A Phase II, Single Center, Open-Label, Single Arm Study to Evaluate the Safety, Tolerability, and Efficacy of Disulfiram and Copper Gluconate When Added to Standard Temozolomide Treatment in Patients with Newly Diagnosed Resected Unmethylated Glioblastoma Multiforme

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Version Date: 01/17/2019

Confidentiality Statement

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Glossary of Abbreviations

ALDH	Aldehyde Dehydrogenase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BVZ	Bevacizumab
CCNU	Lomustine
CR	Complete Response
Cu	Copper Gluconate
DDTC	Diethyldithiocarbamate
DSF	Disulfiram
ECOG	ECOG Performance Status
FDA	Food and Drug Administration
FLAIR	Fluid-attenuated inversion recovery
GBM	Glioblastoma
GSC	Glioma-like stem cells
Me-DDTC	Methyl ester of diethyldithiocarbamate
MGMT	O^6-methylguanine-DNA-methyltransferase
MRI	Magnetic resonance imaging
OBG	O^6-benzylguanine
ORR	Objective response rate
OS	Overall survival
PFS	Progression-free survival
PFS6	Progression-free survival at six months
PR	Partial response
RANO	Response Assessment in Neuro-Oncology Criteria
RT	Radiation therapy
TMZ	Temozolomide
ULN	Upper limit of normal
WHO	World Health Organization

1.0 BACKGROUND AND RATIONALE

1.1 Glioblastoma

Glioblastoma 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 glioblastoma, 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 glioblastoma includes maximal safe resection followed by radiotherapy (RT) and temozolomide. 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 glioblastoma is even more dismal. A meta-analysis of 8 phase II studies of different cytotoxic drugs or cytostatic agents on recurrent glioblastoma 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 glioblastoma (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 temozolomide failed to improve OS (Chinot, et al. 2014; Gilbert, et al. 2014).

New treatments for both newly diagnosed and recurrent glioblastoma are greatly needed.

1.2 Glioblastoma in the Elderly

The median age of patients at the time of diagnosis of glioblastoma is 64, with approximately 6000 new patients over the age of 65 diagnosed each year in the United States with glioblastoma. The prognosis for these patients is worse than for younger patients, both as a consequence of the biologic characteristics of these tumors and comorbidities that may compromise surgery, radiation, or chemotherapy. A retrospective study of Medicare claims data paired with the Surveillance, Epidemiology, and End Results (SEER) database indicated a median overall survival (OS) of 4 months for patients with glioblastoma aged >65 years, inclusive of all treatment modalities. A recent study, reported at ASCO 2016, in patients 70 years of age or older eligible for chemotherapy + radiation therapy, indicated a median survival of 9.2 months and progression-free survival of 5.3 months for patients receiving temozolomide + radiation as compared to 7.6 month median survival and progression-free survival of 3.9 months in the radiation alone arm.

1.3 Incompletely Resected Glioblastoma

Glioblastoma is a highly invasive tumor and, as a consequence, surgical cure of a glioblastoma is not achievable. Gross total resection, defined as resection of contrast-enhancing tumor, can be achieved in approximately 40% of patients and has clear benefit. The majority of glioblastoma patients, however, undergo incomplete resection and some of them receive biopsy only, which is due to diffuse tumor extension, involvement of functionally vital areas, and patient-related risk factors such as increased age and co-morbidities.

1.4 Unmethylated Glioblastoma

Gene methylation is a DNA-based control mechanism that regulates gene expression. In cancer, gene promoter regions can have increased or decreased levels of methylation. This epigenetic modification to DNA blocks gene function, resulting in the protein encoded for by the gene not being produced or being produced at a diminished level.

O^6-methylguanine-DNA methyltransferase (MGMT), is a DNA repair enzyme that plays a critical role in response to therapy. The loss of or silencing of MGMT expression makes glioblastoma cells more sensitive to alkylating drugs such as temozolomide and BCNU. Several studies have shown that approximately 40% to 45% of glioblastoma tumors exhibit MGMT gene methylation with this methylation making the tumor more likely to respond to temozolomide whose damaging effects are reversed by DNA repair enzymes, such as MGMT. Methylation of MGMT results in diminished levels or loss of MGMT expression. In glioblastoma, MGMT gene methylation status is predictive of an improved response to temozolomide, with improved survival and progression-free survival, when compared to patients with unmethylated MGMT.

Glioblastoma patients with unmethylated MGMT respond poorly to temozolomide, with a very minor improvement in median survival and progression-free survival.

1.5 Sensitizing Glioblastoma to Temozolomide

Currently, temozolomide represents the only systemic therapy that has been shown to improve OS for glioblastoma (Stupp, et al. 2005). A phase II study attempted to re-sensitize recurrent temozolomide-resistant glioblastoma (those who would recur while receiving temozolomide or within 2 months of the last treatment of temozolomide) 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 temozolomide, a MGMT inhibitor is thought to be a rational agent to re-sensitize glioblastoma to temozolomide. Unfortunately, combination of O^6-benzylguanine and temozolomide 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).

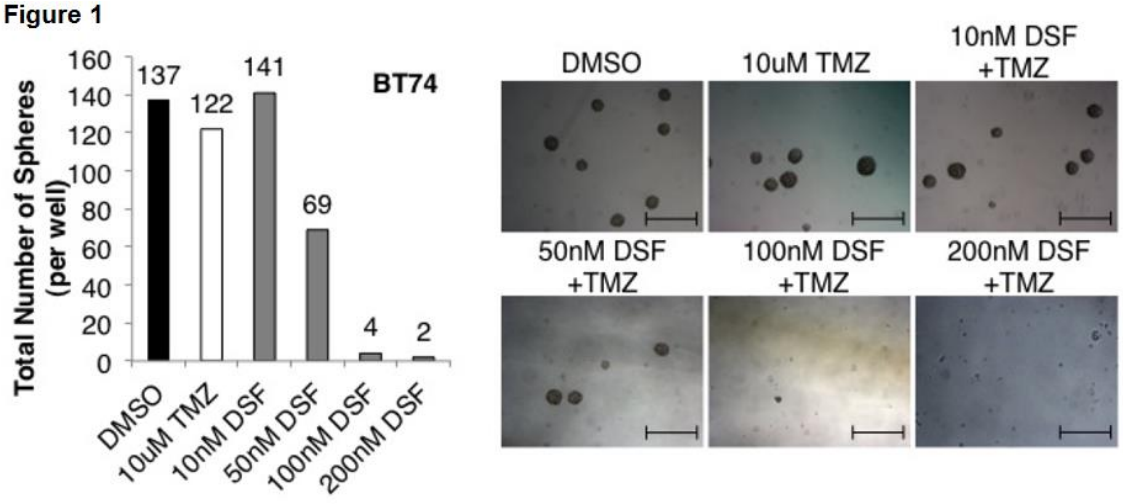
1.6 Preclinical Data of Disulfiram for Temozolomide Sensitization

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 glioblastoma 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 glioblastoma tumor cells that have been shown to be more resistant to RT and chemotherapy) (Hothi, et al. 2012; Lun, et al. 2016).

1.6.1 *In Vitro* Data of Disulfiram for Temozolomide Sensitization

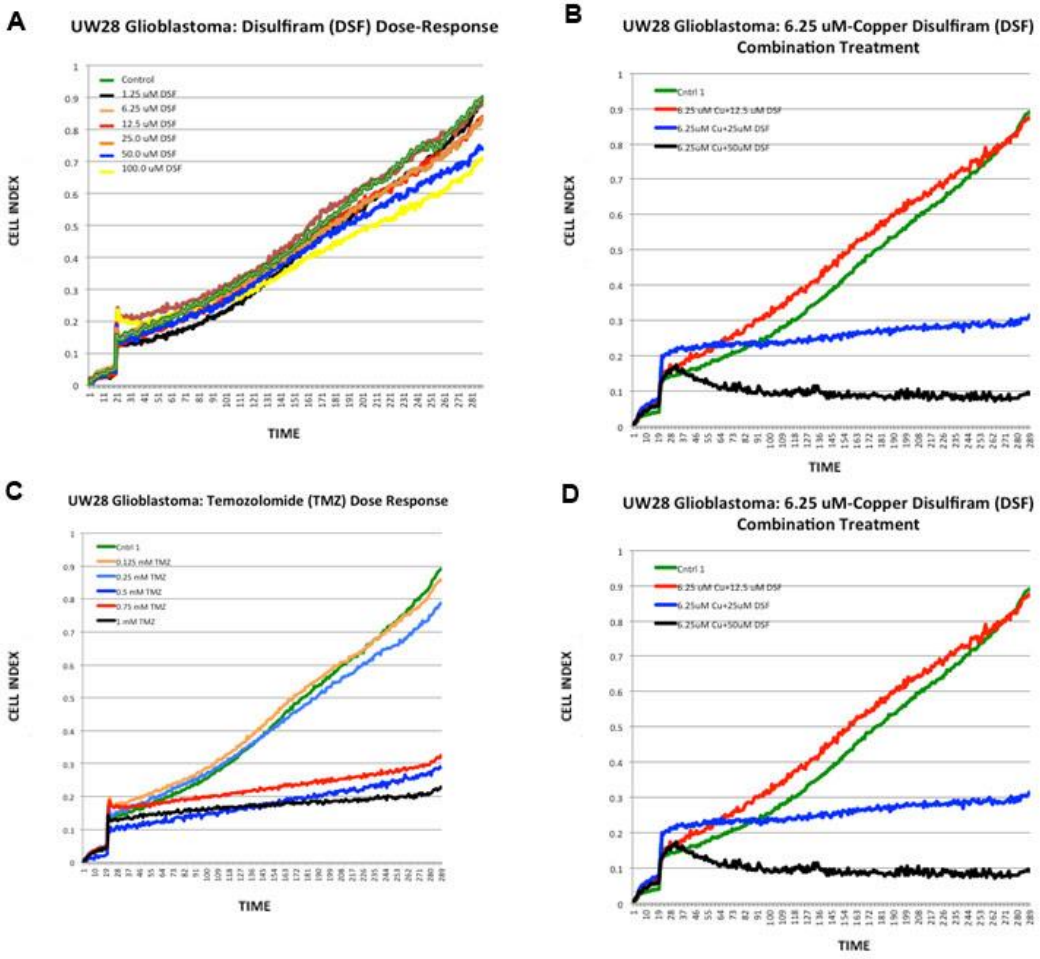
In addition to promising cytotoxicity against glioblastoma cells, DSF has also appeared to synergize with temozolomide. Triscott *et al.* showed that DSF is active against temozolomide-resistant glioblastoma cell lines and provided synergistic activity when combined with temozolomide. As seen in Figure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

Figure 1



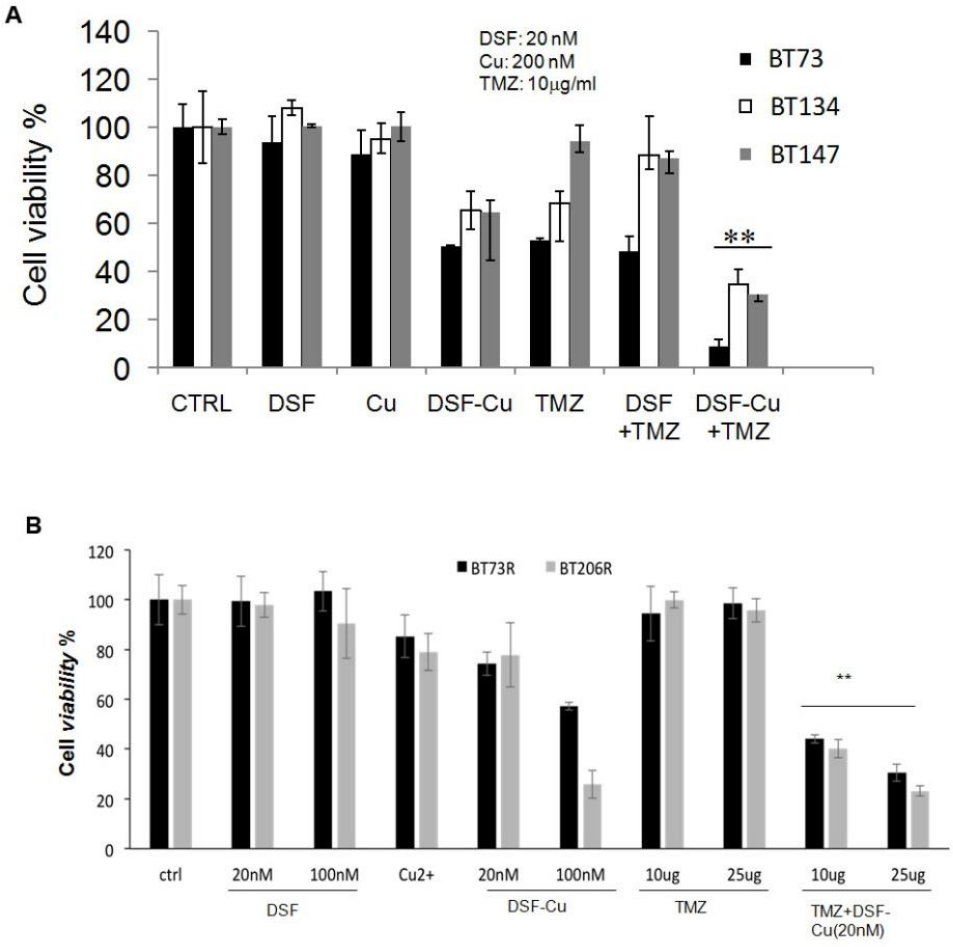
Using low passage patient-derived glioblastoma cells, Dr. Francis Ali-Osman at Duke University observed that copper (Cu) could significantly enhance the cytotoxicity of DSF (Figure 2A-B) and confirmed that the DSF-Cu combination could dramatically increase the sensitivity of glioblastoma cells to temozolomide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

Figure 2



Lun *et al.* recently demonstrated that DSF-Cu not only could increase the sensitivity temozolomide-naïve GSCs to temozolomide (Figure 3A) but also recurrent temozolomide-resistant GSCs (Figure 3B). In Figure 3B, BT73R and BT206R represented two temozolomide-resistant GSC models that were derived from recurrent orthotopic glioblastoma tumors after serial temozolomide exposure, which simulated the evolutionary development of recurrent temozolomide-resistant glioblastomas in clinical practice. Of great interest, DSF-Cu was able to produce synergistic effect in combination with temozolomide on these temozolomide-resistant cells (Figure 3B), suggesting that DSF-Cu can re-sensitize temozolomide-resistant GSCs to temozolomide 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).

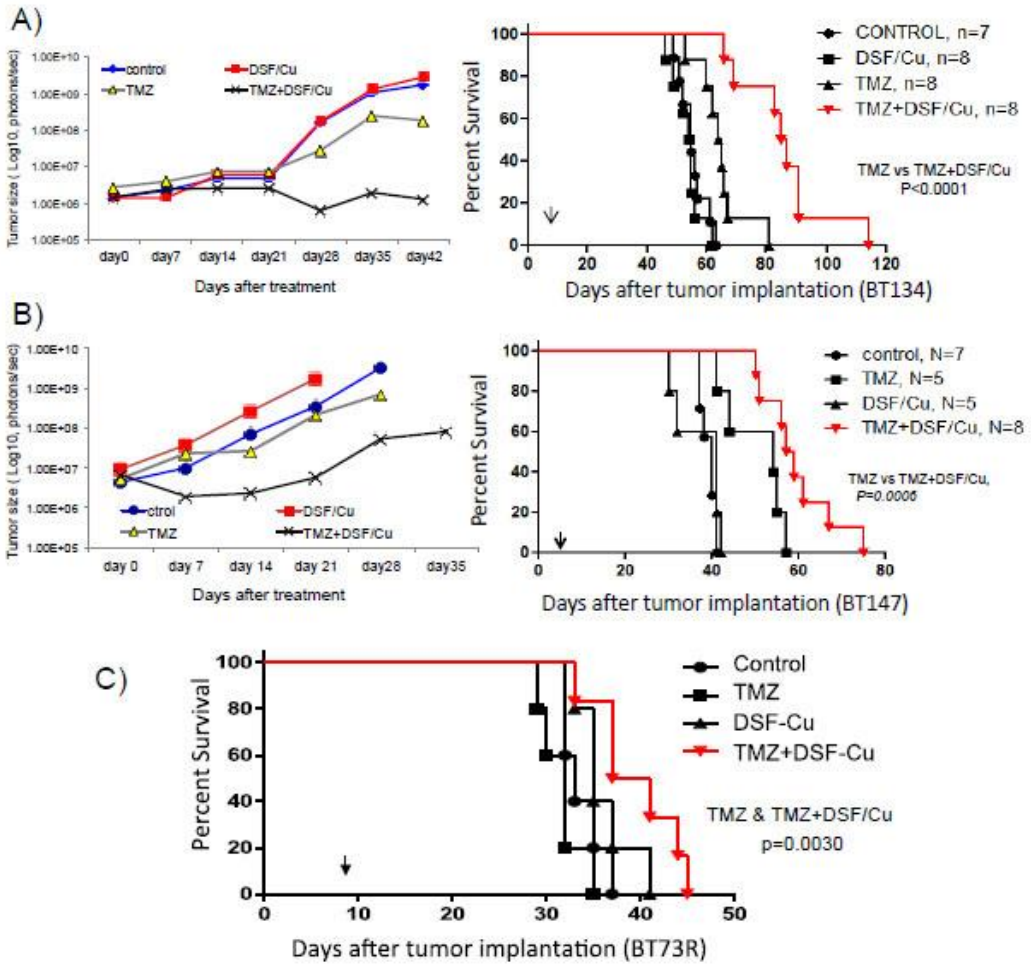
Figure 3



1.6.2 *In Vivo* Data of Disulfiram for Temozolomide Sensitization

Lun *et al.* further verified the synergistic effect of DSF-Cu using orthotopic glioblastoma models that were produced from the GSCs. As seen in Figure 4A and 4B, DSF-Cu alone had limited effect on mice implanted with temozolomide-sensitive GSCs. However, when given with temozolomide, the combination significantly improved survival of mice with orthotopic tumors as compared to temozolomide alone. Furthermore, as seen in Figure 4C, the combination of DSF-Cu and temozolomide also significantly improved survival of mice implanted with temozolomide-resistant BT73R cells as compared to temozolomide 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 temozolomide-resistant glioblastoma tumors to temozolomide and the combination of both would be required to observe the maximal anti-tumor effect.

Figure 4



1.6.3 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 to trigger intrinsic apoptosis of glioblastoma cells through activation of c-Jun amino-terminal kinases (JNK) and p38 pathways (Liu, et al. 2012). **Paranjpe *et al.* showed that DSF increased the sensitivity of glioblastoma cells to temozolomide through inhibition of MGMT and demonstrated that DSF preferentially inhibited tumor MGMT in glioblastoma 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 glioblastoma may be multi-factorial and may be more likely to overcome temozolomide-resistance than a pure MGMT inhibitor such as O^6-benzylguanine.

1.7 Clinical Trial of Repurposing Disulfiram for Glioblastoma

A phase I, dose-escalation pharmacodynamics study of DSF in combination with adjuvant temozolomide for newly diagnosed glioblastoma after standard RT and concurrent temozolomide determined that the maximum tolerated dose (MTD) of DSF with adjuvant temozolomide 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 temozolomide 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 temozolomide followed by 500 mg of DSF and adjuvant temozolomide was 8.1 months (Huang, et al. 2016). In comparison, previous randomized studies showed only a median PFS of 5.5 to 7.3 months with adjuvant temozolomide alone after chemoradiotherapy (Gilbert, et al. 2014; Gilbert, et al. 2013; Stupp, et al. 2005). However, in the Huang et al study, the relative contributions of RT and temozolomide could not be distinguished; therefore, the single-agent efficacy of DSF was not clearly defined (Huang et al, 2016). Logical next steps would be to test DSF and Cu against recurrent temozolomide-resistant glioblastoma and against unmethylated glioblastoma to elucidate their single-agent efficacy and their potential for temozolomide sensitization.

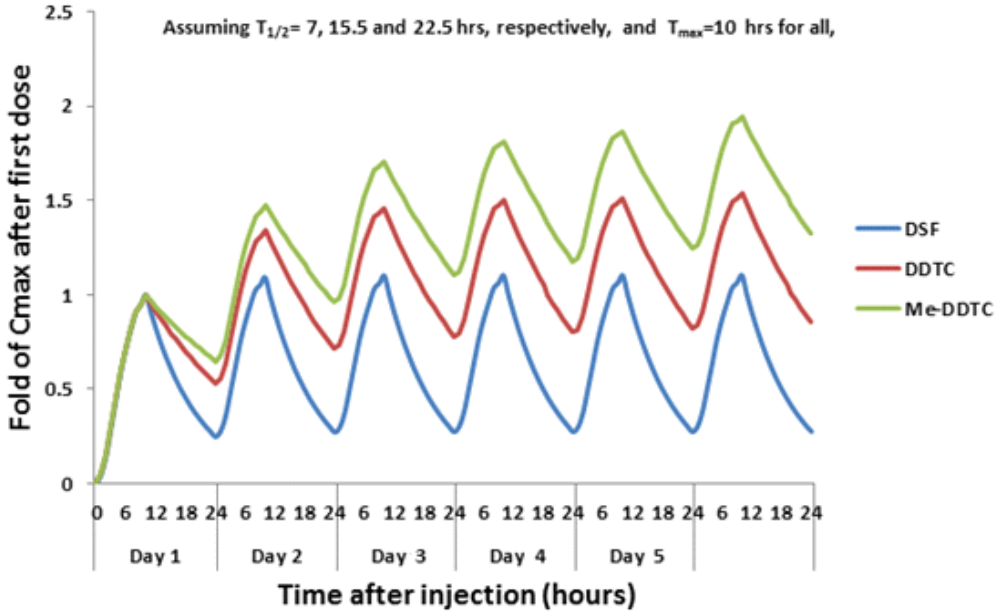
1.8 Drug Metabolism and Pharmacokinetics

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 x 10^4 and 6.1 x 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.

1.9 Toxicology and Safety

DSF has been used clinically to treat alcoholism for more than 60 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 temozolomide in newly diagnosed glioblastoma 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 temozolomide. 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 temozolomide

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 National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v. 4.0 (2009) that were possibly or probably related to DSF and that were not present at baseline.
†Not dose-limiting toxicity as occurred after the first month of DSF.
§Dose-limiting toxicity as occurred within the first month of DSF.

1.10 Disulfiram and Copper Administration

Although the MTD of DSF in combination with adjuvant temozolomide was established at 500 mg once per day, continuous administration of this dose over time was associated with reversible delirium or neuropathy that prompted discontinuation of therapy in some patients. Furthermore, the pharmacodynamics 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 glioblastoma cells (Huang et al 2016). 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).

A recent double-blinded, randomized phase II study compared chemotherapy with and without DSF for metastatic non-small cell lung cancer. 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) (Nechushtan, et al. 2015). In that study, DSF was given at 40 mg TID. In contrast to the Huang phase I study, Nechushtan *et al.* did not report any significant neurological toxicity except fatigue. This study suggests that lower DSF daily dosing may be more effective than higher dose. Given the results of this study and our ANII patient derived primary culture data which suggests that lower DSF dose may be more effective this study will limited DSF total daily dose to 250 mg split into two 125 mg doses given on a BID schedule.

1.11 Study Rationale

Patients with unmethylated glioblastoma respond poorly to current standard treatment and have a poor prognosis.

Although standard post resection radiation therapy may provide a survival benefit of approximately two months, delayed radiation at or after 8 weeks has been shown to not increase overall survival. This study proposes standard of care treatment with 6 – 61/2 weeks of radiation with concurrent temozolomide at 75mgs / m2 along with DSF – Cu followed by adjuvant temozolomide at 150mgs / m2 for 6 -12 cycles in patients with previously untreated, (aside from surgical resection), unmethylated glioblastoma.

Preclinical studies have identified DSF-Cu as having promising activity in glioblastoma cells and can potentially sensitize temozolomide-resistant cells to temozolomide.
A recent phase I study has demonstrated the feasibility and safety of combining DSF with adjuvant temozolomide in newly diagnosed glioblastoma patients after chemoradiotherapy (Huang, et al. 2016). This clinical trial will evaluate whether the addition of DSF-Cu to temozolomide in patients expected to be temozolomide-resistant can induce anti-tumor responses and validate the potential of DSF-Cu to sensitize glioblastoma to temozolomide.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

1. To determine evidence of an antitumor effect in patients with unmethylated glioblastoma treated concurrently with DSF-Cu and concurrent radiation and temozolomide. To determine 6 *month PFS and 12 month OS of patients with unmethylated glioblastoma treated with* DSF-Cu in combination with concurrent radiation and temozolomide.

2.2 Secondary Objectives

2. To evaluate the safety and tolerability of DSF-Cu administered BID when given concurrently with temozolomide.
3. To determine the impact of DSF + Cu on the quality of life (QOL) for patients with newly diagnosed GBM undergoing standard concurrent radiation and temozolomide treatment followed by adjuvant temzolomide.

3.0 PATIENT SELECTION

Approximately 14-40 patients will be enrolled – depending on the preliminary subset analysis data (when accrual of 14 patients is reached) the study may conclude when 14-20 patient mark is reached.

3.1 Inclusion Criteria

1. Age 18 or older
2. Diagnosis of histologically confirmed glioblastoma (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 glioblastoma is made
3. Patients whose tumor is determined to be unmethylated
4. Patients with incomplete resection as determined by residual, measurable gadolinium or contrast-enhancing lesion or lesions
5. Recent resection of glioblastoma. Patients who have only had a tumor biopsy and who are considered unresectable are eligible (but based on the study accrual this subset of patients with unreseactable tumor may be considered for separate analysis)
6. ECOG PS of ≤ 2 (see appendix A)
7. Willing to remain abstinent from consuming alcohol while on DSF
8. No prior radiation or chemotherapy
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 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. Able to understand and willing to sign an institutional review board (IRB)-approved written informed consent document (legally authorized representative permitted)
12. MRI with and without contrast prior to enrollment.

3.2 Exclusion Criteria

1. Radiographic evidence of leptomeningeal dissemination, extensive intraparenchymal dissemination, 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 prior radiation therapy or chemotherapy for glioblastoma
4. History of allergic reaction/hypersensitivity to DSF (without alcohol) or copper.
5. Treatment with the following medications that may interfere with metabolism of DSF: warfarin (unless otherwise chosen by the study PI who will actively adjust Coumadin dose to consistently maintain a safe, therapeutic INR < 3), 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 severe hepatic or renal disease
7. Grade 2 or higher peripheral neuropathy or ataxia per NCI CTCAE version 4.0 (2009)
8. History of idiopathic seizure disorder schizophrenia, or psychosis unrelated to glioblastoma, corticosteroid, or anti-epileptic medications
9. History of Wilson's or Gilbert's disease
10. Current excessive use of alcohol.

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 study is a single arm, open-label, phase II study of DSF-Cu in combination with concurrent temozolomide and radiation therapy followed by DSF – Cu and adjuvant temozolimide for patients with unmethylated tumors.

4.1 Pretreatment Evaluation

Prior to enrollment, patient must have a complete history, physical examination including neurological exam, evaluation of performance status using the ECOG, assessment of corticosteroid usage (mg per day), baseline laboratory studies (CBC, CMP, and Cu) and pregnancy test (if applicable), and brain MRI. A baseline MRI must be performed prior to registration.

4.2 Disulfiram and Copper Dosing

DSF will be given at 125 mg (half of a 250 mg capsule) two times daily with meals. Cu gluconate will be taken with each dose of DSF at a dose of 2 mg (one capsule/tablet). A previous phase I study determined that the MTD is 500 mg per day in combination with temozolomide (Huang et al, 2016). However, in this protocol, DSF-Cu will be administered BID. The DSF total daily dose will be 250 mg per day, as lower dose may be associated with a higher MGMT inhibitory effect, which is approximately half of the daily dose of disulfiram (500 mg) given safely and chronically to patients treated for alcoholism. If administration of DSF and Cu together causes gastrointestinal discomfort, then Cu will be administered 30 minutes after 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.

Temozolomide will be administered following the standard Stupp protocol at a dose of 75 mg/m^2 for 42 days with concurrent radiation therapy followed by a two to three week rest. In patients with stable or responding disease, temozolomide dose will resume at maintenance dose of 150 mg/m^2 once daily on Days 1-5 of every 28-day cycle.
Patients demonstrating continued benefit from the adjuvant temozolomide after 6 cycles can continue treatment to a maximum of 12 cycles.

Patients may be treated with IV temozolomide if they are unable to take the oral formulation of the drug or if they do not have insurance coverage for oral formulation. If patients are treated with the IV formulation they will receive the temozolomide Monday

– Friday during concurrent treatment with radiation and then on days 1 -5 every 28 days in the adjuvant phase.

The recommended dose for Temozolomide as an intravenous infusion over 90 minutes is the same as the dose for the oral capsule formulation. Bioequivalence has been established only when Temozolomide for infusion was given over 90 minutes.
Standard Photon irradiation using 3DCRT or IMRT: 46 Gy in 23 fractions followed by a sequential boost for an additional 7 fractions to 60 Gy

Concurrent temozolomide should be taken within an hour of radiation treatment. During weekends without radiotherapy (Saturday and Sunday), the drug will be taken in the morning.

Adjuvant temozolomide should be taken at bedtime on an empty stomach and should not be taken within one hour of DSF-Cu administration. Patients will be instructed to fast at least 2 hours before and 1 hour after temozolomide administration. Water is allowed during the fast period. If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose.

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

If patients receive the IV formulation of Temozolomide, compliance will be assessed by review of the medical record daily administration.

4.3 Study Evaluations

Consistent with standard of care, patients will be seen approximately every 4 weeks. At each visit, a ECOG evaluation and corticosteroid usage (mg per day) will be recorded. Brain MRI will be obtained every 8 weeks to assess for radiological response. Quality of Life (QOL) will be assessed using the Edmonton Symptom Assessment System – Revised (ESAS-r) questionnaire. Subjects will be monitored for copper levels on Day 1 (+/- 7 days) of Cycle 1 and Cycle 4 of adjuvant temozolomide.

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. Patients will also be removed from the study for non-compliance.

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 temozolomide)
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
Reproductive system	*Less likely*: impotence
General disorders	*Likely*: Fatigue

4.4 General Concomitant Medication/Treatment and Supportive Care Guidelines

Treatment with Optune® may be initiated by Day 1 of Cycle 1, but can begin at any time prior to this as long as it is not started until after completion of radiation therapy. If patient did not declare intent for Optune® to start on Day 1 of Cycle 1, treatment with Optune® may be initiated on Day 1 of Cycle 6, otherwise it may not be initiated until all protocol therapy is completed.

The following medications and procedures are prohibited during the study:

- Any antineoplastic therapy (other than those already used for the treatment of autoimmune disorders) other than temozolomide and DSF
- Any investigational therapy other than DSF or Cu

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.

4.4.1 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.

4.4.2 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.

4.4.3 Central Nervous System Effects

Doses of 500-1000 mg of DSF per day with adjuvant temozolomide 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 125 mg (half of 250 mg tablet) once a day 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.

4.4.4 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. Potassium levels should be monitored, particularly in patients on digitalis, since hypokalemia has been reported.

4.5 Duration of Therapy

Patients demonstrating continued benefit from the adjuvant temozolomide after 6 cycles can continue treatment to a maximum of 12 cycles.

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 will be documented.

In the absence of treatment delays due to adverse events, DSF-Cu 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
- Patient develops unacceptable toxicity deemed possibly, probably, or definitely related to drug that does not resolved to grade 1 or to the patient's baseline status
- Patient requires more than 1 dose reductions
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Serious noncompliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The investigator, or the investigator's institution decides to close the study

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

4.6 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 approximately 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,

First Progression	Definition
	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. • 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.

Response	Criteria
	• 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 ECOG scale 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 Radiation therapy Dose Modicications

Radiation therapy modification is at the discretion of the treating physician as per routine clinical care.

Concomitant temozolomide, if radiotherapy is interrupted
If radiotherapy has to be temporarily interrupted for technical or medical reasons unrelated to the temozolomide administration, then treatment with daily temozolomide should continue. If radiotherapy has to be permanently interrupted then treatment with daily temozolomide should stop. Temozolomide can resume with the initiation of the adjuvant phase of treatment.

6.2 Temozolomide Dose Modifications

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

On day 1 of each cycle (+/- 7 days), ANC ≥ 1.5 x 109/L, platelet count ≥ 100 x 109/L and all treatment-related grade 3 or 4 non-hematologic AEs (except nausea and vomiting unless the patient has failed maximal antiemetic therapy and fatigue) must have resolved (to grade ≤ 1).

6.3 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 125 mg BID to 125 mg qd – and when taken with temozolomide to be administered within 6-8 hours prior to temozolomide dose) 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 temozolomide.

6.3.1 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.

6.3.2 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 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 (2009) 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.htm

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
- any factual suggestion 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 or that impact 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. It is the responsibility of the investigator and Aurora ANII Clinical Research team 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
    - Is life-threatening
    - 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 (Form FDA 3500A) forms will be sent by ANII Clinical Research Team 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

7.8 Timeframe for Reporting Required Events

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

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.
Glioblastoma cells and its stem-like subpopulation (also referred to as CSC) may be more susceptible to the effect of proteasome inhibition than normal brain cells. DSF is an effective MGMT inhibitor. DSF is very lipophilic and readily crosses the blood-brain barrier.

8.1.3 Supplier

ANII Clinical Research Team together with the Aurora Saint Luke's Pharmacy Committee will decide upon a US unique manufacturer / supplier of DSF. Aurora Pharmacy Committee will ensure that for the purposes of the study that Aurora Saint Luke's dispensing pharmacy will ensure cutting DSF 250 mg tabs into halves.

8.1.4 Dosage Form

DSF will be supplied in roughly 125 mg per dose (one half of 250 mg tab).

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 two times daily. It should be taken with meals to improve absorption. It should not be taken within one hour of temozolomide. 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.

8.1.7 Supplier

DSF will be dispensed via the Aurora Saint Luke's Pharmacies and provided by US manufacturer of choice which will be designated following review by the ANII Research Team and by the Aurora Pharmacy Committee

8.1.8 Copper Gluconate Description

Cu gluconate is a dietary food supplement generally recognized as safe (GRAS) under §21CFR184.1260. Cu is an important nutrient in the human body and plays an essential role by participating in numerous metabolic reactions in the body (Lecyk 1980; Stern et al. 2007). Exposure to Cu usually occurs from consumption of food and drinking water. Several studies have concluded that chronic dietary intakes of Cu less than 10 mg/day pose no significant health risk

8.1.9 Dosage Form

Copper gluconate is supplied as 2 mg capsules/tablets.

8.1.10 Storage and Stability

Copper gluconate 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.

8.1.11 Copper Gluconate Administration

Copper gluconate is taken by mouth two times daily. It should be taken with meals and at the same time with DSF.

8.1.12 Supplier

A sufficient supply of Copper Gluconate will be purchased by Aurora Research Institute and will be provided to subjects participating in this study at no cost to the subject.

9.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 4 weeks (+/- 7 days) prior to start of protocol therapy. Scans will be performed prior to the start of the protocol therapy. Each treatment cycle is 28 days.

	Screening / Baseline	**Weekly during radiation treatment**	**Start of Fourth Week of radiation therapy**	**Day 1 of Each Cycle (+/- 7 days)**	**End of Every Even-Numbered Cycle**	**Day 1 of Cycle 1 (+/- 7 days)**	**Day 1 of Cycle 4 (+/- 7 days)**	**End of Treatment**	**Follow-Up[1]**
Informed consent	X								
H&P incl. neurologic exam, ECOG	X			X				X	
Evaluation of corticosteroid usage	X		X	X					
CBC	X	X		X					
CMP	X			X					
Copper, blood	X					X	X		
Pregnancy testing (if applicable)	X								
Edmonton Symptom Assessment System – Revised (ESAS-r) QOL Questionnaire	X		X	X				X	
Brain MRI prior to enrollment	X				X[2]			X	
Temozolomide				Concurrent radiation with temozolomide on days 1-42 at 75 mg/m^2.[3] Responding patients will subsequently receive adjuvant TMZ 150 mg/m^2 Days 1-5 of each following 28 day cycle[3]					
Disulfiram				Days 1-28 of each cycle[4]					
Copper gluconate				Days 1-28 of each cycle[4]					
AE assessment			X	X	X	X	X	X	X

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. A confirmatory MRI should be considered in 8 weeks after documentation of complete response (CR) or partial response (PR) if consistent with the institutional standard of care
3. To be taken at bedtime on an empty stomach. Must not be taken within an hour of DSF.
4. To be taken two times a day with meals.
5. AEs will be tracked for 30 days after the last dose of DSF.

10.0 STATISTICAL CONSIDERATIONS

10.1 Definition of Primary Endpoints and Analytical Plan

The co-primary endpoints will be ORR and PFS6. ORR will be defined as the percentage of patients with CR or 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 unmethylated glioblastoma receiving temozolomide without DSF-Cu, an ORR of less than 10% and PFS6 of less than 20% (Quinn, et al. 2009).

To justify future use of or drug development of DSF-Cu for glioblastoma, we hypothesized that the addition of DSF-Cu to temozolomide 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, 14 -20 patients will detect a proportion of patients who have ORR significantly higher than 5% or PFS6 significantly higher than 10%. Of note, previous phase II study testing addition of O^6-benzylguanine (a MGMT inhibitor) to temozolomide for temozolomide-resistant glioblastoma 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.

10.2 Definition of Secondary Endpoints and Analytical Plans

The secondary endpoints will be toxicity, and OS.

1. Toxicities that are possibly/probably/definitively related to DSF will be graded by NCI CTCAE version 4.0 (2009) 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.

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APPENDIX A:

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

APPENDIX B – Comparison of the ECOG Performance Status to the Karnofsky Performance Status

ECOG PERFORMANCE STATUS	KARNOFSKY PERFORMANCE STATUS
	100—Normal, no complaints; no evidence of disease
0—Fully active, able to carry on all pre-disease performance without restriction	90—Able to carry on normal activity; minor signs or symptoms of disease
	80—Normal activity with effort, some signs or symptoms of disease
1—Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	70—Cares for self but unable to carry on normal activity or to do active work
	60—Requires occasional assistance but is able to care for most of personal needs
2—Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours	50—Requires considerable assistance and frequent medical care
	40—Disabled; requires special care and assistance
3—Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours	30—Severely disabled; hospitalization is indicated although death not imminent
	20—Very ill; hospitalization and active supportive care necessary
4—Completely disabled; cannot carry on any selfcare; totally confined to bed or chair	10—Moribund
5—Dead	0—Dead

APPENDIX C: 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 (___capsule) of disulfiram two times a day with meals with breakfast and dinner). Please also take 2 mg of copper gluconate supplement two 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								
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APPENDIX D: Concurrent Temozolomide Medication Diary

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

Patient Name: _____

INSTRUCTIONS TO THE PATIENT:

1. **Complete this form. Take _____mg (___capsules) of temozolomide daily as instructed by your oncologist.** Temozolomide should be taken within one hour of your radiation appointment each day on an empty stomach and should not be taken within one hour of your disulfiram or copper supplement. During weekends without radiation treatments (Saturday and Sunday), temodar will be taken in the morning.
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				
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12				
13				
14				
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APPENDIX E: Adjuvant Temozolomide Medication Diary

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

Patient Name: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete this form. 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				
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