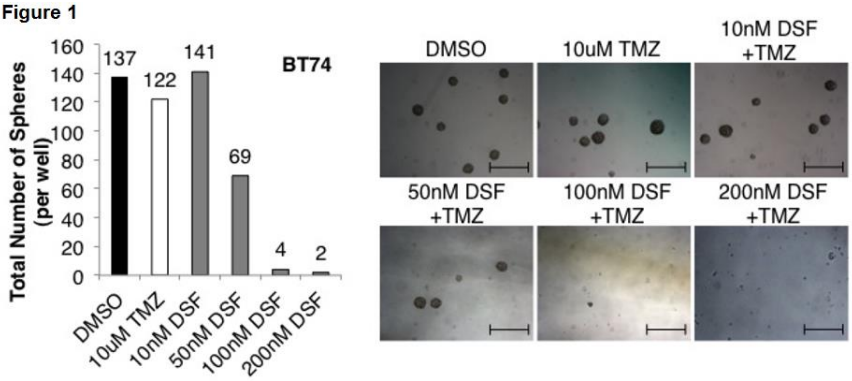
1.3 Preclinical Data of Disulfiram for Temozolomide Sensitization

Disulfiram (DSF) is a FDA-approved oral medication that has been used for treating alcoholism since 1951. It inhibits aldehyde dehydrogenase (ALDH), which leads to accumulation of acetaldehyde in the blood after ingestion of alcohol. It has well-known safety profile for up to 3000 mg per day in the absence of alcohol consumption and has been shown to readily cross the blood-brain barrier (Faiman, et al. 1978; Suh, et al. 2006). Multiple preclinical *in vitro* studies have demonstrated promising activity against GBM cells (Hothi, et al. 2012; Liu, et al. 2012; Lun, et al. 2016; Triscott, et al. 2012). Specifically, two independent high-throughput drug screening studies have identified DSF with potent and selective activity against large panels of patient-derived glioma stem-like cells (GSCs, a subset of GBM tumor cells that have been shown to be more resistant to RT and chemotherapy) (Hothi, et al. 2012; Lun, et al. 2016).

1.3.1 *In Vitro* Data of Disulfiram for Temozolomide Sensitization

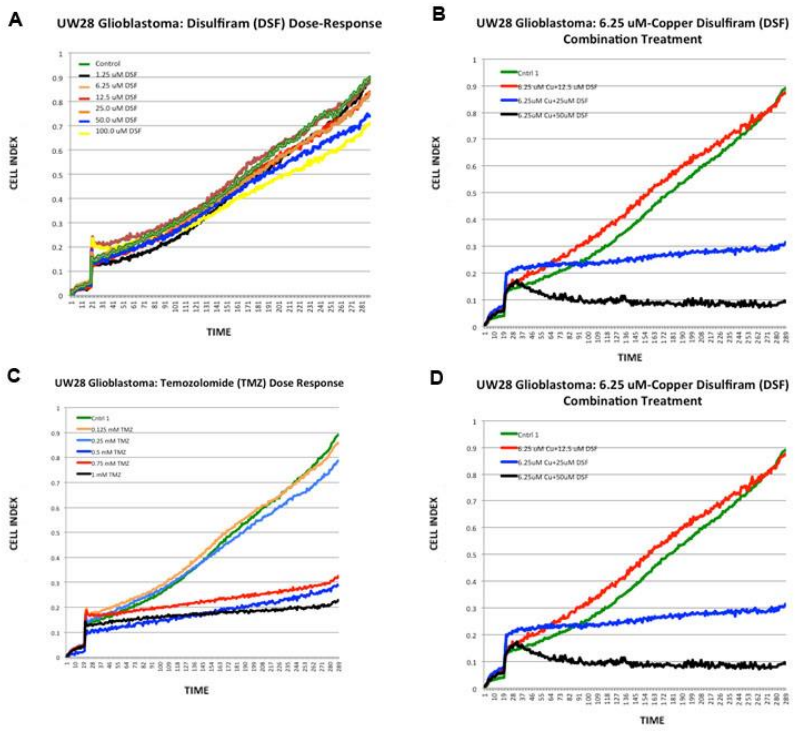
In addition to promising cytotoxicity against GBM cells, DSF has also appeared to synergize with TMZ. Triscott *et al.* showed that DSF is active against TMZ-resistant GBM cell lines and provided synergistic activity when combined with TMZ. As seen in Figure 1, DSF remarkably sensitized GBM cells that were relatively resistant to TMZ treatment (Triscott, et al. 2012).

Figure 1



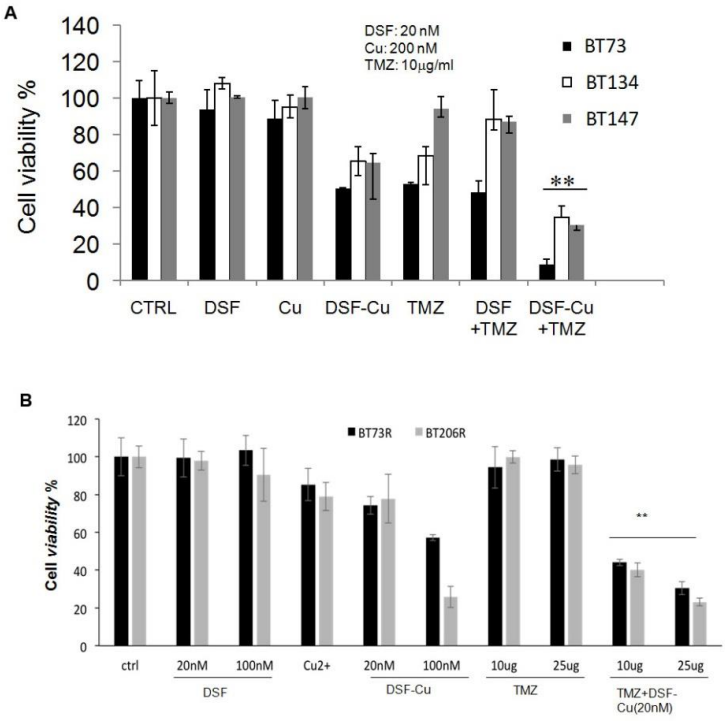
Using low passage patient-derived GBM cells, Dr. Francis Ali-Osman at Duke University observed that copper (Cu) could significantly enhance the cytotoxicity of DSF (Figure 2A-B) and confirmed that the DSF-Cu combination could dramatically increase the sensitivity of GBM cells to TMZ (Figure 2C-D) (personal communications).

Figure 2



Lun *et al.* from University of Calgary recently demonstrated that DSF-Cu not only could increase the sensitivity TMZ-naïve GSCs to TMZ (Figure 3A) but also recurrent TMZ-resistant GSCs (Figure 3B). In Figure 3B, BT73R and BT206R represented two TMZ-resistant GSC models that were derived from recurrent orthotopic GBM tumors after serial TMZ exposure, which simulated the evolutionary development of recurrent TMZ-resistant GBMs in clinical practice. Incredibly, DSF-Cu was able to produce synergistic effect in combination with TMZ on these TMZ-resistant cells (Figure 3B), suggesting that DSF-Cu can re-sensitize TMZ-resistant GSCs to TMZ again (Figure 3B). Lun *et al.* also confirmed that Cu is crucial for the anti-tumor effect of DSF (Figure 3A-B) (Lun, et al. 2016).

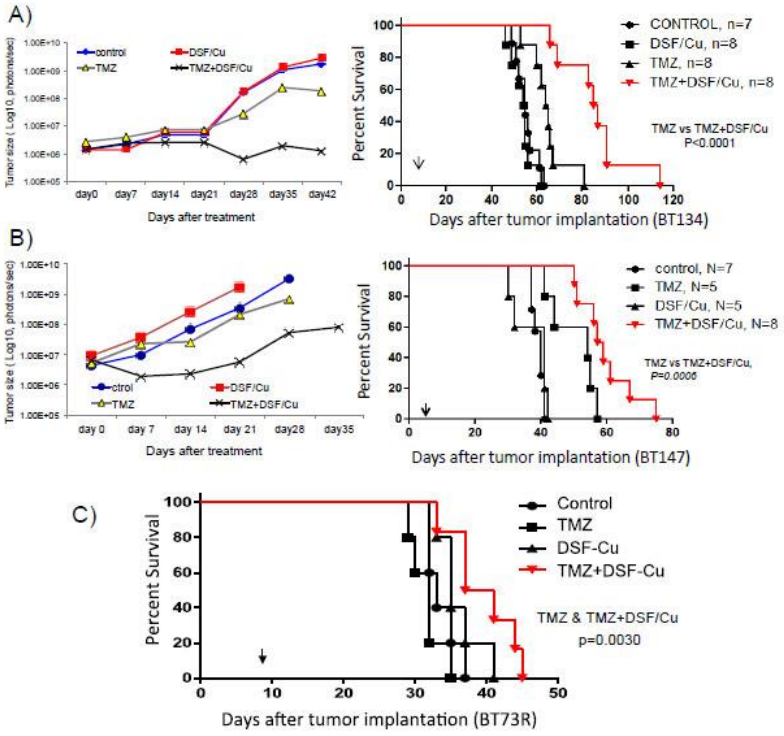
Figure 3



1.3.2 *In Vivo* Data of Disulfiram for Temozolomide Sensitization

Lun *et al.* further verified the synergistic effect of DSF-Cu using orthotopic GBM models that were produced from the GSCs. As seen in Figure 4A and 4B, DSF-Cu alone had limited effect on mice implanted with TMZ-sensitive GSCs. However, when given with TMZ, the combination significantly improved survival of mice with orthotopic tumors as compared to TMZ alone. Furthermore, as seen in Figure 4C, the combination of DSF-Cu and TMZ also significantly improved survival of mice implanted with TMZ-resistant BT73R cells as compared to TMZ alone or DSF-Cu alone (either treatment alone was ineffective) (Lun, et al. 2016). Altogether, these experiments provided proof of concept that DSF-Cu can re-sensitize TMZ-resistant GBM tumors to TMZ and the combination of both would be required to observe the maximal anti-tumor effect.

Figure 4



1.3.3 Mechanism of DSF's Anticancer Activity

The mechanism of DSF's anti-cancer property is not definitively understood. Multiple studies have suggested that it may be due to the inhibition of chymotrypsin-like proteasome activity (Chen, et al. 2006; Cvek, et al. 2008; Hothi, et al. 2012). Others have showed that DSF-Cu complex can induce intracellular reactive oxygen species (ROS) to trigger intrinsic apoptosis of GBM cells through activation of c-Jun amino-terminal kinases and p38 pathways (Liu, et al. 2012). Paranjpe *et al.* showed that DSF increased the sensitivity of GBM cells to TMZ through inhibition of MGMT and demonstrated that DSF preferentially inhibited tumor MGMT in GBM xenografts as compared to MGMT in the normal tissue (Paranjpe, et al. 2014). Lun *et al.* demonstrated that proteasome inhibition was not responsible for all the cytotoxicity of DSF-Cu. Complete proteasome inhibition using proteasome inhibitor bortezomib did not produce the same level of cell death as DSF-Cu, while very low concentration of DSF-Cu with minimal proteasome inhibition was able to produce significant cytotoxicity. Lun *et al.* also showed that DSF-Cu inhibited DNA repair pathways and thus enhanced the effects of DNA alkylating agents and radiation to

increase DNA damage (Lun, et al. 2016). These data suggest that the mechanism of DSF against GBM may be multi-factorial and may be more likely to overcome TMZ-resistance than a pure MGMT inhibitor such as OBG.

1.4 Clinical Trial of Repurposing Disulfiram for GBM

Washington University has previously completed a phase I, dose-escalation pharmacodynamic study of DSF in combination with adjuvant TMZ for newly diagnosed GBM after standard RT and concurrent TMZ. The study has determined that the maximum tolerated dose (MTD) of DSF with adjuvant TMZ is 500 mg per day. Dose-limiting toxicities of delirium and ataxia occurred at dose of 1000 mg per day within the first month of administration. The combination of 500 mg of DSF with adjuvant TMZ had an acceptable safety profile but was associated with reversible neurological toxicities of delirium, ataxia, and neuropathy after prolonged use. The median PFS with RT plus concurrent TMZ followed by 500 mg of DSF and adjuvant TMZ was promising at 8.1 months (Huang, et al. 2016). In comparison, previous randomized studies showed onlymedian PFS of 5.5 to 7.3 months with adjuvant TMZ alone after chemoradiotherapy (Gilbert, et al. 2014; Gilbert, et al. 2013; Stupp, et al. 2005). However, as the relative contributions of RT and TMZ cannot be distinguished from Washington University's previous phase I study, single-agent efficacy of DSF cannot be clearly defined. A logical next step would be to test DSF and Cu against recurrent TMZ-resistant GBM to elucidate their single-agent efficacy and their potential for TMZ re-sensitization.

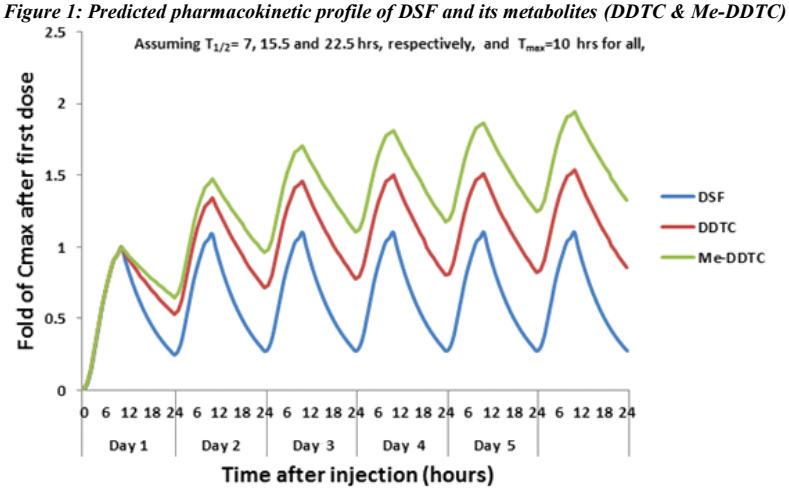
1.5 Drug Metabolism and Pharmacokinetics

After oral ingestion, DSF is partially reduced to diethyldithiocarbamate (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. Enteric formulation and oil may enhance absorption. After absorption, DSF is again reduced to DDTC and then $Cu(DDTC)_2$. Downstream metabolites also include diethylamine, carbon disulfide, diethyldithiomethylcarbamate (Me-DDTC), and glucuronic acid of DDTC. Me-DDTC is biotransformed into active inhibitors of ALDH (Johansson 1992). 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 (Faiman, et al. 1978).

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 inter-subject variability in the plasma concentrations (Faiman, et al. 1984; Jensen, et al. 1982). Doses as low as 100 mg of DSF could produce detectable plasma concentrations of Me-DDTC and complete blockade of ALDH activity in erythrocytes

(Johansson, et al. 1991). 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 disulfide is mostly eliminated via the lungs (Johansson 1992).

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 one 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 (Johansson 1990), which support their ability to cross the blood brain barrier.

1.6 Toxicology and Safety

DSF has been used clinically for more than 50 years, so its safety profile is well known. 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 (Child and Crump 1952). Interestingly, long-term administration of high doses of DSF to rats did not induce any laboratory or histological signs of liver damage (Milandri, et al. 1980). 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.

The current FDA-recommended dose of DSF is 250-500 mg daily for alcohol abstinence. In early clinical practice, a much higher dose of 1000-3000 mg per day was used (Fuller and Gordis 2004). 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 (Jacobsen 1952). At such high dosages, there were also case reports of psychosis in the absence of alcohol ingestion (Guild and Epstein 1951; Martensen-Larsen 1951). A phase I study administered a single dose of oral DSF prior to cisplatin every 3 weeks and encountered dose-limiting reversible confusion at 3000 mg/m^2 (approximately 4800 mg) (Stewart, et al. 1987). High doses of DSF may inhibit cerebrospinal dopamine B-hydroxylase (Nilsson, et al. 1987), and people with very low activity of dopamine hydroxylase may be prone to transient psychosis with such inhibition (Major, et al. 1979). In another phase I study of non-metastatic recurrent prostate cancer patients treated with 250-500 mg of DSF alone, grade 3 toxicities included double vision, hearing loss, LFT abnormality, diarrhea, constipation, and ataxia (Schweizer, et al. 2013).

In a previous phase I study of 500-1000 mg DSF in combination with adjuvant TMZ in newly diagnosed GBM patients after chemoradiotherapy, grade 2-3 toxicities that were attributed to DSF included delirium, ataxia, dizziness, neuropathy, and fatigue (Table 1). Higher dose of 1000 mg DSF per day could not be tolerated due to relatively fast onset of delirium and ataxia within the first month of therapy. Therefore, it was determined that 500 mg per day of DSF is the MTD in combination with adjuvant TMZ. However, even at the MTD of 500 mg per day, 2 of 7 patients eventually stopped DSF 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 (Huang, et al. 2016).

Table 1: Possible DSF-related toxicity during Adjuvant TMZ

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.

1.7 Disulfiram Dosing and Copper Administration

Although Washington University's previous phase I study of DSF in combination with adjuvant TMZ established the MTD at 500 mg once per day, continuous administration of this dosage over time was associated with reversible delirium or neuropathy that prompted discontinuation of therapy in some patients. Furthermore, the pharmacodynamic study showed that 500 mg DSF per day (without concurrent Cu) produced limited proteasome inhibition on peripheral blood cells, one of the presumed mechanisms against GBM cells. Given that Cu has been shown to be essential for DSF's anti-cancer effects, co-administration of Cu with DSF may be necessary to maximize its efficacy (Hothi, et al. 2012; Lun, et al. 2016). A previous phase I study for patients with advanced liver metastasis showed that 6 mg of elemental Cu in the form of copper gluconate is well tolerated when given with 250 mg of DSF daily. In this study, Cu was given in the morning half hour before breakfast, and DSF was given with the evening meals to avoid gastro-intestinal irritation from complexation of DSF and Cu in the stomach (Grossmann, et al. 2011). Currently, at Washington University, a phase I expansion cohort study is being conducted in which patients take 500 mg of DSF every morning along with 2mg of Cu three times a day (TID). The combination of DSF and Cu has been well tolerated to date.

A recent double-blinded, randomized phase II study compared chemotherapy with and without DSF for metastatic non-small cell lung cancer (Nechushtan, et al. 2015). Patients who received concurrent DSF had significantly better PFS and OS than those who received chemotherapy alone (5.9 vs. 4.9 months, and 10.0 vs. 7.1 months, respectively). In that study, DSF was given at 40 mg TID. In contrast to Washington University's phase I study, Nechushtan *et al.* did not report any significant neurological toxicity except fatigue. Given the half-life of DSF is approximately 7 hours, TID administration may be superior to improve its bioavailability and tolerability. By dividing DSF into three doses, it may also be more tolerable to take Cu at the same time to optimize its anti-GBM effect. Given the above considerations, this phase II study will administer DSF at 80 mg TID together with 1.5 mg of Cu TID.

1.8 Study Rationale

Recurrent TMZ-resistant GBM currently lacks any effective treatment. Preclinical studies have identified DSF-Cu as having promising activity in GBM cells and can potentially re-sensitize TMZ-resistant cells to TMZ again. DSF has a well-established safety profile, excellent penetration across the blood brain barrier, relatively low cost, and ease of administration with an oral formulation. If its activity against GBM is validated, its therapeutic and economic impact will be enormous. A recent phase I study has demonstrated the feasibility and safety of combining DSF with adjuvant TMZ in newly diagnosed GBM patients after chemoradiotherapy (Huang, et al. 2016). A clinical trial of continuing TMZ with the addition of DSF-Cu for TMZ-resistant GBM would provide valuable single-agent efficacy data regarding DSF and would prospectively validate its potential to re-sensitize GBM to TMZ. The results of this study will provide important insights on whether further clinical trials for DSF for GBM are warranted or not.

2.0 OBJECTIVES

2.1 Primary Objective

To determine if treating patients who have TMZ-resistant GBM with DSF-Cu concurrently with TMZ would re-sensitize their disease to TMZ.

2.2 Secondary Objectives

1. To evaluate the safety and tolerability of DSF-Cu administered TID when given concurrently with TMZ.
2. To determine OS of TMZ-resistant GBM patients treated with DSF-Cu in combination with TMZ.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. At least 18 years of age.

2. Diagnosis of histologically confirmed GBM (WHO grade IV). Subjects with an original histologic diagnosis of low grade glioma or anaplastic glioma (WHO grade II or III) are eligible if a subsequent histological diagnosis of GBM is made.
3. The subject must have completed RT with concurrent TMZ at least 3 months prior to the planned start of treatment on this study UNLESS there is pathological verification of recurrent GBM and at least 4 weeks have elapsed since the end of RT with concurrent TMZ.
4. Experienced first unequivocal progression of GBM by magnetic resonance imaging (MRI) [as assessed via Radiologic Assessment in Neuro-Oncology (RANO) criteria] within 3 months from the last dose of TMZ.
5. Recent resection of recurrent or progressive tumor will be allowed as long as at least 2 weeks have elapsed from the date of surgery and the subject has recovered from the effects of surgery. Evaluable or measurable disease following resection of recurrent tumor is mandated for eligibility into the study.
6. Karnofsky performance status (KPS) of at least 70% (see Appendix A).
7. Willing to remain abstinent from consuming alcohol while on DSF.
8. Recovered from the toxic effects of prior therapy to < grade 2 toxicity per NCI CTCAE version 4 prior to study registration (except lymphopenia). The minimum duration required between prior therapy and initiation of study drug treatment is as follows:
    a. 12 weeks from completion of radiation therapy UNLESS there is histological confirmation of tumor recurrence in which case at least 4 weeks from completion of RT will suffice

b. 4 weeks from prior cytotoxic therapy
c. 4 weeks from prior experimental drug
d. 6 weeks from nitrosoureas
e. 3 weeks from procarbazine
f. 1 week for non-cytotoxic agents, such as interferon, tamoxifen, and cis-retinoic acid

9. Meets the following laboratory criteria:
   a. Absolute neutrophil count ≥ 1,000/mcL
   b. Platelets ≥ 75,000/mcL
   c. Hemoglobin > 10.0 g/dL (transfusion and/or ESA allowed)
   d. Total bilirubin and alkaline phosphatase ≤ 2x institutional upper limit of normal (ULN)
   e. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) < 3 x ULN
   f. Blood urea nitrogen (BUN) and creatinine < 1.5 x ULN
10. Able to take oral medication.
11. Females of childbearing potential (defined as a female who is not 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.
12. Able to understand and willing to sign an institutional review board (IRB)-approved written informed consent document (legally authorized representative permitted).

3.2 Exclusion Criteria

1. Radiographic evidence of leptomeningeal dissemination, gliomatosis cerebri, infratentorial tumor, or metastatic disease to sites remote from the supratentorial brain.
2. Enrolled in another clinical trial testing a novel therapy or drug.
3. Received more than one course of radiation therapy or more than a total dose of 75 Gy. Subjects may have received radiosurgery as part of the initial therapy (i.e., in addition to one course of radiation therapy); however, the dose used for the radiosurgery counts against the total dose limit listed above.
4. History of allergic reaction to DSF or Cu.
5. Treatment with the following medications that may interfere with metabolism of DSF: warfarin, theophylline, amitriptyline, isoniazid, metronidazole, phenytoin, Phenobarbital, chlorzoxazone, halothane, imipramine, chlordiazepoxide, diazepam. (Note: lorazepam and oxazepam are not affected by the P450 system and are not contraindicated with DSF).
6. Active infection including known Acquired Immunodeficiency Syndrome (AIDS) or Hepatitis C or with a fever ≥ 38.5°C within 3 days prior to the study enrollment.
7. Active or severe hepatic disease.
8. Grade 2 or higher peripheral neuropathy or ataxia per NCI CTCAE version 4.
9. History of idiopathic seizure disorder schizophrenia, or psychosis unrelated to glioblastoma, corticosteroid, or anti-epileptic medications.
10. History of Wilson's disease.
11. History of hemochromatosis.

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12. Pregnant and breastfeeding women will be excluded because of the unknown effect of DSF on fetal development (and because of use of temozolomide).

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 TREATMENT PLAN

This multi-center study is a single arm, open-label, phase II study of DSF-Cu in combination with TMZ for recurrent TMZ-resistant GBM patients after their first relapse from TMZ.

4.1 Pretreatment Evaluation

Prior to enrollment, patient must have a complete history, physical examination including neurological exam, evaluation of performance status using the KPS, assessment of corticosteroid usage (mg per day), baseline laboratory studies (CBC, BMP, and LFTs), and brain MRI. A baseline MRI scan must be performed within 21 days prior to registration. Women of childbearing potential must have a negative pregnancy test within 14 days of initiation of treatment.

Commented [SM2]: Is this really necessary? It may mandate additional costly MRI's.

4.2 Disulfiram and Copper Dosing

Two study drugs will be provided: disulfiram in 40 mg capsules and copper gluconate in 1.5 mg capsules. DSF will be given at 80 mg (two capsules) three times daily with meals. Copper gluconate will be taken with each dose of DSF at a dose of 1.5 mg (one capsule) to improve the potential effect of DSF on GBM tumors. Although Washington University's previous phase 1 study has demonstrated MTD of DSF to be 500 mg per day in combination with TMZ, the current protocol uses a TID administration with concurrent Cu, which may improve absorption and bioavailability. Thus, the DSF dose will be a total of 240 mg per day. If administration of DSF and Cu together causes gastrointestinal discomfort, then Cu will be administered 30 minutes before the DSF dose. If a 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 B) to each study visit for assessment of compliance.

4.3 Temozolomide Dosing

TMZ should be continued according to the standard adjuvant dose that the patient has been receiving. TMZ will not be provided by the study sponsor. Dosage is typically 150-200 mg/m^2 PO per day on Days 1-5 of every 28-day cycle. If the patient was receiving adjuvant TMZ with an experimental agent, only TMZ should be continued. TMZ should be taken at bedtime on an empty stomach and should not be taken within one hour of DSF

administration. Patients will be instructed to bring their completed medication diary (Appendix C) to the study visit for assessment of compliance.

4.4 Study Evaluations

Consistent with institutional standard of care, patients will be seen approximately every 4 weeks and typically within 10 days before the start of the next cycle. At each visit, a KPS evaluation and corticosteroid usage (mg per day) will be recorded. Brain MRI will be obtained every 8 weeks to assess for radiological response.

Patients are evaluated for adverse events for 30 days from the last treatment of DSF-Cu, 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.

Table 2: 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	*Rare*: 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 temozolomide)
Hepatobiliary disorders	*Rare*: Hepatitis
Skin and subcutaneous tissue disorders	*Less Likely*: Allergic dermatitis
Musculoskeletal	*Rare*: Arthralgia, myalgia
Renal and urinary disorders	*Rare*: Dysuria, hematuria

MedDRA Term	Frequency: *Likely: greater than 10%* *Less Likely: 1-10%* *Rare: 1% or less*
Reproductive system	*Less likely*: impotence
General disorders	*Likely*: Fatigue

4.5 General Concomitant Medication and Supportive Care Guidelines

The following medications and procedures are prohibited during the study:

- Any antineoplastic therapy other than TMZ 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.

4.5.1 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, the use of benzodiazepines for antiemetic prophylaxis should be reserved for patients who cannot be satisfactorily managed otherwise.

4.5.2 Diarrhea

Antidiarrheal medications will not be used prophylactically; however, patients will be instructed to take loperamide, 4 mg, 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.

4.5.3 Central Nervous System Effects

Doses of 500-1000 mg of DSF per day with adjuvant TMZ produced neurological toxicities in some patients, including delirium/psychosis, ataxia, and peripheral neuropathy, especially at the 1000 mg dose. Patients should be carefully monitored for early signs of these symptoms. Once other causes such as tumor progression are ruled out, dose reduction of DSF to 40 mg (one capsule) three times daily should be considered if the toxicity is grade 2 or greater (refer to Section 6.2). 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 measurement, computerized tomography, MRI scans, or other appropriate medical assessment.

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.

4.5.4 Management of Disulfiram-Alcohol Reaction

In severe reactions caused by the patient's excessive ingestion of alcohol, supportive measures to restore blood pressure and treat shock should be instituted. 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.

4.6 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.

4.7 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 form.

In the absence of treatment delays due to adverse events, DSF treatment may continue until:

- 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 sponsor (Cantex), the investigator, or the investigator's institution decides to close the study

In the case of tumor progression, both TMZ and DSF-Cu should be discontinued, and patient should be considered for second-line therapy. If TMZ is temporarily withheld due to toxicity, DSF-Cu should be continued until TMZ may be resumed again. If TMZ is discontinued, DSF-Cu may be continued as per the discretion of the treating physician. If DSF-Cu is discontinued due to toxicity, TMZ may be continued as per the discretion of the treating physician.

4.8 Duration of Follow-up

Patients are evaluated for adverse events for 30 days after the last dose of DSF-Cu 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 the study participation will be per routine clinical care. Two years after the patient goes off study, the patient's chart will be reviewed to collect data on progression and survival.

5.0 RESPONSE ASSESSMENT

For the purposes of this study, patients should be evaluated for response every 8 weeks

Response and progression will be evaluated in this study using the updated RANO response assessment criteria for high-grade gliomas (Wen, et al. 2010).

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 post-radiotherapy 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 non-enhancing 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 (Wen, et al. 2010).

Response	Criteria
Complete response	• Requires all of the following: complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. • No new lesions; stable or improved non-enhancing (T2/FLAIR) lesions. • Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically. Note: Patients with non-measurable 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 non-measurable disease.

Response	Criteria
	• Stable or improved non-enhancing (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 non-measurable 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 non-enhancing (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 non-enhancing 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 non-measurable disease.

- NOTE. All measurable and non-measurable 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.

5.1 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 (Wen, et al. 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.

5.2 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for complete response (CR) or partial response (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.

5.3 Neurological Exam and Performance Status

Patients will be graded using the KPSscale and their neurological function evaluated as improved, stable or deteriorated in addition to objective measurement of tumor size. These parameters will be used to determine the overall response assessment.

5.4 Progression-Free Survival

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

6.0 DOSE MODIFICATIONS

6.1 Temozolomide Dose Modifications

TMZ dosage modification is at the discretion of treating physician as per routine clinical care.

6.2 Disulfiram Dose Modifications

If a patient experiences any grade 2 or higher non-hematologic toxicity considered by the investigator to be possibly, probably, or definitely related to DSF, the dose of DSF may be decreased by 50% (i.e., from 80 mg TID to 40 mg TID) once. 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 dose reduction. No second dose reduction is allowed—if a second dose reduction is required, DSF and Cu will be discontinued and the patient will be taken off study. 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.

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 not be re-challenged.

7.0 REGULATORY AND REPORTING REQUIREMENTS

7.1 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:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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

7.2 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.

7.3 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.

7.4 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.

7.5 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.

Protocol exceptions are not expected or encouraged, and should be discussed first with the Study Sponsor.

7.6 Reporting to the Institutional IRB

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.

7.7 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences associated with use of the drug (i.e., there is a reasonable possibility that the experience may have been caused by the drug) by telephone or fax no later than **7 calendar days** after initial receipt of the information. A life-threatening adverse experience is defined as 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.
- Report any serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. A serious adverse drug experience is defined as 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

An unexpected adverse drug experience is defined as 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).

All MedWatch forms will be sent by Cantex to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

Secondary sites must submit a completed MedWatch form to Cantex within **4 calendar days** (for fatal or life-threatening adverse experiences) or **11 calendar days** (for serious, unexpected adverse experiences). Cantex will be responsible for submitting all MedWatch forms to the FDA within the timeframes specified above.

7.8 Timeframe for Reporting Required Events

Reportable adverse events will be tracked for 30 days after the last dose of DSF.

8.0 PHARMACEUTICAL INFORMATION

8.1 Disulfiram

8.1.1 Disulfiram 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 will be manufactured and provided by Cantex Pharmaceuticals for the study participants.

8.1.4 Dosage Form

DSF is supplied as 40 mg capsules.

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 Disulfiram Administration

DSF is taken by mouth three times daily. It should be taken with meals to improve absorption. It should not be taken within one hour of TMZ. Patients 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.

9.0 CORRELATIVE STUDIES

Blood samples will be collected for possible future correlative studies. The correlative studies will not be mandated for this protocol. The correlative studies may include assay of inhibition of proteasome activity and analysis of gamma-H2AX via Western blot. Approximately 40 mL of peripheral blood (10 mL into each of 4 green top tubes containing sodium heparin) will be drawn at the baseline, at Week 2 (+2 days/-4 days), and after 1 month of DSF (but before the start of next cycle of TMZ). For patients at Washington University, the blood should be submitted at room temperature to the laboratory of Albert Kim, M.D, Ph.D. at Washington University. Blood samples should be centrifuged at 6600g for 10 min at 4 ºC. Resulting whole blood cell pellets and supernatant should then be stored separately at -80 ºC. For other participating institutions outside of Washington University, the whole blood cell pellets and supernatant should be prepared as

above and then frozen at -80 ºC. The frozen samples can be shipped to Dr. Kim's laboratory in dry ice.

10.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done no more than 4 weeks prior to the start of the protocol therapy. Each treatment cycle is 28 days.

	Screening / Baseline	**Within 10 Days Before Each Cycle**	**End of Every Even-Numbered Cycle**	**End of Treatment**	**Follow-Up[1]**
Informed consent	X				
H&P incl. neurologic exam, KPS	X	X		X	
Evaluation of corticosteroid usage	X	X			
CBC	X				
LFTs	X				
Brain MRI	X		X[3]	X	
Temozolomide		Days 1-5 of each cycle[4]			
Disulfiram		Days 1-28 of each cycle[5]			
Copper gluconate		Days 1-28 of each cycle[5]			
AE assessment	X ---------- X[6]				

1. Follow-up will be as clinically indicated. A chart review will be done at 2 years after the last dose of DSF to look for progression and survival.
2. Women of childbearing potential only.
3. A confirmatory MRI should be considered in 4-8 weeks after documentation of complete response (CR) or partial response (PR) if consistent with the institutional standard of care
4. To be taken at bedtime on an empty stomach. Must not be taken within an hour of DSF.
5. To be taken three times a day with meals.
6. AEs will be tracked for 30 days after the last dose of DSF.

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
Registration Form Eligibility Form On-Study Form	Prior to starting treatment
Treatment Form	Every cycle
Toxicity Form	Continuous
Treatment Summary Form	Completion of treatment

Follow Up Form	Day 30 Year 2
Tumor Measurement Form	Baseline End of every even numbered cycle End of treatment
MedWatch Form	See Section 7.0 for reporting requirements

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

12.0 STATISTICAL CONSIDERATIONS

12.1 Definition of Primary Endpoint and Analytical Plan

The co-primary endpoints will be objective response rate (ORR) and progression-free survival at 6 months (PFS6). ORR will be defined as the percentage of patients with complete response (CR) or partial response (PR) according to the RANO criteria as described in Section 6.0. PFS6 will be defined as proportion of patients that are free from progressive disease (PD) as per RANO criteria or death at 6 months from the date of the first dose of DSF/Cu. PFS6 and median PFS will be estimated using the Kaplan-Meier product-limit method. For recurrent TMZ-resistant GBM, continuing TMZ should yield ORR of less than 5% and PFS6 of less than 10% (Quinn, et al. 2009). To justify future drug development of DSF for GBM, we hypothesized that the addition of DSF/Cu to TMZ would improve ORR to 20% or increase PFS6 to 30%. The sample size is calculated based on the above hypotheses with one-sided exact test at an approximate α level of 0.10 and 80% statistical power. At these type I and II error rates, 20 patients will detect a proportion of patients who have ORR significantly higher than 5% or PFS6 significantly higher 10%. Of note, previous phase II study testing addition of OBG (a MGMT inhibitor) to TMZ for TMZ-resistant GBM used similar hypothesis and observed a disappointing ORR of 3% and PFS6 of 9% (Quinn, et al. 2009). This negative phase II study has detailed information on ORR, PFS, and OS as well as associated estimated 95% CIs and will serve as a historical comparison for this study.

12.2 Definition of Secondary Endpoints and Analytical Plans

The secondary endpoints will be toxicity, OS, and pharmacodynamic effect of proteasome inhibition.

1. Toxicities that are possibly/probably/definitively related to DSF will be graded by NCI CTCAE version 4.0 and will be tabulated by type and grade.
2. OS will be measured from the date of the first dose of DSF/Cu to the date of death or, otherwise, the last follow-up date on which the patient was reported alive.

Median OS and OS12 (proportion of patients that are alive at 12 months) will be estimated using the Kaplan-Meier product-limit method.

3. Proteasome inhibition of DSF/Cu will be performed on peripheral blood cells using fluorometric 20S proteasome assay as described in section 10.0. They will be normalized to the baseline activity and reported as mean and SD (standard error).
4. Gamma-H2AX expression, a marker of DNA damage, will be analyzed by Western blot. The expression level will be normalized to the baseline activity and reported as mean and SD.

13.0 REFERENCES

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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: Concurrent Disulfiram and Copper Medication Diary

Today's Date: _____ Agent: Disulfiram/Copper Month: ____

Patient Name: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month. Take __ mg (___capsules) of disulfiram three times a day at with each meal l). Please also take 1.5 mg of copper gluconate supplement three times a day with each meal. Do **not** take disulfiram or copper within one hour of your dose of temozolomide.
2. Record the date, and how many times you took disulfiram and copper 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	Check each time you take your disulfiram dose			Check each time you take your copper dose			Comments
		Breakfast	Lunch	Dinner	Breakfast	Lunch	Dinner	
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
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28								

APPENDIX C: Concurrent Temozolomide Medication Diary

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

Patient Name: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form during radiation therapy. Take _____mg (___capsules) of temozolomide daily as instructed by your oncologist. **Temozolomide should be taken at bedtime on an empty stomach and 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 midnight, 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
1				
2				
3				
4				
5				
6				
7				
8				
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12				
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