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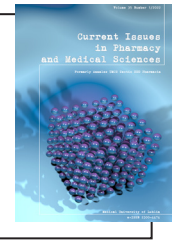

# Disulfiram and the challenges of modern medicine – new directions and possibilities

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**ABSTRACT**

Disulfiram, originally developed for the treatment of alcohol dependence, has recently gained attention for its potential applications in oncology and infectious diseases. In breast and pancreatic cancers, disulfiram forms copper complexes that inhibit tumor growth, induce oxidative stress, and enhance the efficacy of chemotherapy and radiotherapy. It targets cancer stem cells and modulates key enzymes such as aldehyde dehydrogenase (ALDH) and O6-methylguanine-DNA methyltransferase (MGMT), thereby increasing tumor sensitivity to agents such as gemcitabine and temozolomide. In glioblastoma, disulfiram also inhibits polo-like kinase 1 and proteasome activity, promoting apoptosis. In HIV therapy, disulfiram contributes to the "*shock-and-kill*" strategy by reactivating latent viral reservoirs without significant immune activation. In COVID-19, it may inhibit viral proteases (Mpro, PLpro) and reduce neutrophil extracellular trap (NET) formation, potentially mitigating disease severity. Observational studies suggest a reduced risk of infection and symptom development among disulfiram users. Beyond these areas, disulfiram has demonstrated *in vitro* activity against a broad spectrum of pathogens, including *Borrelia burgdorferi*, vancomycin-resistant bacteria, hepatitis C virus, and various parasites. It has also been explored as an adjunct in the treatment of cocaine dependence. Although most findings remain preclinical, the breadth of disulfiram's biological activity supports continued investigation into its therapeutic potential across diverse medical domains.

## INTRODUCTION

Disulfiram (tetraethylthiuram disulfide; Figure 1) was first synthesized in 1881 by the German chemist Grodzki.

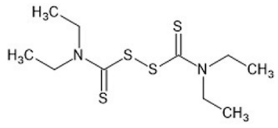

***Figure 1.*** Chemical structure of disulfiram [1]

Initially, it was used in the rubber industry as a vulcanization accelerator [1].

The first observations of its effects on alcohol tolerance were made in 1937 by E.E. Williams, who noted reduced alcohol tolerance among factory workers occupationally exposed to disulfiram, although this finding initially received little attention. In the 1940s, Danish researchers Erik Jacobsen and Jens Hald revisited disulfiram after studies investigating its antiparasitic activity in animals. The compound was shown to form copper chelates within parasitic organisms, disrupting essential biochemical processes and leading to parasite death [2,3]. During self-administration, Jacobsen experienced a pronounced intolerance to alcohol, which led to the hypothesis that disulfiram could be repurposed for the treatment of alcohol dependence and prompted further clinical investigations [4].

In the early 1950s, disulfiram began to be widely used in clinical practice as an aversive agent for the treatment of alcohol dependence [4]. In alcohol metabolism, alcohol dehydrogenase plays a central role by converting ethanol into acetaldehyde, which under normal conditions is rapidly oxidized to acetic acid. However, when disulfiram is administered, this process is disrupted because disulfiram inhibits aldehyde dehydrogenase (ALDH). Inhibition

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of ALDH results in the accumulation of acetaldehyde, leading to unpleasant physiological effects following alcohol consumption [5,6].

Although the exact incidence of adverse effects remains difficult to determine, disulfiram has been associated with a wide range of adverse reactions and variable efficacy outcomes. The therapeutic effect of disulfiram in aversion therapy is largely attributed to its psychological component, with better outcomes observed primarily among patients who adhere to the treatment regimen or who are monitored under supervised conditions. Nevertheless, scientific findings regarding its overall effectiveness remain inconsistent, contributing to ongoing debate [7,8].

In Poland, the oral formulation contains 500 mg of the active substance in divisible tablets, with typical maintenance doses ranging from 125 to 500 mg daily, most commonly 250 mg [7]. In addition, implantable disulfiram tablets administered subfascially have been used to provide sustained drug release over several months [9]. Consequently, disulfiram is currently classified as a second-line pharmacological option in the treatment of alcohol dependence and is recommended only for selected patients under appropriate clinical supervision [10].

In the present review, we focus on two major therapeutic areas: oncology and viral infection, because they represent different mechanistic contexts in which disulfiram has been most extensively investigated. Both fields are characterised by a high global disease burden and persistent limitations of current therapies, underscoring the need for innovative or repurposed treatment strategies. This conceptual framing also provides context for the later discussion of factors that may limit the clinical translation of disulfiram-based therapies.

## THE USE OF DISULFIRAM IN BREAST CANCER TREATMENT

Breast cancer remains the leading cause of cancer-related mortality among women worldwide. Although the disease predominantly affects women over the age of 50, one in five cases is diagnosed before the age of 50, according to the European Society for Medical Oncology. Moreover, the incidence of breast cancer among younger women continues to rise, and the disease course in this population tends to be more aggressive [11]. Over the past several decades, both *in vitro* and *in vivo* studies have demonstrated the potent anticancer activity of disulfiram. In addition to its strong antineoplastic properties, disulfiram has also shown radioprotective effects in healthy cells, suggesting a potentially beneficial role during radiotherapy.

A retrospective study conducted by a Danish-Czech-American research team, which analyzed nationwide demographic data and health registry records, revealed a notable correlation in female patients who received disulfiram in combination with conventional anticancer therapy. The findings indicated that among women treated with disulfiram, mortality was 34% lower compared with those who underwent standard oncological treatment alone [12]. Research conducted across multiple centers has demonstrated that this effect is associated with the chelating activity of disulfiram's metabolite, diethyldithiocarbamate acid (DDC; Figure 2A), toward copper ions. The resulting complex (Figure 2B) has been shown to function as a potent proteasome inhibitor, effectively halting cell proliferation and inducing apoptosis, thereby enhancing the efficacy of radiotherapy [1,12].

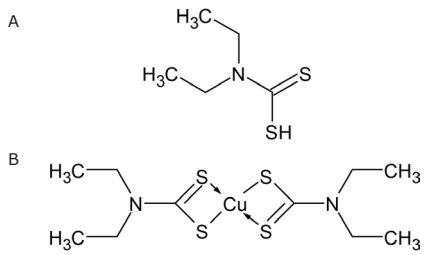


***Figure 2.*** Structures of diethyldithiocarbamate acid [A] and the copper-diethyldithiocarbamate complex [B] [according to 1,12]

During radiotherapy, patients are exposed to a wide range of adverse side effects, many of which are attributed to the generation of highly reactive free radicals. In a mouse model experiment conducted more than fifty years ago, administration of disulfiram demonstrated strong antioxidant properties of its metabolite, diethyldithiocarbamate (DDC) [13]. These properties enable the protection of deoxyribose in healthy cells, making DDC a highly promising radioprotective agent.

Efforts are ongoing to develop a therapeutic approach capable of maximizing the dose of ionizing radiation delivered directly to cancer cells while simultaneously protecting surrounding healthy tissues. Studies have shown elevated copper levels in both tumor cells and in the serum of oncology patients. Further research has confirmed that copper contributes to carcinogenesis and metastasis formation. By chelating copper ions and forming stable complexes, disulfiram prevents their utilization by cancer cells.

Previous studies have demonstrated that the disulfiram-copper complex (DSF/Cu) inhibits breast cancer cell proliferation without significant systemic toxicity. Moreover, the DSF/Cu complex exhibits a synergistic effect with radiotherapy, enhancing its overall efficacy. In breast cancer cell lines resistant to radiation, disulfiram increased the effectiveness of radiotherapy in a copper-dependent manner. While radiotherapy alone induces only low levels of immunogenic cell death, the DSF/Cu complex has been shown to significantly enhance radiation-induced immunogenic cell death [13,14].

A subsequent study conducted by researchers at the Berlin Institute of Health aimed to investigate the role of disulfiram in enhancing the sensitivity of cancer cells to cisplatin, as well as its potential synergistic effects. To evaluate this synergy, low doses of disulfiram were administered. According to later observations, apoptosis and necrosis in the human breast adenocarcinoma cell line MCF-7 increased from 45.9% to 61.6% when cisplatin was combined with disulfiram. These findings demonstrated a significant enhancement of apoptosis with the combined treatment. Cell viability in the MCF-7 line decreased by up to 50%, whereas in MDA-MB-435S (a human cancer cell line with disputed origin, historically classified as breast carcinoma) and SK-BR-3 (a human breast cancer cell line overexpressing the human epidermal growth factor receptor 2), viability declined by 20-30%. These results indicate that

disulfiram can sensitize breast cancer cells to cisplatin therapy, even at low, non-toxic doses.

Furthermore, disulfiram appears capable of overcoming cisplatin resistance in ALDH-positive cells (which express aldehyde dehydrogenase). In this study, both ALDH− and ALDH+ MCF-7 cells were exposed to varying doses of cisplatin, with or without disulfiram, for 72 hours. The experiment revealed notable differences in the survival rates of ALDH− and ALDH+ cells treated with cisplatin alone. ALDH+ cells exhibited pronounced resistance to cisplatin compared with ALDH− cells. However, upon the addition of even small amounts of disulfiram, the survival rate of previously resistant ALDH+ cells decreased by 40-50%, and the difference in cell death between ALDH− and ALDH+ populations became statistically insignificant [15].

## THERAPEUTIC PERSPECTIVES ON DISULFIRAM IN PANCREATIC CANCER

Pancreatic cancer is among the three leading causes of cancer-related deaths in European Union countries. This highly unfavorable statistic stems from several factors, including the fact that the disease often develops without specific symptoms, resulting in diagnosis at an advanced stage in most cases. Moreover, treatment effectiveness has not significantly improved over the past decade, and the five-year survival rate remains extremely low [16]. An additional challenge is the intrinsic resistance of pancreatic cancer cells to both chemotherapy and radiotherapy [17].

Therefore, it is particularly important to explore therapeutic strategies that are more effective than those currently available, or to improve existing therapeutic approaches. Numerous studies suggest that disulfiram may be a potentially effective agent in the treatment of pancreatic cancer [18]. It has been confirmed that the DSF/Cu complex induces both autophagy and apoptosis not only in breast cancer cells but also in pancreatic cancer cells under *in vitro* conditions [19]. *In vivo* studies using a mouse xenograft model of human pancreatic ductal adenocarcinoma have demonstrated that disulfiram also possesses antiproliferative properties against these cells [20]. Furthermore, disulfiram has been shown to selectively target cancer stem cells, which are responsible for chemoresistance and metastasis formation [21].

Numerous studies have investigated the efficacy of disulfiram in combination therapies. Both *in vitro* and *in vivo* experiments have demonstrated that disulfiram, when combined with a low dose of gemcitabine, effectively inhibits the growth of pancreatic ductal adenocarcinoma to a degree comparable with that achieved using a tenfold higher dose of gemcitabine alone. This approach may reduce the need for high-dose chemotherapy and lower the risk of metastasis development [22].

Disulfiram may also enhance the effectiveness of combined chemotherapy and immunotherapy. In a mouse allograft model, a therapeutic regimen comprising disulfiram, anti-PD1 (anti-programmed cell death protein 1) antibodies, and gemcitabine demonstrated potent antitumor activity, suppressing tumor growth and favorably modulating the tumor microenvironment [18]. Another promising strategy involves combining disulfiram with proto-oncogene tyrosine-protein kinase inhibitors such as PP2 (1-tert-butyl-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidin-4-amine) or dasatinib, a drug primarily used in the treatment of chronic myeloid leukemia and acute lymphoblastic leukemia. This combination has shown stronger antitumor effects than monotherapy with either agent in animal models [20].

According to Xu *et al*., disulfiram may enhance the radiosensitivity of pancreatic cancer cells. This was confirmed *in vitro* using PANC-1 (human pancreatic ductal adenocarcinoma cell line) and SW 1990 (human pancreatic adenocarcinoma cell line derived from splenic metastasis), as well as *in vivo* in a mouse model. The increased radiosensitization was attributed to disulfiram-induced progression of DNA damage, suggesting potential for combining disulfiram with radiotherapy in pancreatic cancer treatment [23]. Combined chemotherapy and radiotherapy following disulfiram administration has also proven more effective. *In vivo* studies confirmed that this treatment regimen inhibited tumor growth more efficiently than chemoradiotherapy alone, without causing additional systemic toxicity [24].

Disulfiram may also be integrated into therapeutic regimens that combine radiotherapy with CAR-T (chimeric antigen receptor T) cell immunotherapy. These T cells can be reprogrammed *in vivo* through tumor cells exposed to oxidative stress induced by DSF/Cu. The combined approach, immunotherapy, radiotherapy, and disulfiram, induces a robust and durable immunological memory response against pancreatic tumors, suggesting that this strategy may offer protection against disease recurrence [25].

To date, clinical studies have not conclusively confirmed the efficacy of disulfiram in the treatment of pancreatic cancer. In one clinical trial, the drug failed to halt tumor progression in the patient under investigation. Although a single case does not rule out potential effectiveness in the broader patient population, no positive clinical outcome was observed [26]. Other clinical trials were terminated due to limited funding or insufficient participant enrollment [27]. Based on the reviewed studies, disulfiram appears to hold promise as an adjuvant agent in pancreatic cancer therapy. It may become a valuable component of future therapeutic strategies for this malignancy; however, further research is necessary to definitively establish its clinical efficacy.

## DISULFIRAM IN GLIOMA TREATMENT: EFFICACY AND NOVEL DELIVERY APPROACHES

Glioma is the most common malignant tumor of the central nervous system and is characterized by low patient survival rates. It originates from glial or glial-precursor cells. According to the World Health Organization (WHO) classification, gliomas are graded based on malignancy: grades I and II are categorized as low-grade gliomas, whereas grades III and IV are classified as high-grade gliomas. Despite ongoing advancements in molecular cancer therapies, improving the effectiveness of glioma treatment remains challenging. This is primarily due to the requirement for therapeutic agents to cross the blood-brain barrier (BBB),

which significantly restricts the range of available treatment options [28].

Current standard therapy typically involves surgical tumor resection followed by radiotherapy and chemotherapy with temozolomide (TMZ). Nevertheless, gliomas are characterized by a high recurrence rate. Recurrent tumors often display a more aggressive phenotype due to the adaptive capabilities of glioma cells within the human brain microenvironment. These cancer cells interact with various components of the brain, including stromal cells and immune cell populations, which contributes to increased aggressiveness and resistance to standard therapies [29]. It is estimated that approximately 50% of patients are resistant to TMZ, underscoring the need for strategies that can enhance treatment efficacy [28,29].

Disulfiram has high lipid solubility, enabling the drug and its metabolites to cross the blood-brain barrier (BBB). It has been shown to exert multiple anticancer effects, ranging from inhibition of cell division and tumor growth to the induction of malignant cell death [3,28]. Glioma stem cells exhibit elevated expression of ALDH, an enzyme that protects DNA from genotoxic injury and contributes to treatment resistance. Consequently, ALDH inhibition increases tumor sensitivity to therapy by disabling a key protective mechanism in cancer cells. Importantly, disulfiram does not inhibit fibroblast stem cells or neuronal cells [28].

The effects of disulfiram on enzymes directly involved in the glioma cell cycle have also been investigated. Disulfiram has been shown to reduce the expression of pololike kinase 1 (PLK1), a key regulator of cell cycle progression, thereby disrupting mitosis in cancer cells [29]. It also inhibits the activity of O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein responsible for glioma cell resistance to temozolomide (TMZ). Both *in vitro* and *in vivo* studies have demonstrated that disulfiram induces the loss of MGMT protein in human glioblastoma cells, rendering them sensitive to TMZ treatment and leading to cell cycle arrest and apoptosis [28,30].

Disulfiram also exhibits anticancer activity against glioma in the form of a copper complex-copper diethyldithiocarbamate ($Cu(DDC)_2$), which further expands its therapeutic potential. Cancer cells demonstrate an increased demand for copper due to their high proliferation rate, angiogenic activity, and metastatic capacity. As a result, they express elevated levels of copper transporter 1 (CTR1) on their surface. Administration of disulfiram together with copper facilitates drug delivery into tumor cells via CTR1 transporters located in the cell membrane [31].

The resulting $Cu(DDC)_2$ chelate complexes undergo redox reactions, including Fenton-type reactions, generating reactive oxygen species that induce oxidative stress and promote glioma cell apoptosis. Simultaneously, disulfiram inhibits superoxide dismutase and competes with glutathione reductase, weakening the cells' natural defense mechanisms against reactive oxygen species. In response to oxidative stress, tumor cells activate nuclear factor κB (NF-κB), which may promote tumor growth. However, disulfiram blocks NF-κB activity, thereby reducing tumor volume and limiting invasion into healthy tissue [28].

The DSF/Cu complex also inhibits proteasome activity, resulting in the accumulation of misfolded, polyubiquitinated proteins within cancer cells. Studies have shown that even low concentrations of disulfiram can bind to the 20S proteasome subunit, effectively blocking its function. Due to their proliferative nature, cancer cells are particularly sensitive to proteasome inhibition, which can trigger apoptosis in glioma cells [28,31]. Importantly, DSF/Cu formulations have demonstrated minimal or no cytotoxicity toward healthy cells [31].

Despite promising *in vitro* and *in vivo* results, a randomized clinical trial conducted in 2022 by Swedish and Norwegian research centers found that disulfiram did not improve survival outcomes in patients with recurrent glioblastoma multiforme. This multicenter, open-label, phase II/III randomized trial used a parallel-group design and included one cohort receiving standard-of-care alkylating chemotherapy (SOC; 45 patients) and another cohort treated with SOC combined with disulfiram and copper (43 patients).

The trial assessed two endpoints: six-month survival and overall survival from the time of randomization. At the six-month survival endpoint, results were comparable: 62% of patients (26 of 42) in the SOC group reached six-month survival, compared with 44% (19 of 43) in the SOC + disulfiram/copper group. More pronounced differences were observed for overall survival: median survival in the SOC group was 246 days (95% CI: 163-307 days), whereas median survival in the SOC + disulfiram/copper group was 164 days (95% CI: 117-278 days). These findings indicate that combination therapy with disulfiram and copper does not improve prognosis in recurrent glioblastoma multiforme and is associated with significantly higher toxicity compared with standard treatment [32]

Oral administration remains the standard route for disulfiram, with a maximum daily dose of 500 mg. An alternative method is intranasal delivery, which bypasses the blood-brain barrier (BBB). *In vitro* studies have shown that encapsulating disulfiram in hydroxypropylβcyclodextrin (HP-β-CD) increases its solubility more than 2,000-fold, enabling safe intranasal application. In animal models of glioma, intranasal administration of the DSF/HP-β-CD/Cu complex improved survival outcomes. Additionally, intravenous delivery of $Cu(DDC)_2$ in liposomal formulations has been explored in mice [28].

Nanoparticle-based delivery using a biodegradable mPEG-PLGA (methoxy poly(ethylene glycol)-block-poly(d,l-lactide-co-glycolide)) matrix further enhances disulfiram's therapeutic potential by prolonging its half-life and release time. *In vitro* studies on glioblastoma (T98G) and medulloblastoma (DAOY) cell lines showed that nanoparticle uptake was significantly higher than that of the free drug. *In vivo* experiments confirmed that these nanoparticles can cross the BBB, as evidenced by fluorescent dye tracking within brain tissue up to 24 hours post-administration. Disulfiram nanoparticles also demonstrated enhanced induction of oxidative stress in tumor cells, as measured by standard reactive oxygen species markers. The nanoparticle formulation generated greater levels of reactive oxygen species than the free drug in both T98G and DAOY cells, suggesting improved cytotoxicity at lower concentrations [33].

## DISULFIRAM IN THE CONTEXT OF HIV INFECTION THERAPY

Human immunodeficiency virus (HIV) is a positive-sense single-stranded RNA virus ((+)ssRNA) of the *Retroviridae* family that targets host CD4+ T cells (helper T lymphocytes), leading to their progressive dysfunction and ultimately to immunodeficiency. The development of antiretroviral therapy (ART) has significantly improved survival among individuals living with HIV [34]. However, complete eradication of the virus remains unattainable due to the persistence of latent viral reservoirs. To address this challenge, the "*shock-and-kill*" strategy has been proposed, aiming to reactivate latent HIV and subsequently eliminate infected cells through immune-mediated mechanisms [35].

Disulfiram is considered a potential agent capable of reactivating latent HIV within host CD4+ cells, thereby offering an opportunity to enhance the effectiveness of HIV treatment [36]. The mechanism involves promoting HIV transcription through activation of the classical NF-κB signaling pathway and downregulation of PTEN (phosphatase and tensin homolog). PTEN deficiency activates the PI3K/Akt signaling pathway, which in turn stimulates NF-κB transcription factors [37]. This mechanism has been demonstrated in the U1 cell line and in resting CD4+ T cells isolated from HIV-negative individuals [38].

Further studies using primary CD4+ T cells genetically modified to express the *BCL2* gene, known to promote cell survival, showed that disulfiram can reactivate latent HIV without significantly activating T cells. This lack of broad T-cell activation, a common limitation of many other latency-reversing agents, suggests that disulfiram may offer a favorable safety profile [36].

Pilot studies have shown that in HIV-positive individuals undergoing ART, administration of 500 mg of disulfiram daily for 14 days leads to reactivation of latent HIV reservoirs. However, the reservoir size did not change significantly, with only a 1.16-fold increase compared with baseline levels, and the small sample size and short duration limit the generalizability of these findings [39]. Given its established safety profile in humans, disulfiram remains a promising candidate for further dose-escalation studies aimed at enhancing latency-reversal efficacy.

A study conducted between 2013 and 2014 further investigated the latency-reversing potential of disulfiram in HIV-infected individuals. Administration of disulfiram at doses of 500 mg, 1000 mg, and 2000 mg resulted in a measurable increase in the activation of latent HIV reservoirs. Specifically, the estimated rise in cell-associated unspliced HIV RNA ranged from 1.6- to 1.9-fold, depending on the dose administered. These findings reinforce the notion that disulfiram can effectively induce viral transcription from latent reservoirs. Notably, the treatment was well tolerated across all dosage levels, with no adverse events reported, underscoring its favorable safety profile. This study contributes to the growing body of evidence supporting disulfiram as a viable component of combination latencyreversing therapy [40].

In the context of HIV eradication strategies, disulfiram requires further optimization with respect to dosing and treatment duration. It is increasingly recognized that monotherapy may be insufficient, and that effective latency reversal likely necessitates coadministration with other latency-reversing agents (LRAs). A Phase I clinical trial involving patients on ART who received both vorinostat, a class I/II histone deacetylase (HDAC) inhibitor, and disulfiram was prematurely terminated due to the emergence of Grade 3 neurological toxicity in two participants. Reported symptoms included confusion, emotional lability, lethargy, ataxia, and paranoia. Although partial latency reversal was observed, the adverse effects led to the conclusion that vorinostat is unsuitable for combination therapy with disulfiram [41].

Romidepsin, a class I-selective HDAC inhibitor, has demonstrated superior efficacy in inducing HIV expression compared with vorinostat [42]. However, despite its potency, the combination of romidepsin and disulfiram failed to produce synergistic effects in both *in vitro* and *ex vivo* models [43].

Given its well-characterized safety profile and broad clinical availability, disulfiram remains a promising candidate for future implementation in "*shock-and-kill*" strategies. Its potential integration with immunotherapeutic agents offers a compelling avenue toward the complete eradication of HIV infection, although further investigation is required to validate this therapeutic approach.

## DISULFIRAM AS A POTENTIAL TREATMENT FOR COVID-19

COVID-19 is an infectious respiratory disease caused by the SARS-CoV-2 virus. The clinical spectrum ranges from asymptomatic infection to severe illness, including the development of acute respiratory distress syndrome, which is associated with high mortality. The rapid escalation of cases in 2020 prompted an urgent search for effective therapeutic interventions. In addition to the development of novel agents, substantial attention was directed toward repurposing existing drugs with well-established safety profiles, enabling accelerated clinical evaluation and deployment [44]. Among the repurposed agents investigated, disulfiram emerged as a potential candidate due to its known pharmacological properties and favorable tolerability. Its proposed mechanism of action in the context of coronavirus infection involves the inhibition of neutrophil extracellular trap (NET) formation [45].

Disulfiram acts as a cysteine-reactive compound, targeting zinc-coordinated cysteine residues within the catalytic domains of viral proteases. It has been shown to inhibit papain-like protease (PLpro) and the main protease (Mpro), both of which are essential for SARS-CoV-2 replication. These proteases cleave the viral polyprotein to release RNA-dependent RNA polymerase (RdRp), a key enzyme required for viral genome transcription and replication. Molecular studies suggest that disulfiram suppresses the enzymatic activity of PLpro and Mpro, thereby impairing viral RNA synthesis and replication [45].

NETs are web-like extracellular DNA structures formed by neutrophils in response to infection. They function to trap and neutralize pathogens of considerable size or abundance. However, their surfaces are coated with cytotoxic proteins, including proteases and histones, which can damage host

tissues. In severe COVID-19, excessive NET formation has been observed, contributing to perivascular fibrosis in the lungs, suppression of innate immunity, and activation of the complement cascade. Together, these processes lead to extensive pulmonary injury, thrombosis, and cardiovascular complications [46,47].

The search for agents capable of reducing NET formation has revealed a mechanistic link between disulfiram and inhibition of gasdermin D, a key effector of pyroptosis. Gasdermin D forms pores in nuclear and cytoplasmic membranes, facilitating the release of NETs. *In vivo* studies using murine models have demonstrated that disulfiram inhibits gasdermin D activity in macrophages and reduces NET formation in golden Syrian hamsters [48].

Additionally, disulfiram has shown potential to inhibit SARS-CoV-2 replication by preventing cleavage of the viral polyprotein. Molecular studies indicate that disulfiram may impair or inhibit the interaction between the viral spike protein and the ACE2 (angiotensin-converting enzyme 2) receptor, thereby interfering with viral entry into host cells [45].

A retrospective study conducted during the first wave of the COVID-19 pandemic in Italy, involving 1,297 patients, observed a reduced risk of developing symptomatic disease, particularly fever and dyspnea, among individuals with alcohol use disorder who were receiving disulfiram. Although no significant difference in infection rates was found, the results suggest that disulfiram may attenuate the clinical severity of COVID-19 [44].

In another retrospective cohort study involving U.S. veterans, disulfiram use was associated with a 34% reduction in the risk of SARS-CoV-2 infection. The study cohort consisted of 944,127 veterans, of whom 2,233 had received at least one prescription for disulfiram between February 20, 2019, and February 1, 2021. Among these individuals, 100,873 had a documented diagnosis of alcohol use disorder. Notably, no COVID-19-related deaths were reported among the 188 patients with confirmed infection who were receiving disulfiram, whereas an estimated 5-6 deaths would typically be expected in a comparable untreated cohort [49].

Two clinical trials investigating the effects of disulfiram in COVID-19 have been registered on ClinicalTrials.gov. The first trial (DISCO) evaluates the impact of a 5-day course of disulfiram on symptom severity and inflammatory biomarkers [50]. The second trial involves hospitalized patients with moderate COVID-19 and assesses whether disulfiram can reduce inflammatory responses and prevent progression to severe disease. Participants received either oral disulfiram or placebo for 14 days, with follow-up on day 28 post-treatment [51]. As of now, results from these trials have not been published

While *in vitro* studies have yielded promising results [45], animal experiments have been limited by small sample sizes [48], and clinical trial data remain unavailable. An additional challenge is posed by emerging SARS-CoV-2 variants, which include mutations in viral proteins such as the main protease (Mpro) and papainlike protease (PLpro), both key molecular targets of disulfiram. Mutations in these proteases may alter the structural configuration of their active sites, potentially affecting the binding affinity and inhibitory efficacy of disulfiram. Despite these limitations, disulfiram remains an attractive candidate for drug repurposing in COVID-19 therapy due to its antiviral activity, particularly through inhibition of NET formation. However, further research is required to confirm its clinical efficacy, especially in the context of variant-specific viral mechanisms and across broader patient populations.

## OTHER POTENTIAL APPLICATIONS OF DISULFIRAM

In addition to its investigated roles in oncology and virology, disulfiram has attracted growing scientific interest for its potential activity in a range of other therapeutic areas. Most of these applications remain in the preclinical or early exploratory clinical stages, yet they reflect the compound's broad biological activity and pharmacological versatility. *In vitro* studies have demonstrated bactericidal effects against *Borrelia burgdorferi*, the causative agent of Lyme disease. The proposed mechanism involves disruption of metal ion metabolism, particularly zinc and manganese, which are essential for bacterial survival. Despite encouraging laboratory findings, clinical evidence is currently limited to a single small randomized trial and two case series, with reports of serious adverse events [52,53].

Disulfiram has also been investigated for its activity against the hepatitis C virus (HCV) [54] and for its antibacterial effects, particularly against vancomycin-resistant strains of *Staphylococcus aureus* and *Enterococcus* spp. [3,54-57]. Furthermore, preclinical data suggest that disulfiram has antiparasitic properties, with observed activity against *Plasmodium*, *Leishmania* and *Giardia* spp. When used in combination with benzyl benzoate, it has also been shown to be effective against *Sarcoptes scabiei* and *Pediculus humanus capitis* [3,58-61]. Disulfiram has also been explored as a potential adjunct in the treatment of cocaine dependence [62].

## TRANSLATIONAL CHALLENGES

Despite the anticancer activity observed in preclinical models, the clinical performance of disulfiram-based therapies has been inconsistent, suggesting the presence of several barriers to translation.A key issue is the substantial difference between experimental pharmacokinetics and human physiology. Disulfiram is rapidly metabolised *in vivo* and the efficiency of copper-dependent complex formation and activity is therefore poorly understood in clinical settings [31,63]. In many preclinical studies, copper is either exogenously supplemented or readily available, whereas in humans, copper homeostasis is tightly regulated, potentially limiting efficient complex formation at the tumor site [19,64].

Another limitation relates to the physicochemical properties of $Cu(DDC)_2$ itself; its extremely poor solubility complicates direct *in vivo* administration. Although several nanoformulation strategies, including liposomal systems, have demonstrated promising characteristics in animal studies, their translational relevance has yet to be validated in humans [65,66].

Moreover, the tumor microenvironment of highly aggressive malignancies, such as recurrent glioblastoma or pancreatic adenocarcinoma, introduces additional barriers that are not adequately reproduced in standard *in vitro* or *in vivo* models. Hypoxia, abnormal vascular permeability, and, in the case of glioblastoma, the presence of the BBB may substantially limit drug penetration and attenuate therapeutic efficacy despite encouraging preclinical results [67,68]. Clinical outcomes are further influenced by the fact that many trials have enrolled heavily pretreated or late-stage patients, where therapeutic windows are inherently narrow and toxicity more pronounced [27,32]. Several studies have also been constrained by small sample sizes and heterogeneous patient populations, reducing the statistical power needed to detect meaningful therapeutic effects and complicating interpretation of clinical efficacy signals [26].

Collectively, these factors help explain why the strong anticancer potential observed in controlled experimental systems has not translated into clear clinical benefit, and they suggest that optimized delivery strategies, improved copper availability, and earlier-stage intervention may be required to fully assess the therapeutic capacity of disulfiram.

## CONCLUSIONS

Disulfiram, originally known for its use in the treatment of alcohol dependence, has recently gained attention as a potential anticancer and antiviral agent. By forming complexes with copper, it interferes with carcinogenic processes in breast, pancreatic, and glioblastoma tumors, inducing oxidative stress and apoptosis in cancer cells. Through inhibition of ALDH, disulfiram sensitizes tumor cells to therapy, while its ability to block MGMT enhances the efficacy of temozolomide. In HIV treatment, disulfiram fits into the "*shock-and-kill*" strategy by reactivating latent viral reservoirs without significant T-cell activation. In the context of COVID-19, it inhibits viral proteases and reduces the formation of NETs, potentially mitigating disease severity. Disulfiram also exhibits activity against other pathogens, including bacteria, viruses, and parasites, and shows promise as an adjunct in the treatment of substance use disorders. Despite its broad biological activity, most studies remain limited to *in vitro* and *in vivo* models, and clinical data are still scarce.

Drug repositioning, while offering a promising shortcut to new therapeutic indications, is not without challenges. As a compound no longer under patent protection, disulfiram is inexpensive and widely accessible, which facilitates academic research. However, its low commercial value reduces interest from the pharmaceutical industry. The high cost of clinical trials, limited sponsor availability, and regulatory hurdles associated with registering new indications mean that the path from encouraging laboratory findings to clinical application can be long and uncertain. Early scientific enthusiasm does not guarantee therapeutic success. Rigorous validation in well-designed clinical studies is still essential.

## List of abbreviations

ACE2 – angiotensin-converting enzyme 2
ART – antiretroviral therapy
ALDH – aldehyde dehydrogenase
anti-PD1 – anti-programmed cell death protein 1 antibody
BCL2 – antiapoptotic B-cell lymphoma 2 gene
CAR-T – chimeric antigen receptor T cells
CTR1 – copper transporter 1
$Cu(DDC)_2$ – copper diethyldithiocarbamate
DAOY – medulloblastoma cell line
DDC – diethyldithiocarbamate
DSF/Cu – disulfiram-copper complex
DSF/HP-β-CD/Cu – disulfiram-hydroxypropyl-β-cyclodextrin-copper complex
HDAC – histone deacetylase
HIV – human immunodeficiency virus
HP-β-CD – hydroxypropyl-β-cyclodextrin
LRAs – latency-reversing agents
MCF-7 – human breast adenocarcinoma cell line
MDA-MB-435S – human cancer cell line originally classified as breast cancer
MGMT – O6-methylguanine-DNA methyltransferase
mPEG-PLGA – methoxy poly(ethylene glycol)-block-poly(d,l-lactide-co-glycolide)
Mpro – main protease
NET – neutrophil extracellular trap
NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells
PANC-1 – human pancreatic ductal adenocarcinoma cell line
PI3K/Akt – phosphoinositide 3-kinase/protein kinase B
PLK1 – polo-like kinase 1
PLpro – papain-like protease
PP2 – 1-tert-butyl-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine, a selective inhibitor of the proto-oncogene tyrosine-protein kinase Src
PTEN – phosphatase and tensin homolog
RdRp – RNA-dependent RNA polymerase
SK-BR-3 – human breast cancer cell line overexpressing the human epidermal growth factor receptor 2
SOC – standard-of-care alkylating chemotherapy
(+)ssRNA – positive-sense single-stranded RNA
SW 1990 – human pancreatic adenocarcinoma cell line derived from splenic metastasis
T98G – human glioblastoma multiforme cell line
TMZ – temozolomidee

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