Concise Review: Bullseye: Targeting Cancer Stem Cells to Improve the Treatment of Gliomas by Repurposing Disulfiram

JOANNA TRISCOTT, MARY ROSE PAMBID, SANDRA E. DUNN

Key Words. Brain tumor • Disulfiram • Clinical trials • Cancer stem cells

ABSTRACT

Cancer stem cells (CSCs) are thought to be at the root of cancer recurrence because they resist conventional therapies and subsequently reinitiate tumor cell growth. Thus, targeting CSCs could be the bullseye to successful cancer therapeutics in the future. Brain tumors are some of the most challenging types of cancer to treat and the median survival following the initial diagnosis is 12–18 months. Among the different types of brain tumors, glioblastoma (GBM) is considered the most aggressive and remains extremely difficult to treat. Despite surgery, radiation, and chemotherapy, most patients develop refractory disease. Temozolomide (TMZ) is a chemotherapy used to treat GBM however resistance develops in most patients. The underlying mechanisms for TMZ resistance (TMZ-resistant) involve the expression of DNA repair gene O(6)-methylguanine-DNA methyltransferase. CSC genes such as Sox-2, BMI-1, and more recently Y-box binding protein-1 also play a role in resistance. In order to develop novel therapies for GBM, libraries of small interfering RNAs and off-patent drugs have been screened. Over the past few years, several independent laboratories identified disulfiram (DSF) as an off-patent drug that kills GBM CSCs. Reportedly DSF has several modes of action including its ability to inhibit aldehyde dehydrogenases, E3 ligase, polo-like kinase 1, and NFkB. Due to the fact that GBM is a disease of heterogeneity, chemotherapy with multitargeting properties may be the way of the future. In broader terms, DSF kills CSCs from a range of different cancer types further supporting the idea of repurposing it for “target practice.”

INTRODUCTION

Brain Tumors: General Overview and Need for New Therapies

Brain tumors are difficult to treat in general given their location and the lack of targeted therapies. In adults, glioblastoma (GBM) is the most common type of brain tumor, and while they do occur in children to a lesser extent, most patients are faced with living 12–18 months after diagnosis. There are several characteristics of GBM that hinder therapeutic development. These include a heterogeneous morphology and the presence of subpopulations of cancer stem cells (CSCs) that appear undifferentiated, have a functional capacity for self-renewal, and are drug resistant [1–3]. Evidence of CSCs in pediatric tumors questions whether these may also be responsible for the initial formation of these malignancies [4]. There are several genes associated with GBM CSCs including CD44 [5], CD133 [1], Nanog, Oct4, Sox-2, Mushashi, BMI-1, and more recently the transcription and translation factor YB-1 (Y-box binding protein-1) [6]. Maximal safe resection and radiation therapy are used in the treatment of GBM. As well, temozolomide (TMZ) has been incorporated into standard care procedures with the establishment of the Stupp protocol [7, 8]. With a reported 1.9% 5-year survival rate for patients treated with radiotherapy alone, the addition of TMZ with radiotherapy only increased 5-year survival to 9.8% of patients [8]. Unfortunately, the toxicity of TMZ is often not well tolerated by patients, and the number of clinically available compounds that are capable of crossing the blood-brain barrier (BBB) is limited. As well, additional modalities of TMZ-resistant such as O(6)-methylguanine-DNA methyltransferase (MGMT) expression further complicate the problem. This repair enzyme can remove the alkyl groups added by TMZ to the O6 position of guanine in DNA, therefore preventing interstrand cross-linking and block TMZ-induced apoptosis of proliferative cells [9, 10]. Other proteins that have received less attention, such as YB-1, also convey TMZ resistance [11], and its expression was linked to maintaining GBM in a stem cell state [6].
Table 1. Clinical trials involving disulfiram in cancer

<table>
<thead>
<tr>
<th>Clinical trial identifier</th>
<th>Disease</th>
<th>Study phase</th>
<th>Sponsor</th>
<th>Enrollment</th>
<th>Status</th>
<th>Start/completion dates</th>
</tr>
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<td>July 2008 to March 2013</td>
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<td>15</td>
<td>Terminated</td>
<td>September 2006 to August 2012</td>
</tr>
<tr>
<td>NCT00256230</td>
<td>Metastatic melanoma</td>
<td>Phase I/II</td>
<td>University of California, Irvine</td>
<td>7</td>
<td>Completed</td>
<td>January 2002 to August 2007</td>
</tr>
<tr>
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<td>Glioblastoma</td>
<td>Phase II</td>
<td>Olympong Medical Center</td>
<td>TBD</td>
<td>Not active yet</td>
<td>September 2015 to September 2018</td>
</tr>
<tr>
<td>NCT00312819</td>
<td>NSCLC</td>
<td>Phase II/III</td>
<td>Hadassah Medical Organization</td>
<td>60</td>
<td>Completed</td>
<td>March 2006 to December 2009</td>
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<tr>
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<td>Prostate cancer</td>
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<td>Johns Hopkins University</td>
<td>19</td>
<td>Completed</td>
<td>October 2013 to December 2017</td>
</tr>
<tr>
<td>NCT01907165</td>
<td>Glioblastoma</td>
<td>Phase II</td>
<td>Washington University School of Medicine</td>
<td>TBD</td>
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TBD, to be determined.

*Identifiers in reference to www.clinicaltrials.gov online database.

Disulfiram (DSF), also known as Antabuse, has been used for treatment of substance abuse and in addiction studies [13, 14]. Initially, the compound had been used in the process of rubber manufacturing. In 1937, it was discovered that factory workers, who were regularly exposed to DSF, would experience flu-like symptoms when they ingested alcohol [14]. The first clinical trials for use of DSF as an antialcoholic treatment began in 1948, and it has been used in patients for more than 60 years [13]. With the more recent discovery of a stem cell population in cancer, scientists are once again finding new purposes for DSF. Currently, there are two ongoing clinical trials for DSF in GBM (www.clinicaltrials.gov, identifiers NCT01907165 and NCT01777919) (Table 1). There are no pediatric brain tumor trials with DSF reported; however, this would be a logical next step pending positive outcomes from GBM trials in adults.

We and other groups as well report that DSF inhibits the growth of glioma cell lines and blocks self-renewal. DSF important inhibits the growth of TMZ-resistant cells isolated from patients [15, 16]. To illustrate this point, we compared TMZ to DSF in two primary GBM isolates (Fig. 1A). While TMZ had no effect on the growth of these cells, they were highly sensitive to DSF. Importantly, DSF is effective regardless of MGMT expression because ABT011 cells express high levels of MGMT while ABT015 cells express low levels of this enzyme (data not shown).

In T98G GBM cells that are TMZ-resistant, Paranjpe et al. recently reported that DSF downregulates MGMT in xenografts implanted subcutaneously [17]. They suggest that DSF could, therefore, be used to treat gliomas because it crosses the BBB however they did not perform intracranial injections of T98G cells. Choi et al. recently published an elegant study in atypical teratoid rhabdoid tumors (AT/RT) that demonstrated DSF crosses the BBB in mice and can reduce AT/RT CSCs [18]. AT/RT are a rare yet deadly type of pediatric brain tumor where improved therapies are most certainly needed. Of note, they reported that DSF reduced aldehyde dehydrogenase (ALDH) in vitro by ~75% and in tumors. It also inhibited EdU incorporation and tumor cell proliferation based on Ki67 staining. AT/RT CSCs were more sensitive to DSF than clinically used drugs such as ifosfamide (IFO). Likewise, IFO

![Infographic](image-url)
Potential Mechanisms of DSF Anticancer Activity

DSF could be a way to hit the “Bullseye” given the fact that it not only kills CSCs, but seems to do so by targeting multiple pathways operative in these refractory cells (Fig. 1B). DSF is most widely known as an inhibitor of ALDH. ALDH is a family of metabolic enzymes that catalyze the oxidation of aldehydes, which are toxic products of alcohol metabolism [19]. A relationship between high ALDH activity and stem cell behavior prompted the use of an ALDH-based fluorescence assay, Aldefluor, to identify undifferentiated populations both within cancer and normal tissues [20–22]. In cancer studies, high ALDH expressing cells have been associated with enhanced xenograft tumor formation in mice and chemotherapeutic resistance [23, 24]. ALDH enzyme activity is thought to be involved with cell detoxification, and Aldefluor active cells are associated with resistance to cisplatin, docetaxel, and doxorubicin [25].

The drug cyclophosphamide is a fundamental chemotherapeutic in many pediatric brain tumor treatment protocols. High levels of ALDH have been directly shown to intervene with cyclophosphamide metabolism and decomposition making this a potential mechanism for chemotherapeutic resistance [26, 27]. The ALDH1a1 isoform has previously thought to have the strongest association with the CSC phenotype. For example, Marcato et al. [28] suggest expression of ALDH1a3 to have greater CSC correlation and prognostic importance compared to ALDH1a1 in breast cancer. With a family of 19 total ALDH isoforms it is difficult to pinpoint complete functional independence due to redundancy in activity.

There are additional anticancer properties of DSF. For example, it also suppresses the proteasome and NFκB pathways [29–33]. More specifically, DSF suppresses ubiquitin NFκB ligase activity [34]. In the body, the DSF molecule is converted into a smaller metabolite called diethyldithiocarbamate. This metabolite has been shown to chelate into complexes when in combination with copper or zinc ions. These complexes are suggested to inhibit proteasome activity and elevate radical oxygen species [33]. Under this premise, many cancer studies use DSF in combination with copper [33, 35, 36]. While some studies report increased efficacy of DSF in combination copper in killing cancer cells, this increased copper-mediated cytotoxicity is apparent in normal cells as well [18]. Choi et al. (2014) discuss the potential danger of using additional copper and zinc in treatment regimens, as they are teratogenic, and could result in developmental defects [18]. It is crucial that potential metal ion toxicities are considered in the design of clinical studies.

DSF has also been shown to impinge on epigenetic pathways. It is suggested that DSF contains functional groups that are extremely thiol reactive, and this chemistry is effective in blocking the active site of certain enzymes. In prostate cancer, DSF can act as a DNA demethylating agent through inhibition of DNA methyltransferase 1 [37]. Aberrant methylation in cancer genomes can potentiate overexpression of oncogenes or inhibit the expression of tumor suppressors, therefore, targeting epigenetic controls allows reprogramming of cell pathways. More recent studies on the fusion protein NUP98-PHF23 show DSF treatment can reduce its chromatin-modifying potential and induce cell death in acute myeloid leukemia. In addition, transcriptional availability of CSC signature genes, such as Hoxa, Hoxb, and Meis1, is blocked by DSF [38]. These observations further exemplify the anti-CSC activity of DSF.

Interestingly, treatment of primary GBM cells with DSF in vitro reduced the expression of kinases such as polo-like kinase 1 (PLK1) protein and mRNA [16]. The exact mechanism driving DSF induced PLK1 downregulation still requires further investigation. However, these findings suggest DSF to be capable of targeting aggressive PLK1 high cell populations, which may be responsible for driving tumor relapse. Out of all the cancer pathways affected by DSF, PLK1 is the one that stands out as an interesting molecular target because it is a well-established drug candidate for cancer.

Repurposing DSF for Other Cancers

DSF was identified in several studies as an agent that inhibits CSCs for cancers of the breast, ovary, pancreas, lung, and blood [15, 16, 39, 40]. A recent study reported that the liposomal packaging of DSF (Lipo-DSF) inhibited breast cancer CSCs in part by disrupting the NFκB pathway [29]. Hypoxia rendered the cells resistant to chemotherapy and expanded the CSCs population as defined by the markers CD24, CD44, Oct4, Sox2, and Nanog. However, DSF blocked the hypoxia induced CSC expansion. Of note, in vivo they combined Lipo-DSF [a novel liposomal formulation] with Copper and demonstrated that it suppressed the growth of MDA-MB-231 breast cancer cells. Lipo-DSF also reduced the ALDH+ CSC population in mice. Importantly, the treatment did not have any obvious adverse effects on the major vital organs based on histopathological evaluations. DSF has also been formulated into micelles where this new delivery system reduced the metastatic potential in the 4T1 model of breast cancer [41]. In both instances, they show that DSF inhibits breast cancer cells that are refractory to conventional therapies because MDA-MB-231 and 4T1 cells are reportedly resistant to chemotherapy. Thus, this introduces two examples where altering the formulation of DSF could improve drug delivery. Therefore, it is reasonable to consider that changes to the formulation of DSF could further improve its delivery to the brain.

DSF is also promising for pancreatic cancer. Again, the suggested mechanism relates to triggering the proteasome pathway leading to degradation of NFκB [42]. Likewise, DSF inhibited the growth of chronic lymphocytic leukemia cells through a similar mechanism [43]. As well, DSF had little effect on peripheral blood mononuclear cells at doses that are clinically achievable in patients [43]. A potential application of DSF is also suggested for hepatocellular carcinomas where it reportedly reduces CSCs by disrupting the p38 MAPK pathway [44]. Considered together, there is gaining momentum for CSC inhibition with DSF in a wide-range of cancers placing it in a unique category as a potential anticancer agent.
Metastases are a major problem in many types of cancers. Bone metastases are a particular problem with sarcomas. Greco et al. reported that ALDH is present in bone metastases from patients with sarcomas [45]. In a small number of cell-based models they were also able to show that DSF suppressed the growth of cells that had metastasized to the lymph nodes and lungs [45]. Consistent with this study, DSF was identified in a large screen for antimetastatic agents using a model of fibrosarcoma [46]. Given that 90% of all cancer deaths are due to metastases it is encouraging that DSF could provide some benefit in cancers that have spread.

CONCLUDING REMARKS

In oncology, kinase inhibitors such as those that block PLK1 are attractive for many reasons, but their downside can be dose-limiting toxicities. The main side effect is often neutropenia. DSF, conversely, is not commonly associated with neutropenia suggesting that its mode of action has a better safety profile. Despite the safety profile of DSF that has been put to practice for decades, researchers are attempting to establish which dosing schedule and chemotherapeutic combination will deliver the greatest response from tumor cells. The only adverse side effect reported is hepatotoxicity when DSF is prescribed at high doses. Since the exact mechanism behind the potent efficacy of DSF on tumor cells in vitro remains vague, the question of whether additional copper supplements are necessary for efficacy still needs exploration.

Treatment of malignant brain tumors offers unique challenges due to the sensitive nature of neural tissues, the BBB, and CSC subpopulations. While current standard of care regimens like TMZ are often ineffective and can be hard for patients to tolerate, there are limited options for clinicians to offer patients. DSF provides a means to deliver a multitargeting agent that kills CSCs. Other advantages of DSF include that it is inexpensive, accessible worldwide, and has potential efficacy against the chemo-resistant CSC population. DSF may offer hope for pediatric cases that are in dire need for novel treatments that reduce adverse side effects. Fighting cancer with what we already have may help pave the way for future targeted therapeutic options. CSCs are positioned as the bullseye for developing advanced cancer therapeutics in the next decade.

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