




STUDY PROTOCOL

Disulfiram repurposing combined with nutritional copper supplement as add-on to chemotherapy in recurrent glioblastoma (DIRECT): Study protocol for a randomized controlled trial [version 1; referees: 2 approved]

Asgeir Store Jakola ¹⁻³, Katja Werlenius^{4,5}, Munila Mudaisi⁶, Sofia Hylin⁷, Sara Kinhult⁸, Jiri Bartek Jr.^{9,10}, Øyvind Salvesen¹¹, Sven Magnus Carlsen^{12,13}, Michael Strandéus¹⁴, Magnus Lindskog^{15,16}, David Löfgren¹⁷, Bertil Rydenhag^{1,3}, Louise Carstam^{1,3}, Sasha Gulati^{2,18}, Ole Solheim^{2,18}, Jiri Bartek^{19,20}, Tora Solheim^{21,22}

¹Department of Neurosurgery, Sahlgrenska University Hospital, Gothenburg, Sweden

²Department of Neurosurgery, St. Olavs Hospital, Trondheim, Norway

³Institute of Neuroscience and Physiology, Department of Clinical Neuroscience, University of Gothenburg, Sahlgrenska Academy, Gothenburg, Sweden

⁴Department of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden

⁵Institute of Clinical Sciences, University of Gothenburg, Sahlgrenska Academy, Gothenburg, Sweden

⁶Department of Oncology, Linköping University Hospital, Linköping, Sweden

⁷Department of Neurology, Karolinska University Hospital, Stockholm, Sweden

⁸Department of Oncology, Skåne University Hospital, Lund, Sweden

⁹Department of Neurosurgery, Karolinska University Hospital, Stockholm, Sweden

¹⁰Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden

¹¹Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway

¹²Department of Cancer Research and Molecular medicine, Norwegian University of Science and Technology, Trondheim, Norway

¹³Department of Endocrinology, St. Olavs Hospital, Trondheim, Norway

¹⁴Department of Oncology, County Hospital Ryhov, Jönköping, Sweden

¹⁵Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

¹⁶Section of Oncology, Akademiska University Hospital, Uppsala, Sweden

¹⁷Department of Oncology, Örebro University Hospital, Örebro, Sweden

¹⁸Department of Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway

¹⁹Department of Medical Biochemistry and Biophysics, Division of Genome Biology, Science for Life Laboratory, Karolinska Institute, Stockholm, Sweden

²⁰Research Center, Danish Cancer Society, Copenhagen, Denmark

²¹European Palliative Care Research Centre (PRC), Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

²²Cancer Clinic, St. Olavs Hospital, Trondheim, Norway

v1 First published: 15 Nov 2018, 7:1797 (<https://doi.org/10.12688/f1000research.16786.1>)

Latest published: 15 Nov 2018, 7:1797 (<https://doi.org/10.12688/f1000research.16786.1>)

Abstract

Background: Disulfiram (DSF) is a well-tolerated, inexpensive, generic drug

Open Peer Review

Referee Status:  

Invited Referees

that has been in use to treat alcoholism since the 1950s. There is now independent preclinical data that supports DSF as an anticancer agent, and experimental data suggest that copper may increase its anti-neoplastic properties. There is also some clinical evidence that DSF is a promising anticancer agent in extracranial cancers. In glioblastoma, DSF induced O⁶-methylguanine methyltransferase (MGMT) inhibition may increase response to alkylating chemotherapy. A recent phase I study demonstrated the safety of DSF in glioblastoma patients when DSF was administered at doses below 500 mg/day together with chemotherapy. We plan to assess the effects of DSF combined with nutritional copper supplement (DSF-Cu) as an adjuvant to alkylating chemotherapy in glioblastoma treatment.

Methods: In an academic, industry independent, multicenter, open label randomized controlled phase II/III trial with parallel group design (1:1) we will assess the efficacy and safety of DSF-Cu in glioblastoma treatment. The study will include 142 patients at the time of first recurrence of glioblastoma where salvage therapy with alkylating chemotherapy is planned. Patients will be randomized to treatment with or without DSF-Cu. Primary end-point is survival at 6 months. Secondary end-points are overall survival, progression free survival, quality of life, contrast enhancing tumor volume and safety.

Discussion: There is a need to improve the treatment of recurrent glioblastoma. Results from this randomized controlled trial with DSF-Cu in glioblastoma will serve as preliminary evidence of the future role of DSF-Cu in glioblastoma treatment and a basis for design and power estimations of future studies. In this publication we provide rationale for our choices and discuss methodological issues.

Trial registration: The study underwent registration in [EudraCT 2016-000167-16](#) (Date: 30.03.2016,) and [Clinicaltrials.gov NCT02678975](#) (Date: 31.01.2016) before initiating the study.

Keywords

Randomized controlled trial, glioma, glioblastoma, disulfiram, alkylating agents, brain tumor

| | 1 | 2 |
|--------------------------|--------|--------|
| version 1 | | |
| published 15 Nov 2018 | | |
| | report | report |

- Kalkunte S. Srivenugopal**, Texas Tech University Health Sciences Center, USA
- Frantz R. Poulsen** , University of Southern Denmark, Denmark

Discuss this article

Comments (0)

Corresponding author: Asgeir Store Jakola (legepost@gmail.com)

Author roles: **Jakola AS:** Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Werlenius K:** Investigation, Methodology, Writing – Review & Editing; **Mudaisi M:** Investigation, Writing – Review & Editing; **Hyllin S:** Investigation, Writing – Review & Editing; **Kinhult S:** Investigation, Writing – Review & Editing; **Bartek Jr. J:** Investigation, Writing – Review & Editing; **Salvesen Ø:** Formal Analysis, Methodology, Visualization, Writing – Review & Editing; **Carlsen SM:** Methodology, Software, Writing – Review & Editing; **Strandéus M:** Investigation, Writing – Review & Editing; **Lindskog M:** Investigation, Writing – Review & Editing; **Löfgren D:** Investigation, Writing – Review & Editing; **Rydenhag B:** Methodology, Resources, Writing – Review & Editing; **Carstam L:** Conceptualization, Methodology, Writing – Review & Editing; **Gulati S:** Conceptualization, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Solheim O:** Conceptualization, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Bartek J:** Conceptualization, Supervision, Writing – Review & Editing; **Solheim T:** Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The sponsor, Department of Neurosurgery, Sahlgrenska University Hospital, has no role in the research planning or execution of the trial. The data belongs to the investigator and is guaranteed for by the principal investigator. Further, the sponsor or principal investigator has no connection with the pharmaceutical industry. The study is financed by grants from: • The Swedish state under the agreement between the Swedish government and the county councils concerning , the ALF-agreement (ALFGBG-716671) • AG Foundation. • Sahlgrenska Foundation. • Nordic Cancer Union. • The Swedish Society of Medicine (SLS-776901)

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2018 Jakola AS *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Jakola AS, Werlenius K, Mudaisi M *et al.* **Disulfiram repurposing combined with nutritional copper supplement as add-on to chemotherapy in recurrent glioblastoma (DIRECT): Study protocol for a randomized controlled trial [version 1; referees: 2 approved]** *F1000Research* 2018, 7:1797 (<https://doi.org/10.12688/f1000research.16786.1>)

First published: 15 Nov 2018, 7:1797 (<https://doi.org/10.12688/f1000research.16786.1>)

Introduction

Glioblastoma is a highly malignant brain tumor associated with limited treatment responses and population based series report median survival of only 10 months¹. Standard initial treatment is extensive surgical resections followed by postoperative fractionated radiotherapy, and concomitant and adjuvant temozolomide treatment. Unfortunately, initial treatment prolongs survival only modestly and in principle all glioblastomas eventually recur². Initial responses to chemotherapy are more often seen in patients with O⁶-methylguanine methyltransferase (MGMT) hypermethylation, and consequently this is an important predictive biomarker³. At time of recurrence there is no international treatment consensus^{4,5}. A common approach is to reintroduce temozolomide as long as the progression occurs more than 3–6 months after end of the initial treatment. In patients with tumor progression early after primary treatment or seen on ongoing temozolomide therapy, lomustine (also known as CCNU)⁶ or the combination regimen procarbazine, CCNU and vincristine (PCV) are often the treatment of choice. However, effect of second line treatment is often limited and new improved treatments are therefore imperative.

‘Repurposing’ of drugs for new indications has gained increased interest⁷. Disulfiram (DSF) is an inexpensive, generic drug that has been used since 1947 to treat alcohol dependency⁸. When alcohol is avoided, the drug is well tolerated in doses below 500 mg daily even with concomitant administration of temozolomide⁹.

DSF has demonstrated a wide range of antineoplastic effects, potentially also in gliomas^{10–16}. The interaction of DSF with copper (DSF-Cu) is likely responsible for the anti-cancer effect where DSF-Cu inhibit so-called glioma initiating cells at very low concentrations¹⁷. DSF-Cu may further inhibit the proteasome system^{10,17,18} and potentiate alkylating DNA damage through impaired DNA repair in glioma cells, an effect that may be mediated through inhibition of MGMT^{17,19}. Finally, a recent paper demonstrated benefit of cancer patients using disulfiram and suggested ditiocarb–copper complex to be the metabolite of disulfiram with anti-cancer effects through effects on protein turnover²⁰. As a consequence, it is now suggested that DSF-Cu should be implemented in clinical trials^{17,21}.

We seek to study the efficacy and safety of adding DSF combined with nutritional copper supplement (DSF-Cu) to patients with recurrent glioblastomas and concomitant with alkylating chemotherapy. The study is an academic, multicenter, open labeled randomized controlled phase II/III superiority trial with parallel group design. The aim of this paper is to present the intervention, design, endpoint, and power calculations of this study.

Methods and design

Study design

The study is a multi-center, open label randomized controlled phase II/III trial with parallel group design. All patients receive alkylating chemotherapy, and in the experimental arm treatment with DSF-Cu is added. Patients will be in the study at a maximum of 24 months or until death.

Participants, interventions and outcomes

Study setting. The patients will be recruited from and treated at University Hospitals in Norway and Sweden. The study involves eight centers (Gothenburg, Stockholm, Linköping, Lund, Jönköping, Uppsala, Örebro and Trondheim). The study was opened for inclusion January 2017 and per October 14 2018 we have randomized 36 patients.

Eligibility criteria. Adult patients (≥ 18 years) with first recurrence of glioblastoma will be screened for inclusion from the regular clinical practice at regional oncological departments (Figure 1). The full eligibility criteria are presented in Table 1. In short, adult patients intended for treatment with alkylating chemotherapy for recurrent glioblastoma are eligible if they have a Karnofsky Performance status 60–100 (ECOG Grade 0-2)²², are willing to refrain from alcohol, and have no major contraindications towards DSF-Cu.

Interventions. There is no consensus on choice of treatment at first glioblastoma recurrence⁵. In the Nordic countries, temozolomide, lomustine or PCV are commonly applied. A pragmatic comparator is thus needed to reflect clinical practice. Preclinical data suggest that DSF may enhance the effect of alkylating agents through inhibition of DNA repair¹⁷. We therefore use DSF-Cu combination as add-on to “any alkylating chemotherapy” in the intervention group while the comparator group receives “any alkylating chemotherapy” alone.

The administration of DSF and nutritional copper supplement is scheduled to start concomitant with alkylating chemotherapeutic treatment. Patients will take DSF (Antabus®) as a once daily oral dose of 400 mg, based on the known toxicity profile⁹. In case of intolerance, dose reduction to 200 mg per day is allowed. Copper supplementation will be administered separately from DSF with DSF administration in the evening. Copper nutritional supplement will correspond to 2.5 mg of elementary copper, which is not considered a safety concern²³. Adherence to treatment will be assessed at each control with a reminder, self-reporting of drug intake and drug tablet count of remaining DSF. The only surveillance of nutritional copper intake is by self-reporting.

Before study closure no crossover from the control group to DSF-Cu is allowed.

The intervention should continue also after change of chemotherapy, or during temporary chemotherapy withdrawal due to reached maximal cumulative dose or side effects from chemotherapy.

Patients are to discontinue permanently the treatment with DSF-Cu for any of the following reasons:

- Unacceptable adverse effect(s) as judged by the treating doctor.
- No longer indication for tumor targeted therapy.
- The patient wishes to withdraw from treatment.

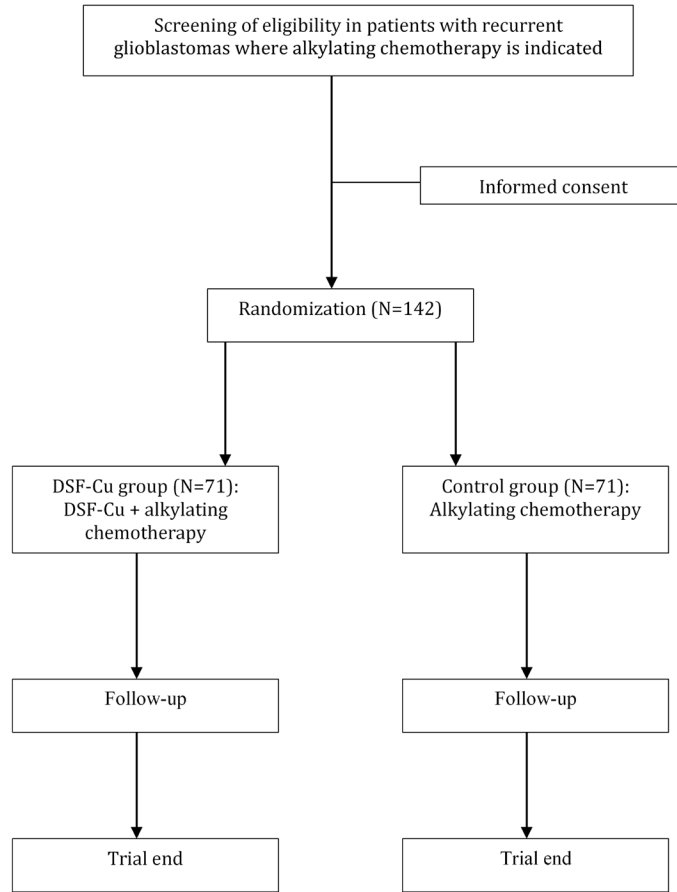


Figure 1. Flow-chart of patient screening, inclusion and follow-up.

Table 1. Full inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|--|--|
| Age 18 years or older. | History of idiopathic seizure disorder, psychosis or schizophrenia. |
| A previous histopathologically verified diagnosis of glioblastoma/gliosarcoma (later referred to as only glioblastoma) now presenting with a first-time progression/recurrence documented by MRI. | Uncontrolled hypertension (i.e. systolic BP > 180 mmHg) and a diagnosis of congestive heart failure |
| Planned treatment of recurrence with chemotherapeutic alkylating agents | Received radiotherapy within the 3 months before the diagnosis of tumor progression to reduce risk of misdiagnosis due to pseudoprogression. |
| Karnofsky performance status of 60 – 100 ²² . | Addiction to alcohol or drugs. |
| At inclusion not receiving another experimental treatment, including compassionate use programs, for glioblastoma | Pregnant and/or breastfeeding and women of childbearing potential who do not have a negative pregnancy test taken no longer than 14 days prior to randomization. |
| Able to take oral medications. | History of liver disease. |
| No known allergy to DSF or copper. | History of Wilson's disease. |
| Absolute neutrophil count ≥ 1,500/mcL and platelets ≥ 100,000/mcL | History of hemochromatosis. |
| Willingness to refrain from ingestion of alcoholic beverages while in the study is a criterion to be randomized. However, once randomized alcohol abstinence only affects the group treated with DSF | Nickel hypersensitivity ⁸ |
| | Need for metronidazole, warfarin and/or theophylline medication since their metabolism may be influenced by DSF. |
| | Patients who are taking medications metabolized by cytochrome P450 2E1 |
| | Unfit for participation for any other reason judged by the including physician |

- If patients develop conditions that contribute to excess risk as judged by the treating physician.

Other experimental therapy, including compassionate use programs, are not allowed in either treatment group as long as the patient is in the trial and receives the assigned treatment.

Outcomes. The primary end-point of the study is survival assessed at 6 months post-randomization.

Secondary end-points are:

- Overall survival from date of randomization.
- Median progression free survival.
- 6 and 12 month progression free survival.
- Health-related generic quality of life²⁴.
- Volumetric growth rate assessed from baseline MRI to first follow-up MRI scan²⁵.
- Safety assessment (toxicities).

Participant timeline. To reduce patient burden, timing of data collection after randomization is scheduled according to choice

of chemotherapy regimen, where patients that receive temozolomide usually are assessed every 4th week while patients treated with PCV/lomustine are assessed every 6th week. Consequently, this may lead to four additional observations in the temozolomide group compared with the lomustine/PCV group. The more detailed clinical control and MRI assessment every 3rd month is independent of chemotherapy regimen. The primary endpoint will not be affected by the differences in timing of assessments during the trial.

Finally, hematological status and liver function will be monitored as long as patients are on active treatment every 4–6 weeks, once again depending on the chemotherapeutic protocol (Table 2).

Sample size and study power. The sample size calculation is based on data from previous trials reporting that 50% of patients treated with lomustine deceased within 8 months⁶. We use a phase III end-point in a phase II setting because this study will be the basis for power calculation of a later more rigid phase III study, and because progression is less relevant in patients with already demonstrated progression/recurrence and with a short expected survival. Due to the phase II/III setting we are relaxing the alpha value to 0.10 (by convention 0.05) since phase II

Table 2. Schematic view of study schedule with timing and content of study visits.

| Visit | Screening | Monthly | Every 3 months |
|---------------------------------------|-----------|-------------------|----------------------|
| Month* | 0 | 1,2,4,5,7,8,10,11 | 3,6,9,12,15,18,21,24 |
| Eligibility | X | | |
| Informed consent | X | | |
| Alcohol screen** | X | | X |
| KPS | X | X | X |
| Demographics | X | | |
| Pregnancy test | X | | |
| Medical history | X | | |
| Euroqol-5D 3L | X | | X |
| Toxicity/AE/SAE/SAR and SUSAR | | X | X |
| Compliance | | X | X |
| Discontinuation | | X | X |
| Consider drug interactions | X | | X |
| MRI Scan | X | | X |
| Blood samples: | | | |
| Hematological and liver monitoring*** | X | X | X |

AE denotes adverse event, SAE; serious adverse event, SAR; serious adverse reaction SUSAR; suspected unexpected serious adverse reaction, MRI; magnetic resonance imaging, KPS; Karnofsky Performance Status

*The interval for patients treated with lomustine/PCV is 6 weeks, while 4 weeks is clinical routine in the case of temozolomide

**Continued alcohol screen only relevant if randomized to the disulfiram group.

***Blood samples as indicated here relevant as long as on active treatment and includes: hemoglobin, thrombocytes, leukocytes with differential count, ASAT, ALAT, G-GT, ALP, bilirubin, INR).

studies are “preliminary assessment of a new intervention before embarking on a larger and expensive randomized controlled trial”²⁶. Thus, we argue that it is good science to slightly increase the chance of false positive results (due to chance) for a relevant end-point instead of using less relevant and difficult to interpret surrogate end-points (such as progression).

Based on the assumption that the treatment group will have an improvement in proportion achieving 6-month survival from 60% to 80% we need a final sample size of 128 in total (64 in each group; alpha 0.1, power 80% and two sided test). Considering 10% attrition, the actual included sample need to be 142 with 71 patients included in each group.

Assignment of interventions. A web-based randomization system (WebCRF 3.0) developed and administered by the Unit of Applied Clinical Research, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU, Trondheim, Norway), will be used. Randomizations are computer generated in a 1:1 ratio with stratification by center. Blocks of varying sizes will be used to make prediction of allocation impossible. Randomization will be performed only after the initial chemotherapy treatment has been selected. Patient enrollment is performed by a study member with appropriate delegation at the local site.

Data collection, management and analysis

Data collection. Patients will be in the trial for a maximum of 24 months or until death (see study schedule in Table 2). Generic quality of life is followed every 3rd month using EQ-5D 3L. Quality of life will be monitored until progression, death or 24 months post-inclusion^{24,27}.

MRI scanning will be performed at a minimum of every 3rd month. MRI assessment may continue up to 24 months post-inclusion and this should be implemented also after documented progression in the DSF-Cu group, as long as the patient continues on only DSF-Cu, or in combination with another established rescue therapy such as surgery, radiation therapy or other alkylating chemotherapy. Consequently, MRI controls are indicated and should be continued as long as tumor targeted therapy is administered (both in control and intervention group). The RANO criteria is used in MRI assessment and for evaluation of progression²⁸.

After withdrawal of tumor target therapy no additional follow-up (including MRI) is planned within the context of this study, in order to minimize patient burden in the end of life setting. Safety assessment are according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0²⁹. Survival is followed until death or 24 months post-inclusion.

Data management. Data will be recorded in the web-based eCRFs (WebCRF 3.0) at The Unit for Applied Clinical Research at the NTNU. All data will be entered in the WebCRF 3.0 at the local study site. All electronic patient information is handled using a study participation number that is not logically connected to any personal data. It is possible to manually “decode”

back to personal data using a name list stored securely at the respective study sites.

All data entered into the WebCRF are stored at two separate servers at NTNU. These servers are protected by the general firewall at the university and then by another firewall dedicated to protect clinical research data. The server that stores the data is not the same server as the one in contact with the Internet. There are several further safety measures not to be exposed. There are backups taken every 24-hour throughout the years. The responsibility for this system lies with the Unit for Applied Clinical Research at NTNU.

Statistical methods. All outcomes will be analyzed according to the intention to treat principle unless otherwise specified in post-hoc analyses. This is an open label trial, however the statistician performing analyses will be blinded for treatment assignment. Also, an investigator blinded for treatment allocation will perform the assessment of radiological end-points.

The 6-month survival (i.e primary end-point) will be assessed with 2x2 tables and analyzed with chi-square test. This analysis will be performed 6-months after last patient inclusion. Concerning the primary end-point we do not expect any missing data.

Concerning the secondary outcomes they will be evaluated as follows:

- Actual survival data at 24 months post-inclusion will be analyzed similar to the primary end-point.
- Overall survival analyses will be analyzed using Kaplan-Meier plot and tested with log-rank test. This analysis will be performed at 6 months after the last patient inclusion together with the assessment of primary outcome. Also, this will be analyzed in a longer-term follow-up study (24 months post-inclusion).
- Overall progression free survival will be analyzed with Kaplan-Meier plot and tested with log-rank test. This analysis will also be performed at 6 months and at 24 months after the last patient inclusion. The assessment of progression will be done in accordance with the latest RANO criteria by the local investigator.
- Actual progression free survival analyses at 6 and 24 months after the last patient inclusion. The assessment of progression will be done in accordance with the latest RANO criteria by the local investigator and analyzed similar to the primary end-point.
- Comparison of general health related quality of life at 6 and 24 months after last included patients. We will use an area under the curve approach (i.e. cumulative quality of life)²⁷.
- Semiautomatic segmentation of contrast-enhancing tumor volume assessed at baseline and first MRI control to assess growth dynamics. The data will be analyzed with

independent samples t-test or Mann-Whitney U test, based on data distribution.

- Safety assessment with comparing proportion of a composite of grade 3, 4 and 5 toxicities according to the common terminology Criteria for Adverse Events (CTCAE).

We stratify only for study center, however important baseline variables like repeated surgery, time since primary treatment, age, functional status, lomustine versus temozolomide treatment, and biomarker status from initial surgery if available (IDH and MGMT) will be registered and adjusted for in post-hoc exploratory analyses. Also, we will explore outcomes in post-hoc as treated analyses if non-compliance to treatment protocol becomes apparent.

Monitoring

All source data will be accessible for auditing and monitoring. The external, independent monitor will maintain patient confidentiality. Authorities have the right to perform inspections as well as the monitor.

An initiation visit from study monitors takes place at each site before the enrolment of study subjects at the specific site can start. The first monitoring visit after patient recruitment has started will be performed after inclusion of 5 participants for the specific site, however a minimum of one contact (by e-mail or telephone) and one visit per year to each site is required. After the last study subject has completed the last visit and all CRFs are completed a close-out monitoring visit will take place at each site.

Harms. Adverse events and toxicities is assessed at each study visit and graded according to the NCI Common Toxicity Criteria for adverse events (NCI-CTCAE) Version 4.0 (<http://ctep.cancer.gov/reporting/ctc.html>).

Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) and Suspected Unexpected Serious Adverse Reactions (SUSAR). A serious adverse reaction (SAR) is a serious adverse event (SAE) that may be related to trial treatment. Suspected unexpected serious adverse reactions (SUSAR) are reactions where DSF-Cu may be suspected as the reason for an unexpected SAR. The assessment of relatedness is the responsibility of Department of Clinical Pharmacology at Sahlgrenska University Hospital (Gothenburg, Sweden) and the local investigator responsible for the patient.

All reportable cases of adverse events are reported to the Department of Clinical Pharmacology, Sahlgrenska University Hospital. They are responsible to notify sponsor, authorities and local investigators when indicated.

Interim analysis and criteria for aborting the trial. At 50% inclusion (71 included patients) an interim analysis on primary end-point and safety will be performed. An independent data monitoring scientific committee (DMSC) will review the results

of this interim analysis. This committee consists of professor Anja Smits (Gothenburg), associate professor Petter Förander (Stockholm) and Dr. Annika Malmström (Linköping). A recommendation signed by all members of the monitoring committee will be handed to the sponsor after this meeting and the final decision is with the sponsor.

Further, the sponsor may terminate the study prematurely for any reasonable cause. The Ethics committees and competent authorities will then be informed. Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study.
- If competent authorities obtain information that raises doubts about the safety or scientific validity of the study, the competent authorities can suspend or prohibit the study.

Reporting and transparency

The reporting of this protocol is consistent with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) recommendation³⁰. Reporting of the trial will be consistent with the consolidated standards of reporting trials (CONSORT) 2010 statement³¹.

Ethical considerations

This study will be conducted in compliance with the protocol and according to Good Clinical Practice guidelines and applicable regulatory standards. Further, the study will be conducted in accordance with the latest Declaration of Helsinki³². The regional committee in Gothenburg approved the study for all centers in Sweden in a multicenter approval (Dnr 332-16). Ethical approval for the Norwegian site was acquired separately from the ethical committee in region Central Norway (2016/283). Further, the study is approved by the Norwegian medicines agency (16/04133) and the Swedish medical product agency (5.1-2016-25235). Protocol modifications will be reported to the local ethical committees and significant modifications will in addition be communicated to the Norwegian medicines agency and the Swedish medical product agency.

Patients will be included only after the informed consent form is signed ([Supplementary File 1](#), Swedish version). The informed consent needs to be explicit and written and may at any time be withdrawn by the participant. Also, the right of the participant to refuse to participate without giving reasons must be respected.

Patients without possibility to provide written, informed consent (e.g. severe cognitive deficit and aphasia) are not to be included via proxies.

Patient confidentiality will be maintained through de-identified and secure storage of data and a secure, local storage of the name lists at the respective study sites.

Dissemination

The principal investigator (ASJ) and the study statistician (ØS) will have access to the final dataset. A cleaned dataset from specific sites may be given back in anonymous form to sites upon request, but only after the final scientific publication (after 24 months follow-up). No other data sharing is planned.

The trial results will be communicated through publication of a scientific report in a peer-reviewed journal. There are no restrictions on publication (e.g. a negative trial will still be published).

Trial status

At the time of submission the recruitment has not been completed (started January 2017).

This paper is based on protocol version 2.0, dated December 12th 2017.

Discussion

This study investigates the role of DSF-Cu in recurrent glioblastoma. To our knowledge this is the first planned, randomized controlled trial with DSF-Cu in glioblastoma. The results from this trial will consequently serve as preliminary evidence of the future role of DSF-Cu in glioblastoma treatment as well as to serve as a basis for power estimations in future studies on the same topic.

The study has several pragmatic features, including an open design and a patient centered primary end-point³³. Due to the nature of our study end-point, we need to recruit more patients than if we had chosen a surrogate marker which is more common in the phase II setting. Nevertheless, we believe that surrogate markers are less relevant in the setting of glioblastoma recurrence since survival is short and image interpretation and response assessment is difficult^{6,28}. Consequently, the trial is best described as a phase II/III trial. Further, the trial is to a high degree integrated within the frame of regular clinical practice, reducing the need of extra resources and thereby cost³³. The study will thus reflect clinical practice and the little deviation from everyday practice also reduce patient burden.

It may be a personal sacrifice for some patients to abstain from alcohol intake completely. Nevertheless, DSF has a well-known risk profile and the major risks of treatment with 400 mg DSF daily is mainly associated with lack of compliance with respect to alcohol intake and infrequently serious idiosyncratic hepatitis may be seen. In addition, there is a risk of neuropathy that is reversible upon drug discontinuation⁹. In sum, the risks of DSF-Cu, also combined with chemotherapy, are presumably

limited⁹. The potential benefit lies in improved response to treatment.

The main strengths of this study are the multi-center setting, a hard primary end-point, secondary endpoints relevant to patients, a randomized controlled design and clinically relevant control group representing present best clinical practice. Further, the study being independent from the pharmaceutical industry is another strength. Limitations include the open design making both patients and investigators aware of treatment assignment. Thus, it is difficult to completely guarantee against unscheduled use of DSF-Cu in the control group. To minimize the risk of bias the statistical analyses both for primary and secondary endpoints will be performed by an investigator blinded for the actual treatment. Endpoints will be analyzed before analyzes of adverse events that may reveal actual treatment in the two groups.

In conclusion, we present a protocol for investigating the efficacy of disulfiram and nutritional copper supplement (DSF-Cu) as a supplement to alkylating chemotherapy in the setting of recurrent glioblastoma. We have provided rationale for our choices and discussed methodological issues of importance for this open labeled, randomized controlled trial.

Data availability

No data is associated with this article.

Grant information

The sponsor, Department of Neurosurgery, Sahlgrenska University Hospital, has no role in the research planning or execution of the trial. The data belongs to the investigator and is guaranteed for by the principal investigator. Further, the sponsor or principal investigator has no connection with the pharmaceutical industry.

The study is financed by grants from:

- The Swedish state under the agreement between the Swedish government and the county councils concerning , the ALF-agreement (ALFGBG-716671)
- AG Foundation.
- Sahlgrenska Foundation.
- Nordic Cancer Union.
- The Swedish Society of Medicine (SLS-776901)

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary material

Supplementary File 1: Consent form, in Swedish.

[Click here to access the data](#)

References

- Rønning PA, Helseth E, Meling TR, *et al.*: **A population-based study on the effect of temozolomide in the treatment of glioblastoma multiforme.** *Neuro Oncol.* 2012; **14**(9): 1178–1184.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Stupp R, Mason WP, van den Bent MJ, *et al.*: **Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma.** *N Engl J Med.* 2005; **352**(10): 987–996.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hegi ME, Liu L, Herman JG, *et al.*: **Correlation of O⁶-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity.** *J Clin Oncol.* 2008; **26**(25): 4189–4199.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Seystahl K, Wick W, Weller M: **Therapeutic options in recurrent glioblastoma--An update.** *Crit Rev Oncol Hematol.* 2016; **99**: 389–408.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Weller M, van den Bent M, Hopkins K, *et al.*: **EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma.** *Lancet Oncol.* 2014; **15**(9): e395–403.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Taal W, Oosterkamp HM, Walenkamp AM, *et al.*: **Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial.** *Lancet Oncol.* 2014; **15**(9): 943–953.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Collins FS: **Mining for therapeutic gold.** *Nat Rev Drug Discov.* 2011; **10**(6): 397.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chick J: **Safety issues concerning the use of disulfiram in treating alcohol dependence.** *Drug Saf.* 1999; **20**(5): 427–435.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Huang J, Campian JL, Gujar AD, *et al.*: **A phase I study to repurpose disulfiram in combination with temozolomide to treat newly diagnosed glioblastoma after chemoradiotherapy.** *J Neurooncol.* 2016; **128**(2): 259–66.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Chen D, Cui QC, Yang H, *et al.*: **Disulfiram, a clinically used anti-alcoholism drug and copper-binding agent, induces apoptotic cell death in breast cancer cultures and xenografts via inhibition of the proteasome activity.** *Cancer Res.* 2006; **66**(21): 10425–10433.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kast RE, Boockvar JA, Bruning A, *et al.*: **A conceptually new treatment approach for relapsed glioblastoma: coordinated undermining of survival paths with nine repurposed drugs (CUSP9) by the International Initiative for Accelerated Improvement of Glioblastoma Care.** *Oncotarget.* 2013; **4**(4): 502–530.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Liu P, Brown S, Goktug T, *et al.*: **Cytotoxic effect of disulfiram/copper on human glioblastoma cell lines and ALDH-positive cancer-stem-like cells.** *Br J Cancer.* 2012; **107**(9): 1488–1497.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Loo TW, Bartlett MC, Clarke DM: **Disulfiram metabolites permanently inactivate the human multidrug resistance P-glycoprotein.** *Mol Pharm.* 2004; **1**(6): 426–433.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rae C, Tesson M, Babich JW, *et al.*: **The role of copper in disulfiram-induced toxicity and radiosensitization of cancer cells.** *J Nucl Med.* 2013; **54**(6): 953–960.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Triscott J, Lee C, Hu K, *et al.*: **Disulfiram, a drug widely used to control alcoholism, suppresses the self-renewal of glioblastoma and over-rides resistance to temozolomide.** *Oncotarget.* 2012; **3**(10): 1112–1123.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Westhoff MA, Zhou S, Nonnenmacher L, *et al.*: **Inhibition of NF- κ B signaling ablates the invasive phenotype of glioblastoma.** *Mol Cancer Res.* 2013; **11**(12): 1611–1623.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lun X, Wells JC, Grinshtein N, *et al.*: **Disulfiram when Combined with Copper Enhances the Therapeutic Effects of Temozolomide for the Treatment of Glioblastoma.** *Clin Cancer Res.* 2016; **22**(15): 3860–75.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hothi P, Martins TJ, Chen L, *et al.*: **High-throughput chemical screens identify disulfiram as an inhibitor of human glioblastoma stem cells.** *Oncotarget.* 2012; **3**(10): 1124–1136.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Paranjpe A, Zhang R, Ali-Osman F, *et al.*: **Disulfiram is a direct and potent inhibitor of human O⁶-methylguanine-DNA methyltransferase (MGMT) in brain tumor cells and mouse brain and markedly increases the alkylating DNA damage.** *Carcinogenesis.* 2014; **35**(3): 692–702.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Skrott Z, Mistrik M, Andersen KK, *et al.*: **Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4.** *Nature.* 2017; **552**(7684): 194–199.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kast RE, Karpel-Massler G, Halatsch ME: **CUSP9* treatment protocol for recurrent glioblastoma: aprepitant, artesunate, auranofin, captopril, celecoxib, disulfiram, itraconazole, ritonavir, sertraline augmenting continuous low dose temozolomide.** *Oncotarget.* 2014; **5**(18): 8052–8082.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Karnofsky DA, Burchenal JH: **The clinical evaluation of chemotherapeutic agents in cancer.** New York: Columbia University Press. 1949; 191–205.
[Reference Source](#)
- European Food Safety Authority: **Tolerable upper intake levels for vitamins and minerals.**
[Reference Source](#)
- EuroQol Group: **EuroQol--a new facility for the measurement of health-related quality of life.** *Health Policy.* 1990; **16**(3): 199–208.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Stensj oen AL, Solheim O, Kvistad KA, *et al.*: **Growth dynamics of untreated glioblastomas in vivo.** *Neuro Oncol.* 2015; **17**(10): 1402–11.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Khan I, Sarker SJ, Hackshaw A: **Smaller sample sizes for phase II trials based on exact tests with actual error rates by trading-off their nominal levels of significance and power.** *Br J Cancer.* 2012; **107**(11): 1801–1809.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sagberg LM, Solheim O, Jakola AS: **Quality of survival the 1st year with glioblastoma: a longitudinal study of patient-reported quality of life.** *J Neurosurg.* 2015; **124**(4): 989–97.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wen PY, Macdonald DR, Reardon DA, *et al.*: **Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group.** *J Clin Oncol.* 2010; **28**(11): 1963–1972.
[PubMed Abstract](#) | [Publisher Full Text](#)
- National Cancer Institute: **Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.** Bethesda, USA. 2010.
[Reference Source](#)
- Chan AW, Tetzlaff JM, G otzsche PC, *et al.*: **SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials.** *BMJ.* 2013; **346**: e7586.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Schulz KF, Altman DG, Moher D, *et al.*: **CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials.** *Trials.* 2010; **11**: 32.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- World Medical Association: **World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.** *JAMA.* 2013; **310**(20): 2191–2194.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ford I, Norrie J: **Pragmatic Trials.** *N Engl J Med.* 2016; **375**(5): 454–463.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Referee Status:  

Version 1

Referee Report 03 January 2019

<https://doi.org/10.5256/f1000research.18352.r42298>



Frantz R. Poulsen 

Department of Clinical Research, University of Southern Denmark, Odense, Denmark

The study protocol describes a randomized controlled trial investigating if addition of disulfiram and copper to the standard Stupp regimen for treatment of glioblastoma will increase survival at 6 months, overall survival, progression free survival etc. The study is limited to patients with first recurrence.

The study is well designed and well planned to answer the research questions. It is, however, more and more common practice to perform surgical resection of the recurrence (if possible) prior to additional chemo therapy. I cannot from the study protocol read if this is also the case here and how the authors will deal with this.

The RANO criteria will be used in the assessment of progression. Although these criteria are well recognized they may not always be that easy to use and conclude from. Therefore it was very nice, the authors also use volumetric measurements to evaluate growth rate. The authors might, however, consider using other measures than contrast enhancement to assess volumetric growth rate (flair, T2 etc).

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Referee Expertise: Neurosurgery

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 23 November 2018

<https://doi.org/10.5256/f1000research.18352.r40689>**Kalkunte S. Srivenugopal**

Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, TX, USA

The alcohol aversion and metal-chelating drug disulfiram (DSF) exhibits strong affinity for cysteines present in various regulatory proteins. The irreversible mixed disulfides thus formed inactivate the biological activities of several signaling, DNA repair, and anticancer targets, through the same mechanism by which the drug inhibits ALDH and prevents binge drinking. The strong anticancer effects of DSF particularly in combination with copper against gliomas, its long-term use, safety and tolerability, penetration through the blood brain barrier have made it attractive to repurpose the drug for brain cancers. DSF's ability to inhibit MGMT, the single most important determinant of glioma resistance to alkylating agents is an additional feature that can increase the efficacy of temozolomide in the trial.

The current protocol to evaluate the use Cu-DSF in a non-alcoholic setting for recurrent glioblastoma is well designed and well justified. First, there is no consensus on treatment for GBM recurrence and the high-risk patient selection is appropriate. The therapeutic outcome between the two arms (Cu-DSF with or without alkylators) should be well-comparable. Whether the Cu-DSF by itself will have anti-glioma effects in a clinical setting is not known and difficult to predict. This is because, the copper chelated with DSF [BisN, N-diethyldithiocarmato) copper II], essentially appears to be an inactivated molecule and lacks the ability to form S-conjugates and fails to inhibit proteasome and other targets. The fate of DDC-Cu and its cytotoxic mechanisms *in vivo* are still unclear. Other comments and suggestions are listed below.

1. Whether the protocol uses copper gluconate or another chelated form is not mentioned. There is one study (Proc Amer Assoc Cancer Res. 58, Abstract 4053, 2017) which describes that the efficacy of DSF-Cu is highly dependent on the time (at least 6 h) and schedule (DSF prior to copper) in brain tumor cell cultures.
2. There is a good chance of myelosuppression, particularly, in patients taking DSF-Cu and temozolomide or other alkylating agents. Therefore, blood cell counts more frequently than 4-6 weeks is suggested.
3. Cu-DSF is likely to sensitize GBMs to radiation as well. If there are a subset of patients getting radiation alone or in combination with DSF-Cu, it is recommended that the outcomes be assessed as well.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Brain tumor chemotherapy, redox-stress signaling and anticancer drug resistance

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research