DISulfiram for COvid-19 (DISCO) Trial:

A Phase 2 Double-Blind, Randomized Placebo-Controlled Trial of Disulfiram Compared to Standard Care in Patients with Symptomatic COVID-19

Clinical Protocol Co-Chairs: Sulggi Lee, Rada Savic

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DISulfiram for COvid-19 (DISCO) Trial

SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements and institutional policies.

Principal Investigator: _____

Print/Type

Signed: _____ Date: _____

Name/Title

Contents

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2 LIST OF ABBREVIATIONS

ADR: Adverse Drug Reaction
ADE: Antibody-mediated enhancement of infection
AE: Adverse Event/Adverse Experience
CBC: Complete Blood Count
CCP: COVID-19 Convalescent Plasm
CDC: United States Centers for Disease Control and Prevention
CFR: Code of Federal Regulations
CLIA: Clinical Laboratory Improvement Amendment of 1988
COI: Conflict of Interest
COVID-19: Coronavirus Disease
CRF: Case Report Form
CRP: C-Reactive Protein
DMC: Data Management Center
DSF: Disulfiram
DSMB: Data and Safety Monitoring Board
EUA: Emergency Use Authorization
FDA: Food and Drug Administration
GCP: Good Clinical Practice
ICF: Informed Consent (Informed Consent Form)
ICH: International Conference on Harmonization
ICU: Intensive Care Unit
IEC: Independent Ethics Committee
IND: Investigational New Drug Application
IRB: Institutional Review Board
LOS: Length of Stay
MERS: Middle East Respiratory Syndrome
NP: Nasopharyngeal
OHRP: Office of Human Research Protections
RT-PCR: Reverse Transcriptase Real-Time Polymerase Chain Reaction
PK: Pharmacokinetic
QC: Quality Control
RDV: Remdesivir
SAE: Serious Adverse Event
SARS: Severe Acute Respiratory Syndrome
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2
SUSAR: Serious and Unexpected Suspected Adverse Reaction
TACO: Transfusion-associated Circulatory Overload
UAE: Unexpected Adverse Event
UP: Unanticipated Problem

TITLE	DISulfiram for COvid-19 treatment (DISCO) Trial
SPONSOR	University of California, San Francisco
PRINCIPAL INVESTIGATOR(S)	Sulggi Lee, MD PhD Rada Savic, PhD
FUNDING ORGANIZATION	Sulggi Lee, M.D. UCSF Departmental Discretionary Funds Mercatur International Fast Grant
NUMBER OF SITES	One (UCSF Fresno)
RATIONALE	The identification of a safe, effective treatment for individuals with early symptomatic COVID-19 that prevents progression to more severe disease would have immediate public health implications. A hallmark of severe COVID-19 disease is immune system dysregulation called cytokine storm. Multiple studies have reported that patients with severe disease demonstrate elevated levels of pro-inflammatory cytokines early in disease, and elevated IL-6 plasma concentrations are predictive of poor clinical outcomes in COVID-19. Several anti-inflammatory trials are now underway in hospitalized patients to determine whether blockade of these cytokines reduce COVID-19-associated mortality, including planned trials to inhibit IL-6/IL-1β signaling (e.g., NCT04320615, NCT04362813). However, even earlier treatment of symptomatic COVID-19 in patients might further reduce COVID-19 morbidity, such as in preventing hospitalizations and ICU admissions. Disulfiram, an FDA-approved drug for the treatment of alcohol dependence disorder is an appealing therapeutic option for COVID-19. It has a good safety profile, easy dosing schedule, and recent data suggesting multiple mechanisms by which disulfiram may act on COVID-19 (both as a direct antiviral agent as well as indirect effects on reducing inflammation). In addition, disulfiram has been studied extensively with detailed available pharmacokinetic data; disulfiram has a short half-life

	~7.5 hours with >90% of drug eliminated within 3 days post-dose, allowing quick reversal of any potential adverse effects.
STUDY DESIGN	This is a double-blind, placebo-controlled trial on a backbone of standard care of disulfiram in patients with early symptomatic COVID-19. Disulfiram 1000 mg (cohort 1) or 2000 mg (cohort) versus placebo will be given once daily for 5 consecutive days. A total of 60 participants will be randomized (2:1) to receive active drug versus placebo in a dose escalation study. The first 30 participants will first be enrolled in the 1000 mg dosing cohort, randomized 2:1 to receive either disulfiram 1000 mg (N=20) or placebo (N=10) daily for 5 consecutive days. As long as safety monitoring criteria are met to proceed to the second dose, the next 30 participants will be enrolled in the 2000 mg dosing cohort, randomized 2:1 to receive either disulfiram 2000 mg (N=20) or placebo (N=10) daily for 5 consecutive days. Drug/placebo will be administered using strict infection control protocols designed to support the study of people with acute COVID-19 infection per the Center for Diseases Control (CDC) guidelines (https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-in-home-patients.html).
PRIMARY OBJECTIVE	To determine the effect of oral disulfiram given as 1000 mg daily (cohort 1) or 2000 mg daily (cohort 2) versus placebo for 5 consecutive days in adults with early symptomatic SARS-CoV-2 infection on: Change in inflammatory biomarkers (plasma cytokine levels – e.g., IL-6, IL-1β) on days 5, 15, and 31.[2]
SECONDARY OBJECTIVES	To determine the effect of oral disulfiram (given as 1000 mg daily (cohort 1) or 2000 mg daily (cohort 2) versus placebo for 5 consecutive days in adults with early symptomatic SARS-CoV-2 infection on: • Safety and tolerability (Grade 2 or higher adverse events on days 5, 15, and 31). • Change in COVID symptom severity (five-point symptom scale[3]) on days 5, 15, and 31. • Change in SARS-CoV-2 PCR quantitative viral load[4] on days 5, 15, and 31.

	• The proportion of detectable versus not detectable levels of SARS-CoV-2 RNA from site-collected nasopharyngeal (NP) swabs at entry, day 5, and day 15 among a subset of participants. • Time to recovery (date of symptom onset to date of complete resolution of symptoms) • If applicable, the proportion of participants hospitalized at day 5, 15, and 31. • If applicable, the proportion of participant deaths at day 5, 15, and 31.
NUMBER OF PARTICIPANTS	Total: N=60 patients ≥ age 18 with confirmed COVID-19 infection diagnosed by molecular diagnostic assay • Cohort 1: N=20 disulfiram 1000 mg, N=10 placebo • Cohort 2: N=20 disulfiram 2000 mg, N=10 placebo
PARTICIPANT SELECTION **CRITERIA**	<u>Inclusion Criteria</u>: • Willing and able to provide written informed consent, and • Age ≥ 18 years, and • Recent symptomatic COVID-19 disease (<14 days from symptom onset) • Recent SARS-CoV-2 positive test by molecular diagnostic assay within the preceding 7 days, and • Not currently hospitalized, and • Willing to abstain from any alcohol during the two-week period in which disulfiram will be administered and during the two-week period immediately after disulfiram administration. • Both male and female participants are eligible. Females of childbearing potential must have a negative pregnancy test at screening and agree to use a double-barrier method of contraception throughout the study period.

	Exclusion Criteria: • Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study • Active malignancy requiring systemic chemotherapy or surgery in the preceding 3 months or for whom such therapies are expected in the subsequent 6 months • Decompensated liver disease as defined by the presence of ascites, encephalopathy, esophageal or gastric varices, or persistent jaundice • Serious illness requiring systemic treatment and/or hospitalization in the 3 months prior to study enrollment • Concurrent treatment with immunomodulatory drugs, and/or exposure to any immunomodulatory drug in the 4 weeks prior to study enrollment (e.g. corticosteroid therapy equal to or exceeding a dose of 15 mg/day of prednisone for more than 10 days, IL-2, interferon-alpha, methotrexate, cancer chemotherapy). NOTE: use of inhaled or nasal steroid or unless if dexamethasone administered as part of inpatient COVID-19 standard of care treatment is not exclusionary. • Serious medical or psychiatric illness that, in the opinion of the site investigator, would interfere with the ability to adhere to study requirements or to give informed consent. • Current alcohol use disorder or hazardous alcohol use (>7 drinks per week for women or > 14 drinks per week for men) as determined by clinical evaluation. • Current use of any drug formulation that contains alcohol or that might contain alcohol. • Current use of oral lopinavir/ritonavir solution (and the inability to switch to a solid dosage form during the entire duration of the study). • Current use of warfarin. • ALT or AST > 2 x the upper limit of normal or total bilirubin outside the normal range.

	• Creatinine clearance (CrCl) < 50 mL/min by Cockcroft-Gault. • Allergy to rubber or thiuram derivatives
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Oral disulfiram given as 1000 mg daily (cohort 1) or 2000 mg daily (cohort 2) versus placebo for 5 consecutive days in adults with early symptomatic SARS-CoV-2 infection will be administered in a double-blind fashion. Placebo tablets will be administered in a double-blind fashion and given as a single oral administration on days 1-5.
DURATION OF PARTICIPATION AND DURATION OF STUDY	Study products will be administered at days 1-5. Participants will be observed on study at days 5, 15, and 31. Participants will be randomized to either of two treatment arms, oral disulfiram or placebo. **Screening**: up to 7 days prior to treatment randomization. **Treatment** (for each arm): 5 days (participants to be observed for a total of 4 hours on the last treatment day, Day 5) **Follow-up** (for each arm): 31 days after the initial dose for that treatment arm. The total duration of the study is expected to be 31 days (approximately 1 month).
CONCOMITANT MEDICATIONS	The following medications are not allowed during the study because of potential interactions with disulfiram: warfarin; any over the counter drug, food or other product that contains or might contain alcohol; if HIV-positive, specific protease inhibitor preparations (oral amprenavir solution, ritonavir solution, lopinavir/ritonavir solution, soft gelatin form of ritonavir or lopinavir/ritonavir, tipranavir).
EFFICACY EVALUATIONS	• Change in plasma inflammatory biomarker levels[2] • Change in SARS-CoV-2 viral load[4] • Change in COVID-19 symptom severity[3] (5-point scale)
PRIMARY ENDPOINTS	• Change in plasma inflammatory biomarker levels[2] (e.g., IL-6, IL-1β) at days 5, 15, and 31.

SECONDARY ENDPOINTS	• Safety and tolerability • Change in SARS-CoV-2 PCR quantitative viral load[4] at days 5, 15, and 31. • Change in COVID-19 clinical stage (five-point symptom severity scale[3]) at days 5, 15, and 31. • Time to recovery (date of symptom onset to date of complete resolution of symptoms) • If applicable, the proportion of participants hospitalized at day 5, 15, and 31. • If applicable, the proportion of participant deaths at day 5, 15, and 31
OTHER EVALUATIONS	• SARS-CoV-2 specific T- and B- cell responses[5] • Pyroptosis (aberrant proinflammatory cell death) assay[6] • High sensitivity SARS-CoV-2 antibody titer (IgM, IgE, IgA, IgG1, IgG2, IgG3, IgG4) • Changes in host gene and protein expression from matched blood and respiratory (NP) samples at the single cell level (B, T, natural killer, and myeloid cells). • Changes in T- and B-cell receptor (TCR/BCR) repertoire from matched blood and respiratory (nasal/oropharyngeal) samples.
SAFETY EVALUATIONS	All participants will be followed for possible adverse events (AEs) throughout their involvement in the study.
PLANNED INTERIM ANALYSES	A Safety Monitoring Committee (SMC) will be convened and will meet biannually to review all adverse events and the conduct of the study. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study. All main analyses will be conducted on an intention-to-treat basis. Models will assess the relationship between the timing of disulfiram treatment initiation relative to symptom onset and study outcomes for key efficacy/activity endpoints. Stratified analyses will be performed based on symptom severity, as well as laboratory values (e.g., hs-CRP, ferritin, D-dimer levels at baseline).

4.1 Background and scientific rationale

The coronavirus-2019 (COVID-19) pandemic has caused devastating morbidity and mortality worldwide.[7-12] But the virus itself does not seem to explain the person-to-person variability in clinical course (ranging from asymptomatic carriage to severe lethal respiratory disease, as well as long-term inflammatory sequelae).[13-15] Variation in host genetics and host-specific immune responses likely play a major role in determining the severity of COVID-19.[13-15] Patients who progress to severe acute respiratory distress syndrome, for example, demonstrate increased expression of interleukin (IL)-6 and IL-1. [16-18] Several anti-inflammatory trials are now underway in hospitalized patients, to determine whether blockade of these pathways reduces COVID-19 associated mortality,[18,19] including planned trials to inhibit IL-6/IL-1b signaling (NCT04320615, NCT04362813, NCT04346355, NCT04356937, NCT04341675).[16] Yet early treatment of mild-to-moderate symptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is likely to play an even greater role in public health efforts to control COVID-19 since over 80% of COVID-19 cases can be asymptomatic.[20] This suggests that early treatment may potentially prevent hospitalizations/ICU admissions by reducing community viral transmission.

Disulfiram is an FDA-approved drug and has been in clinical use for over 60 years. Recent data suggests that it may have both direct antiviral, as well as indirect (anti-inflammatory), effects on SARS-CoV-2 infection (**Figure 1**).[21-24] Disulfiram directly inhibits M^{pro}, the protease that processes the 2 main proteins, pp1a and pp1b, required for SARS-CoV-2 viral replication and transcription.[23] Disulfiram can also act as a zinc ejector to inhibit viral replication.[22] Disulfiram may indirectly reduce inflammation via inhibition of the NF-κB pathway (upstream of IL-1β/IL-6 signaling),[25-27] and recent data demonstrates that disulfiram inhibits gasdermin D pore formation (the final common step of NLRP3 inflammasome-induced pyroptosis and cytokine release).[24]

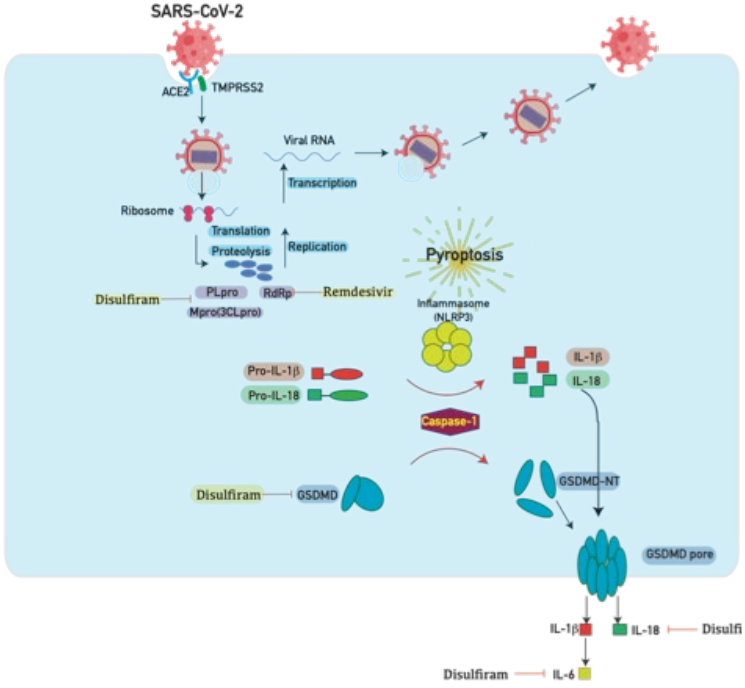

Figure 1. Potential **direct antiviral** (inhibiting M^{pro} protease or zinc moiety to inhibit viral replication) and **indirect anti-inflammatory** (inhibition of NF-Kβ or NLRP3 inflammasome) effects of disulfiram on SARS-CoV-2 infection.

Given its relative safety, easy dosing schedule and putative antiviral and anti-inflammatory effects associated with SARS-CoV-2 infection, disulfiram is an appealing therapeutic option for COVID-19 treatment. Disulfiram is FDA approved for the treatment of alcohol dependence disorder (NCT01286259).[28] More recently, disulfiram has been shown to be safe when administered in the setting of HIV cure (NCT01944371) [29] and cancer trials (NCT02715609, NCT02671890, NCT03323346, NCT00256230, NCT02678975).[30,31]Disulfiram is an oral daily administered drug. Disulfiram given at doses as high as 2000 mg has recently been shown to be safe

and well tolerated in HIV-infected individuals.[29] Additionally, disulfiram has a short half-life (~7.5 hours) with over 90% of the drug eliminated within 3 days post dose.[32,33,34] Now several drug screens have identified disulfiram as an effective antiviral against SARS-CoV and SARS-CoV-2.[22,23]

4.1.1 SARS-CoV-2-associated immune dysregulation

Leveraging safe and readily available FDA-approved drugs that can potentially block inflammation and have antiviral activity against SARS-CoV-2 may help provide additional rationale and safety data for blocking inflammation, especially among mild-to-moderately symptomatic COVID-19 patients in whom early treatment is most likely to confer overall public health benefit. Unlike the first severe acute respiratory syndrome (SARS-CoV) virus, SARS-CoV-2, largely manifests as asymptomatic disease (in over 50-80% of infected individuals tested).[20,35-37] However, the virus itself does not seem to explain the person-to-person variability in clinical course, ranging from asymptomatic carriage to severe lethal respiratory and/or cardiac disease.[13-15] Higher levels of inflammation – e.g., plasma IL-6 levels, predict disease severity.[1,16-18] Uncontrolled clinical trials suggest that blockade of IL-6 may improve COVID-19 clinical outcomes,[38-40] but controlled trial results are still pending.

Hypercytokinemia is regarded as a hallmark of COVID-19. High levels of cytokines including TNF-α, IL-1β, IL-1Ra, sIL- 2Rα, IL-6, IL-10, IL-17, IL-18, IFN-γ, MCP-3, M-CSF, MIP-1a, G-CSF, IP- 10 and MCP-1 have been reported in patients with severe disease.[41,42] Observational studies of COVID-19 patients demonstrate increased levels of acute phase reactants during infection, e.g., elevated erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and ferritin levels, (these markers all have readily available clinical assays) that may represent underlying elevated cytokine levels.[41-43] Initial increases in cytokine levels can subsequently lead to "cytokine storm," excessive cytokine release which can lead to multiple organ failure resulting in rapid deterioration and death, as observed in severe COVID-19 patients.[44,45]

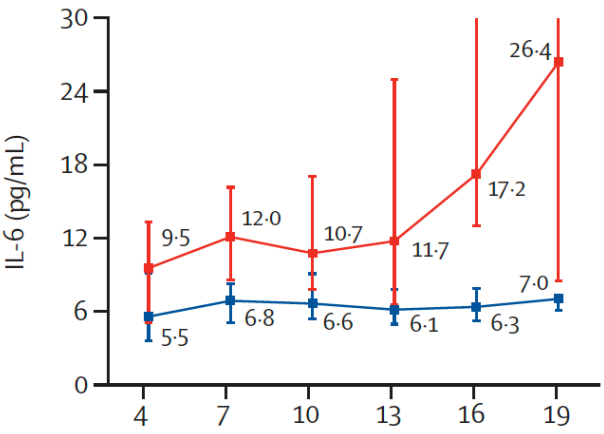

Figure 2. Differences in plasma IL-6 levels between survivors (N=137) and non-survivors (N=54) in Wuhan, China. Non-survivors demonstrated statistically significantly higher plasma IL-6 levels as early as day 4 of symptom onset.[1]

Studies suggest that IL-1β and IL-6, in particular, are major driving factors of poor prognosis.[1,43] A strong correlation was found between higher IL-6 levels and severe disease or the need for mechanical ventilation compared to patients with uncomplicated disease. [43-53] Indeed, IL-6 has been suggested as a predictive biomarker for respiratory failure and death (**Figure 2**), and randomized placebo-controlled clinical trials blocking this pathway are underway (e.g., NCT04320615, NCT04362813, NCT04346355, NCT04356937, NCT04341675).[54] In a non-randomized, single-arm study, tocilizumab (soluble IL-6 receptor monoclonal antibody) was given to patients with severe COVID-19, and demonstrated decreased oxygen requirements, resolution of radiographic abnormalities, and clinical improvement with no adverse events or deaths.[50] Two other non-randomized studies found similar benefit as well.[52,55] Data from placebo-controlled IL-6 blocking trials are still pending. However, there are limited human data on the role of disulfiram on systemic cytokine levels. Prior studies have demonstrated that disulfiram may block IL-18 production in patients with glioblastoma, pancreatitis.[56-58] IL-18 is closely linked to IL-6 as both IL-18 and IL-1β (the key upstream regulator of IL-6) are proteolytically cleaved

via caspase-1 in response to innate immune sensing during pyroptosis.[59] In fact, prior studies have used plasma IL-18 as a surrogate for inflammasome signaling/plasma IL-1β levels, given challenges in measure IL-1β from patient plasma samples (in the picogram/mL range) using older assays.[60-63] Our group has recently demonstrated that IL-1β levels can now be reliably measured in plasma using newer chemiluminescent high sensitivity multiplex cytokine assay (Meso Scale Diagnostics, Rockland, MD) to discriminate differences within and between HIV+ antiretroviral therapy (ART)-suppressed individuals (Figure 3).[64-66]

Additional immune pathways also likely contribute to the hypercytokinemia observed in SARS-CoV-2 infection. For example, innate immune activation during SARS-CoV-2 infection can lead to activation of downstream signaling effectors such as NF-κB.[67-69] NF-κB promotes the transcription of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, [67,69] further triggering Th1 and Th17 production with subsequent secretion of IFN-γ and IL-17. [50,67,69] While the exact mechanism by which SARS-CoV-2 triggers an innate immune response is unknown it is likely similar to that used by SAR-CoV. Previous studies have detailed that SARS-CoV is detected by pattern recognition receptions (PRR) such as melanoma differentiation-associated protein 5 or retinoic acid-inducible gene 1 protein in the host.[70] The PRRs then interact with mitochondrial antiviral-signaling proteins (MAVS) triggering a kinase filled cascade which ultimately activates NF-κB. In turn NF-κB activates cytokine transcription leading to the elevated concentrations noted in COVID-19 patients.[1,47,71] These kinases act to phosphorylate IFN-regulatory factors stimulating production of type 1 interferons (IFNs) which in turn active the JAK-STAT pathway.[70] Both SARS-CoV and Middle Eastern respiratory virus (MERS-CoV) have developed mechanisms to interfere with the host innate response (using papain-like protease (PL^{pro}) and non-structural proteins) potentially leading to the dysregulation seen in severe cases.[72-76]

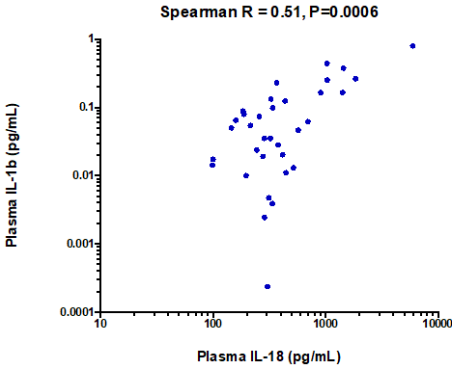

Figure 3. Plasma IL-1β and IL-18 levels performed using the MSD high sensitivity cytokine assay were correlated among 41 HIV+ ART-suppressed participants from the UCSF SCOPE cohort.[17]

4.1.2 Disulfiram and putative SARS-CoV-2 antiviral activity

Human coronaviruses, including the initial SARS-CoV, MERS-CoV, and the recent SARS-CoV-2, are all positive-sense single-stranded RNA viruses. Both SARS-CoV and MERS-CoV have developed mechanisms to interfere with the host innate response as described below .[72-76] Similarly, SARS-CoV-2 appears to promote immune dysregulation, as observed in severe COVID-19 patients who progress to severe acute respiratory distress syndrome (ARDS), with corresponding elevated plasma levels of IL-1 and IL-6.[16-18]

For these coronaviruses, after the virus enters the host cell, two polyproteins, pp1a and pp1ab, are directly translated and then cleaved by two viral proteases, main protease (Mpro) and papain-like protease (PLpro).[24,77,78] Both PLpro and Mpro play pivotal roles in mediating viral replication and transcription by cleavaging non-structural proteins (nsp) 1, 2 and 3 and Mpro cleaves all junctions downstream of nsp4, respectively.[78] In a recent drug screen, disulfiram was shown to inhibit MERS-CoV PLpro and SARS-CoV PLpro.18 Indeed, disulfiram acts as an allosteric inhibitor of MERS-CoV PLpro and as a competitive inhibitor of SARS-CoV PLpro, with a dose-dependent inhibitory effect on both proteases with IC50 values in the micromolar range (7.52 ± 2.13 μM).[79] Disulfiram was also

identified as a candidate for targeting SARS-CoV-2 Mpro by a high-throughput screen, demonstrating an IC50 value of 9.35±0.18 μM.[23] In in vitro cell-based assays, disulfiram demonstrated its anti-SARS-CoV-2 viral activity, reducing the absolute number of viral RNA copies (per mL) from 1.5 $x10^9$ to 1 $x10^9$ compared to positive controls (SARS-CoV-2).[23]

In addition to direct binding to the active site of human corona virus PLpro and Mpro, disulfiram may also act as a zinc ejector.[22] There are four cysteines (Cys) bound to a zinc ion in PLpros 65-68 and disulfiram could inhibit PLpro by binding to the zinc site. Studies using a zinc release assay illustrated that disulfiram may bind not only to the active site but also to the zinc-binding sites in MERS-CoVpro and SARS-CoV PLpro.[22] In addition, prior studies have shown that disulfiram may eject Zn2+ from the Zn-Cysteine-4 site in the hepatitis C virus NS5A protein, inhibiting viral replication.[21] Finally, in one study to identify druggable Zn-sites in SARS-CoV-2, nsp3 in SARS-CoV-2 PLpro, nsp10 transcription factor, and nsp13 helicase were identified as the most attractive drug targets.[79,80] Recent data now suggests that disulfiram may act synergistically with the nucleoside analog, remdesivir (an FDA-approved SARS-CoV-2 antiviral agent shown to reduce time to recovery in severe[81] and moderate[82] COVID-19 in the inpatient setting), by targeting conserved Zn2+-sites in SARS-CoV-2 nsp13 and nsp14 and inhibiting nsp13 ATPase and nsp14 exoribonuclease activities.

4.1.3 Disulfiram and mediation of aberrant inflammation in SARS-CoV-2 infection

The mechanism by which SARS-CoV-2 leads to severe COVID-19 disease in some individuals may be due to aberrant, excessive inflammation caused by activation of the NOD-like receptor protein 3 (NLRP3) inflammasome, a key player in antiviral responses.[83] Disulfiram was recently shown to inhibit gasdermin D (GSDMD) pore formation, the final common step of NLRP3 inflammasome-induced inflammatory cell death ("pyroptosis") and subsequent release of proinflammatory cytokines such as IL-1β and IL-6.[6] In addition, disulfiram inhibits NF-κB signaling, a transcription-factor mediated pathway that enhances NLRP3 inflammasome activation.[84]

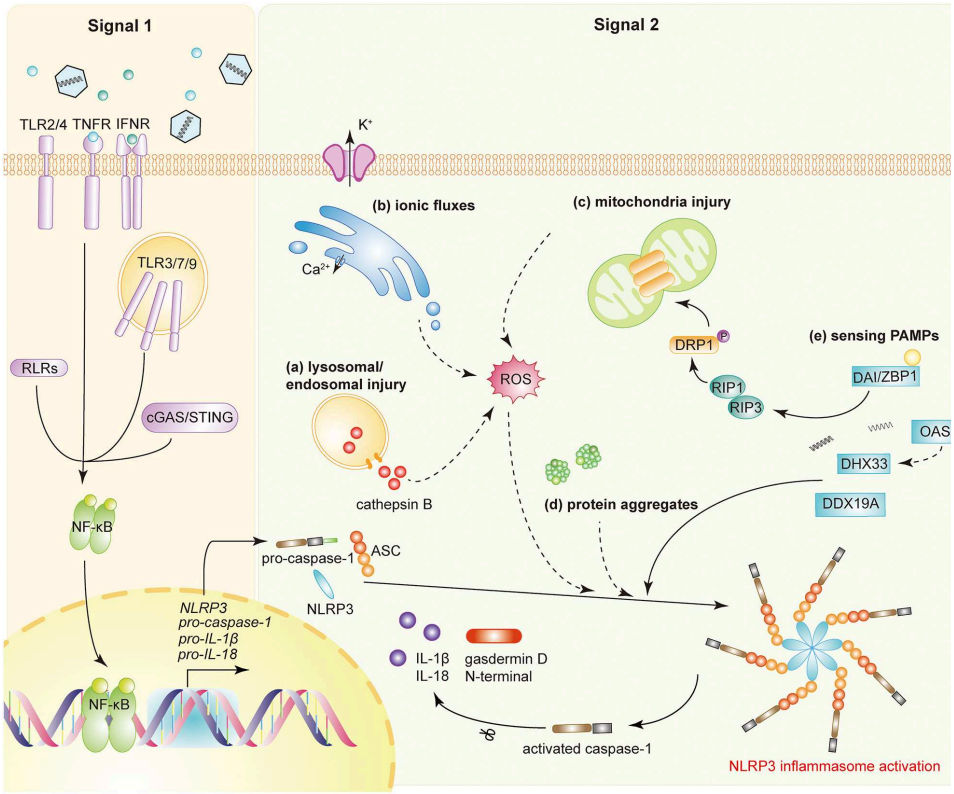

Figure 4. NLRP3 inflammasome activation during viral infections. Activation of the NLRP3 inflammasome requires 2 signals: (1) priming signal: activation of PRRs, TNFR, or IFNR induction of NF-kB activation, which triggers transcription of NLRP3, pro-caspase-1, pro-IL-1b, and pro-IL-18, and (2) activation signal: multiple DAMPs (damage-associated molecular patterns) and PAMPs (pathogen-associated molecular patterns) induce NRLP3 inflammasome assembly and activation. Caspase-1 then mediates the proteolytic processing of pro-IL-1b, pro-IL-18, and gasdermin D (GSDMD, the final common step), to lead to proinflammatory cytokine production. (Zhao et. al., Front Immunol 2020)

The host antiviral response is triggered by highly conserved sensors called pattern recognition receptors (PRRs) that recognize "danger signals" caused by the pathogen (e.g., aberrant ion concentrations, mitochondrial damage, accumulation of misfolded protein aggregates, etc.). Some PRRs form inflammasomes (the most well-studied being the NLRP3 inflammasome), cellular protein complexes that mediate host immune responses to pathogens.[85] Inflammasome assembly triggers ultimately leads to pyroptosis (inflammation-associated cell death) via auto-cleavage of pro-caspase1 which mediates proteolysis of pro-IL-1β, and gasdermin D (GSDMD) (**Figure 4**). GSDMD membrane pore formation then promotes the secretion of IL-1β (which induces IL-6 expression) and IL-18 (which promotes cytotoxic activity of T and NK cells via interferon-γ production).[86]

Unlike apoptosis, pyroptosis occurs faster and is accompanied by the release of large amounts of pro-inflammatory cytokines and chemokines.[87] In SARS-CoV-2 infection, a rise of IL-1β and lymphopenia were observed in patients, strongly suggesting the presence of cell pyroptotic activity.[88] In SARS-CoV infection, a small viral protein which modifies host cell permeability, viroporin 3a, was shown to trigger the NLRP3 inflammasome and subsequent IL-1β release.[89,90] Disulfiram may be able to reduce aberrant inflammation during SARS-CoV-2 infection via inhibition of NLRP3 inflamasome activation – either via inhibition of the final common step (GSDMD pore formation) or via inhibition of NF-κB-mediated enhancement of NLRP3 inflammasome activation and proinflammatory cytokine release.

Disulfiram has recently been shown to strongly inhibit GSDMD pore formation in a large high-throughput biochemical screen.[24] Disulfiram had direct *in vitro* and *in vivo* effects on GSDMD pore formation, with inhibition of liposomal leakage and subsequent reduction of pyroptosis and inflammatory cytokine release. Disulfiram has also been shown to have inhibitory effects on NF-κB and IL-6/STAT3 signaling pathways in cancer cells.[25-27] Therefore, disulfiram may mediate aberrant inflammasome activation via multiple potential mechanisms, a potential mechanism by which severe COVID-19 disease may be prevented if administered early in infection.

4.1.4 Clinical experience and safety of disulfiram

Disulfiram was first advocated as a therapy for alcoholism in 1937 when it was noted that rubber factory workers exposed to the molecule became intolerant of alcohol. Disulfiram has been approved by the FDA since 1951 for the treatment of alcohol dependence. Given its unusual history and early drug development, the quality of evidence supporting its use is limited. Nonetheless, a recent multicenter observational study in Italy found that disulfiram statistically significantly reduced COVID-19-related symptoms (fever and dyspnea) in a cohort of 752 individuals taking disulfiram (dosing range 100-800 mg daily) for the treatment of alcohol dependence compared to 542 COVID-19+ controls.[91] There were a total of only 4 cases and 4 controls who were admitted to be able to determine whether disulfiram prevented hospitalizations due to COVID-19. Reviews of disulfiram outcome studies spanning several decades have found that only one of more than 40 clinical trials have met current adequate research design criteria and only five of 135 disulfiram clinical trials have had a controlled design.[92]

The mechanism for the drug's alcohol deterrent effects lie in its ability to inhibit liver mitochondrial aldehyde dehydrogenases (ALDH).[93] This enzyme converts acetaldehyde to acetate in alcohol metabolism leading to an immediate uncomfortable reaction upon exposure to alcohol and when inhibited leads to the buildup of acetaldehyde. [94] The reaction to alcohol usually consists of symptoms such as flushing, heat sensation, nausea, vomiting, palpitations, headaches, and/or circulatory changes such as decreased blood pressure or increased pulse rate. The time to onset of ALDH inhibition which is sufficient to result in a reaction upon alcohol consumption is 12 hours and ALDH function is completely restored within 6 days of stopping disulfiram in alcoholics. [33,95]

The current recommended dose of disulfiram is 250 to 500 mg once daily. When disulfiram was first used clinically almost sixty years ago, it was common practice to use doses up to 1 to 3 grams.[96] However, these higher doses were associated with reports of severe adverse reactions, particularly in those individuals who continued to be exposed to alcohol.[97] Many of the serious adverse effects of disulfiram, such as psychotic reactions, hypotension and neuropathy, are thought to be dose-related,[96] and most fatal reactions occurred in patients who were taking more than 500 mg a day of disulfiram and who consumed more than 28 grams of alcohol.[98,99]

Given the variability in the clinical and biologic effect of disulfiram (see below), early recommendations by manufacturers and experts were to confirm that the drug was indeed active by challenging patients with alcohol. One recommended approach was to administer four 200 mg tablets (800 mg total) of disulfiram on the first day, reducing by one tablet each subsequent day until the fifth day, when patients were administered a single pill (200 mg) and then challenged with a dose containing 15-30 mL of pure ethanol. [100] In one study of 63 patients using this approach, 33 required 200 to 300 mg, 17 required 400 to 500 mg, 6 required 600 to 700 mg and 7 required 800 to 1500 mg in order to develop a clinical reaction to ethanol. During longer-term dosing, significant side effects were uncommon, but included drowsiness and mild confused states; these side effects waned in one to three days after dose reduction

or cessation.[100] Other approaches to deal with the patient-to-patient variability in activity were to administer very high doses. In one study, doses up to 6 grams per day were given.[101] Disulfiram was reported to be safe, although with chronic dosing rare but significant liver complications occurred that may or may not have been drug-related.[102]

4.1.5 HIV clinical trials of disulfiram

Our group has performed clinical trials of disulfiram in the setting of HIV cure trials, which have demonstrated safety and tolerability at doses as high as 2000 mg.[103,104] In a phase 2b dose escalation trial in HIV-infected virally suppressed participants, we evaluated disulfiram given at 500 mg, 1000 mg or 2000 mg daily for 3 consecutive days (NCT01944371).[29] Disulfiram was well tolerated at all doses studied; there were no drug discontinuations or grade 2-4 adverse events observed. These safety data were consistent with our prior phase 1 trial evaluating disulfiram 500 mg daily administered for 14 consecutive days in HIV-infected virally suppressed participants (NCT01286259).[104] A follow-up study in HIV-infected participants was performed, this time in combination with a histone deacetylase inhibitor, vorinostat (SAHA) (NCT03198559). In this phase 2b trial, HIV-infected virally suppressed participants received 2000 mg of oral disulfiram once daily for 28 days but this time, in combination with 400 mg oral vorinostat once daily on days 8-10 and again on days 22-24 (for a total of 6 days total of vorinostat overlap). Although there was evidence that this combination did reverse HIV latency, the study was halted due to two participants experiencing neurological adverse events including confusion, lethargy ataxia and paranoia. All symptoms resolved after discontinuation of both study drugs, disulfiram and vorinostat. Review of the participants' clinical records was unable to completely rule out that disulfiram administration, in combination with vorinostat, was related to these adverse events. Therefore, for the proposed work, we will only be administering disulfiram alone, as was demonstrated to be safely given in our prior phase 2b dose escalation trial.[29]

4.1.6 Pharmacology of disulfiram

Overview. The metabolism of disulfiram is complex (**Figure 5**).[105-107] Disulfiram is metabolized to diethyldithiocarbamate (DDTC) and then to diethyldithiocarbamate methyl ester (DDTC-Me). DDTC-Me is formed by thiol S-methyl ester transferase from DDTC. DDTC-Me is then metabolized to S-methyl-N-N- diethylthiocarbamate (disulfiram carbamate, or DETC-Me), and then bioactivated to S-methyl-N-N- diethylthiolcarbamate sulfoxide (DETC-MeSO). DETC-MeSO inhibits ALDH and hence is responsible for the anti-alcohol effect. [106,108] With regard to the anti-alcohol activity, most investigators—including Dr. McCanz-Katz, who lead the PK study outlined below—focused on latter metabolites (DETC-Me and DETC-MeSO).

Bioactivation of disulfiram to the active metabolite DETC-MeSO occurs principally via CYP 3A4/5, with contributions by CYP 1A2, 2A6 and 2D6.[107,109] Faiman and colleagues reported that the reduced

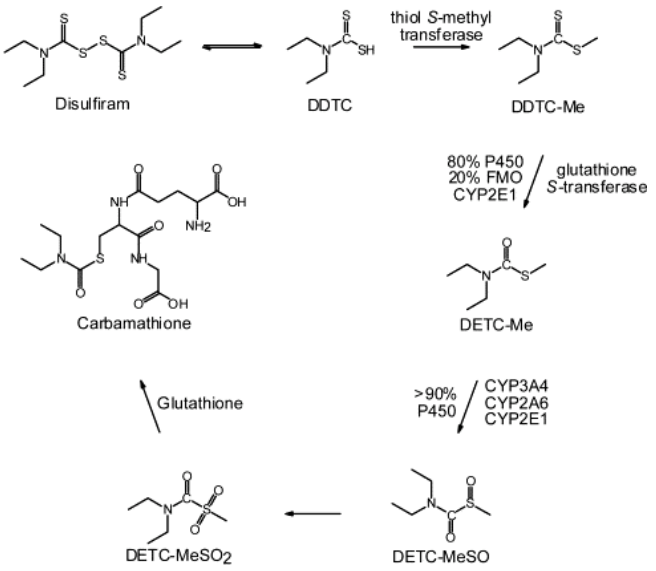

Figure 5. **Disulfiram metabolism**. Disulfiram is rapidly metabolized to its metabolites, including DDTC, DDTC-Me, DETC-Me, DETC-MeSO, and carbamthione.

A formal study of the elimination kinetics of disulfiram was performed by Faiman and colleagues and reported in 1984.[111] Fifteen alcoholics (all men) were given 250 mg as a single dose on day 1 and day 12. The observed half-life of disulfiram, diethyldithiocarbamate (DDTC), diethyldithiocarbamate-methyl ester (DDTC-Me), diethylamine (DEA), and carbon disulfide were found to be 7.3, 15.5, 22.1, 13.9, and 8.9 hours, respectively. Disulfiram is reported to be 90% eliminated in 3 days.[112] Peak plasma levels for disulfiram and its metabolites occurred at approximately 8 to 10 hours after the dose, while peak levels of carbon disulfide occurred 5 to 6 hours after the dose. There was marked variability in plasma levels of disulfiram and its metabolites (see Figure) which was attributed in part to plasma protein binding (disulfiram is 95% protein bound) and/or differences in liver metabolism.[113] It has also been suggested that metabolite of disulfiram, diethyldithiocarbamate (DDTC), inhibits several CYP enzymes including CYP 3A4/4, 2E1, and 2A6 which all play a role in bioactivation of disulfiram to DETC-MeSO.[109] This effect has been postulated as a reason why disulfiram produces variable reactions when alcohol is co-consumed.[109]

The pharmacokinetics and pharmacodynamics of disulfiram are highly variable between individuals. Up to 50% will not have a disulfiram-ethanol reaction on the recommended 250 mg dose.[100] For some, doses up to 500 mg are insufficient to cause a disulfiram-ethanol reaction.[100] This creates a clinical challenge in finding a dose of disulfiram that will be effective to elicit a mild disulfiram- ethanol reaction, while maintaining the safety of the patient should he or she drink alcohol while on the medication.[110]

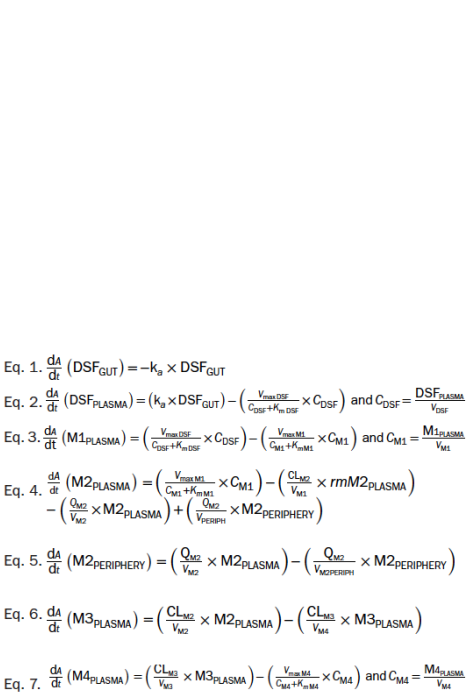

Figure 6. Seven-compartment pharmacokinetic model of disulfiram (DSF) metabolism. CL, clearance (L/hour); DSFGUT, amount of disulfiram in gut (mg); DSFPLASMA, amount of disulfiram in plasma (mg); ka, absorption constant; knon-lin, nonlinear elimination calculated using Km and maximum rate of metabolism (Vmax) terms; M1PLASMA, amount of N,N-diethyldithiocarbamate (DDTC) in plasma (mg); M2PLASMA, amount of diethyldithiocarbamate-methyl ester (DDTC-Me) in plasma (mg); M3PLASMA, amount of S-methyl-N, N-diethylthiolcarbamate sulfoxide (DETC-MeSO) in plasma (mg); M3PERIPHERY, amount of DETC-MeSO in plasma (mg); M4PLASMA, amount of carbamathione in plasma (mg); Q, blood flow (L/hour); V, volume of distribution (L).

disulfiram undergoes enteroheptic recycling. In a separate earlier study reported in 1979, a 600-fold variation in disulfiram plasma concentrations was reported.[114] Other studies have noted high variability in levels of key metabolites.

More recently, our group performed a detailed pharmacokinetic/ pharmacodynamic (PK/PD) modeling study based on our phase 2b dose escalation HIV cure clinical trial.[29,34] Parent drug (disulfiram) and four metabolites (DDTC, DDTC-Me, DETC-MeSO and carbamathione) were quantified and measured over the 31- day study period, as well as viral outcome measures for the PD analysis (cell-associated HIV RNA in CD4+ T cells and plasma HIV RNA). A comprehensive seven-compartment PK/PD model was built to estimate disulfiram metabolism (**Figure 6)**. Higher doses of administered disulfiram were associated with higher plasma concentrations of parent drug and metabolites (**Figure 7**). A linear PK model simultaneously incorporating all metabolites underpredicted plasma concentrations in the 2000 mg group, which initially appeared to be due to supraproportional concentrations of disulfiram in the highest dosing group (**Table 1**). However, after fitting a PK model allowing for nonlinear elimination kinetics, the supraproportional concentrations seemed to be largely explained by reduced elimination (e.g., due to saturation of CYP enzymes at higher concentrations) rather than increased bioavailability at the 2000 mg dose.

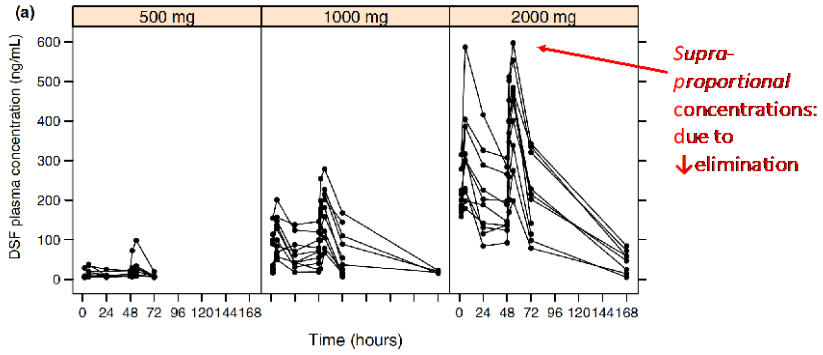

Figure 7. **Plasma disulfiram concentrations by dosing group**. Elevated plasma drug concentrations were observed with higher administered dose of disulfiram, which was safe and well tolerated all doses studied. Supra-proportional concentrations of disulfiram were observed in the 2000 mg dose, which was largely attributed to decreased elimination (rather than increased bioavailability) in the PK model.

4.1.7 Table 1. Final PK model estimates for median AUC_{0-72} exposure to metabolites by dosing cohort.

	DSF (Disulfiram)	M1 (DDTC)	M2 (DDTC-Me)	M3 (DETC-MeSO)	M4 (Carbamathione)
500 mg group	3,186 (3,140–3,281)	2,826 (2,651–3,602)	2,309 (1,563–3,397)	1,563 (1,324–2,544)	4,295 (4,041–4,923)
1000 mg group	8,386 (7,419–12,861)	7,511 (4,974–14,886)	4,154 (3,416–6,000)	3,225 (2,102–5,383)	8,640 (7,232–19,379)
2000 mg group	22,331 (18,482–35,102)	20,362 (15,114–31,421)	7,048 (6,248–9,701)	8,643 (6,279–9,976)	25,167 (16,058–32,086)

DSF, parent drug; disulfiram; M1, metabolite 1; M2, metabolite 2; M3, metabolite 3; M4, metabolite 4; DSF, disulfiram; DDTC, N,N-diethyldithiocarbamate; DDTC, diethyldithiocarbamate-methyl ester; DDTC-MeSO, S-methyl-N,N-diethylthiolcarbamate sulfoxide.

[a]Median AUC_{0-72} estimate with 2.5 to 97.5 percentile range.

Disulfiram concentrations were significantly associated with increases in HIV cell-associated RNA (with an Emax of 78% and AUC_{50} of 1600 mg-hr/L for the parent compound) for all of the dosing cohorts. Similarly, each of the four metabolites were also shown to significantly increase cell-associated RNA. Disulfiram concentrations were also associated with an increase in plasma HIV RNA levels, but only in the highest (2000 mg) dosing group.[29,34]

4.1.8 Disulfiram dose justification

Disulfiram's safety profile has been evaluated in several clinical trials.

In an open-label clinical trial to determine short-term disulfiram on decay of the HIV reservoir in patients on highly active antiretroviral therapy (NCT01286259), patients received 500 mg disulfiram daily via oral administration for 14 days. The safety and tolerability of a two-week course of disulfiram was defined using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification, August 2009). A total of 16 patients were recruited, with no adverse event reported during the treatment course.

In a phase 2 non-randomized trial focusing on short-term disulfiram to reverse latent HIV infection (NCT01944371),[103] patients received disulfiram daily for 5 days at doses of 500 mg, 1000 mg or 2000 mg. The safety and tolerability of disulfiram was defined using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 2.0, November 2014. Thirty patients were recruited, and all participants completed the study. There were no deaths, drug discontinuations, or grade 2-4 adverse events observed. No adverse events related to the study drug were reported in the 500 mg group, which was consistent with the findings described in the previous trial above. Grade 1 clinical adverse events related to study drug were more common in the higher dosing cohorts including 10 events in the 1000 mg group, and 14 events in the 2000 mg group.

The DIVA study (NCT03198559) was a phase 2b trial investigating whether disulfiram in combination with vorinostat could active HIV-1 viral replication. The proposed study design was to enroll 14 virally suppressed HIV-infected patients and to administer 2000 mg disulfiram orally once daily for 28 days and 400 mg vorinostat once daily orally on days 8-10 and again on days 22-24 (6 days total). The primary outcome measure was plasma HIV RNA on day 11 normalize to each patients' baseline value. The safety and tolerability of this combination was defined using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 2.0, November 2014. Although there was evidence that this combination did reverse HIV latency this study was stopped after recruiting two patients. Both patients experienced neurological adverse events including confusion, lethargy ataxia and paranoia. All symptoms resolved after discontinuation of study drugs. Upon review of the patients' clinical records disulfiram was listed as possible and probably related to these adverse events respectively.

4.1.9 SARS-CoV-2 viral load quantification

Serial SARS-CoV-2 viral load quantification will be performed using quantitative reverse transcription polymerase chain reaction (rtPCR) using the Roche MagNA Pure 96 (MP96) System.[4] A modified CDC protocol was used to target the N1 and N2 gene of SARS-CoV-2 RNA, and this assay has been validated across multiple patient tissue types.[115] The assay will be used to evaluate SARS-CoV-2 viral decline in those receiving or not receiving disulfiram. These assays will be performed on nasopharyngeal (NP) swabs, but also from blood samples. In addition, saliva will be collected and stored for potential future quantification of SARS-CoV-2 RNA and anti-SARS-CoV-2 IgA.

4.1.10 Preliminary data demonstrating the ability to enroll acute COVID+ patients

At the onset of the global COVID-19 pandemic, we rapidly developed the acute COVID-19 Host Immune Response Pathogenesis (CHIRP) study to evaluate clinical, demographic, behavioral, immunologic, and genetic risk factors associated with COVID-19 acquisition and severity. In order to minimize the potential risk of SARS-CoV-2 transmission to non-research patients, as well as household members to potentially infected research staff, we developed a study design for example,

allowing outpatient in-person visits using a mobile research van in order to abide by the Centers for Diseases Control guidelines for discontinuation of home isolation (https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-in-home-patients.html). In less than four weeks of cohort development, we obtained study protocol approval, set up the off-campus location and mobile study van, and implemented strict infection control protocols to enroll COVID+ individuals and close household contacts. In order to minimize exposure time during in-person visits, electronic informed consent and release of information are obtained by phone. Longitudinal biospecimen collection are performed at weeks 1, 2, 4, 8, and 24 from baseline visit, collecting blood, urine, stool, saliva, and home expectorated sputum. To date, a total of 13 (26%) of 50 prospective screened participants have been enrolled in the study after just three weeks from study initiation, including four individuals and four family clusters. Among the first 13 participants enrolled, 62% are female, with a median age of 42 years. We will now leverage this intensive sampling cohort infrastructure to rapidly enroll participants for the DISCO trial.

5 INVESTIGATIONAL PLAN:

5.1 Study objectives

5.1.1 Primary objective

- To determine the effect of oral disulfiram given at 1000 mg daily (cohort 1) or 2000 mg daily (cohort 2) versus placebo for 5 consecutive days in adults with early mild-to-moderate symptomatic SARS-CoV-2 infection on inflammation (plasma cytokine levels - e.g., IL-6, IL-1β) using the MesoScale Diagnostics high sensitivity plasma cytokine platform[2]

5.1.2 Secondary objectives

- To determine the effect of oral disulfiram (2000 mg daily given for 5 consecutive days) in adults with early mild-to-moderate symptomatic SARS-CoV-2 infection on:
    - The safety and tolerability of oral disulfiram 2000 mg/day given for 5 consecutive days in adults with symptomatic SARS-CoV-2 infection.
    - SARS-CoV-2 PCR positivity and quantitative viral load using qRT-PCR on the Roche MagNA Pure 96 system.[4]
    - COVID symptom severity (using an adapted SSS-8 five-point symptom scale for common COVID-19 symptoms[3] which abide by the FDA Guidance: Assessing COVID-19-Related Symptoms in Outpatient Adult and Adolescent Subjects in Clinical Trials of Drugs and Biological Products for COVID-19 Prevention or Treatment.[116]
    - The proportion of participants hospitalized at day 5, 15, and 31.
    - The proportion of participant deaths at day 5, 15, and 31.

5.2 Study Definitions

- *Enrolled*: From time consented to participate until designated as (i) ineligible based on the inclusion/exclusion criteria or withdraws, (ii) been discontinued from the study or (iii) completed the study.
- *Randomized*: when a randomization number is assigned.
- *Screen Failures*: signed informed consent, but then determined to be ineligible or withdraws before being randomized.
- *Discontinued*: randomized, but then withdrawn by investigator or participant withdraws consent.
- *Completed:* Participants are considered completed when they are followed through to day 31, had an adverse event or death occurred prior to day 31.

5.3 Study Population

5.3.1 Inclusion Criteria

1. Willing and able to provide written informed consent, and
2. Age ≥18 years, and
3. Recent symptomatic COVID-19 disease (<14 days from symptom onset)

4. Recent SARS-CoV-2 positive test by molecular diagnostic assay within the preceding 7 days, and
5. Not currently hospitalized, and
6. Willing to abstain from any alcohol during the two-week period in which disulfiram will be administered and during the two-week period immediately after disulfiram administration.
7. Both male and female participants are eligible. Females of childbearing potential must have a negative pregnancy test at screening and agree to use a double-barrier method of contraception throughout the study period.

5.3.2 Exclusion Criteria

1. Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study

2. Active malignancy requiring systemic chemotherapy or surgery in the preceding 3 months or for whom such therapies are expected in the subsequent 6 months

3. Decompensated liver disease as defined by the presence of ascites, encephalopathy, esophageal or gastric varices, or persistent jaundice

4. Serious illness requiring systemic treatment and/or hospitalization in the 3 months prior to study enrollment

5. Concurrent treatment with immunomodulatory drugs, and/or exposure to any immunomodulatory drug in the 4 weeks prior to study enrollment (e.g. corticosteroid therapy equal to or exceeding a dose of 15 mg/day of prednisone for more than 10 days, IL-2, interferon-alpha, methotrexate, cancer chemotherapy). NOTE: use of inhaled or nasal steroid is not exclusionary.

6. Serious medical or psychiatric illness that, in the opinion of the site investigator, would interfere with the ability to adhere to study requirements or to give informed consent.

7. Current alcohol use disorder or hazardous alcohol use (>7 drinks per week for women or > 14 drinks per week for men) as determined by clinical evaluation.

8. Current use of any drug formulation that contains alcohol or that might contain alcohol

9. Current use of warfarin.

10. Current use of oral lopinavir/ritonavir solution (and the inability to switch to a solid dosage form during the entire duration of the study).

11. ALT or AST > 2 x the upper limit of normal or total bilirubin outside the normal range.

12. Creatinine clearance (CrCl) < 50 mL/min by Cockcroft-Gault)

13. Allergy to rubber or thiuram derivatives

Note: Receipt of medications with potential anti-COVID activity that are considered standard-of-care at the study site (e.g., small molecules or monoclonal antibodies directed against SARS-CoV-2) is permitted during the trial. Prior receipt of current standard of care inpatient COVID-19 treatment, including remdesivir, convalescent plasma/sera, and dexamethasone,[117] is also permitted and should be recorded as a prior medication.

5.3.3 Participant Withdrawal

Participants can terminate study participation and/or withdraw consent at any time without prejudice. Randomized participants who withdraw from the study will not be replaced. If a participant discontinues study dosing (for example, as a result of an AE), every attempt should be made to keep the participant in the study and continue to perform the required study-related follow-up and procedures (see Section 8.1, Criteria for Discontinuation of Study Treatment). The investigator may withdraw participants if they are non-compliant with study procedures or if the investigator determines that continued participation in the study would be harmful to the participant or the integrity of the study data.

5.3.4 Treatment

A total of 60 participants will be randomized (2:1) to receive active drug versus placebo in a dose escalation study. The first 30 participants will be enrolled in the 1000 mg dosing cohort, randomized 2:1 to receive either disulfiram 1000 mg (N=20) or placebo (N=10) daily for 5 consecutive days. As long as safety monitoring criteria are met to proceed to the second dose, the next 30 participants will be enrolled in the 2000 mg dosing cohort, randomized 2:1 to receive either disulfiram 2000 mg (N=20) or placebo (N=10) daily for 5 consecutive days.

5.3.5 Randomization

Participants enrolled in the study will be randomized using a web-based randomization procedure to receive active drug or no therapy at a 2:1 ratio within each dosing cohort.

5.3.6 Study drug administration

Drug will be administered within 72 hours of randomization.

5.3.7 Concomitant medications

Disulfiram is a substrate of CYP3A4, CYP2A6 and CYP2E1 and an inhibitor CYP2E1, CYP1A2 and CYP2C9, and therefore has potential drug-drug interactions.[118] Concomitant medications will be documented on the Case Report Form (CRF) up to day 31. Most of these interactions can be managed clinically, as discussed below.

1. Amprenavir oral solution: Amprenavir oral solution contains propylene glycol and should be avoided when using disulfiram.
2. Benzodiazepines: Disulfiram may inhibit the metabolism of certain benzodiazepines. Close monitoring for increased benzodiazepine effects is recommended.
3. Metronidazole. Metronidazole can have a disulfiram-like effect when administered with alcohol and should not be co-administered with this drug.
4. Ritonavir: The gelatin capsule and liquid formulations of ritonavir contain alcohol. We will avoid the use of these formulations. The new ritonavir formulation has a small alcohol content, and thus co-administered with disulfiram is not contraindicated.
5. Sertraline: The oral concentrate version of this drug contains alcohol. Thus, disulfiram should not be co-administered with this drug.
6. Any drug which contains or may contain alcohol should be avoided.
7. Theophylline Derivatives: Disulfiram may increase the serum concentration of theophylline.
8. Tipranavir: Disulfiram may enhance the adverse effect of tipranavir and should be avoided.

9. Vitamin K Antagonists (e.g., warfarin): Disulfiram may increase the serum concentration of Vitamin K. We will exclude individuals who are receiving warfarin.
10. Isoniazid: We will monitor for any potential side effects of isoniazid as the interactions between this drug and disulfiram are unknown
11. Tricyclic antidepressants (TCAs): We will monitor for any potential side effects of TCAs as the interactions between these drugs and disulfiram are unknown.

5.3.8 Prohibited medications

For study days 1-31, use of medications that are considered standard-of-care at the study site (including antiviral medications available under Emergency Use Authorization) is permitted. Use of other medications with potential anti-SARS-CoV-2 activity, including off-label, expanded access, or investigational agents, should be discussed with the team.

Note: Participants who are hospitalized during the study are eligible to receive COVID-19 treatment per local standard of care, including remdesivir, immunomodulatory agents and/or convalescent sera/plasma and will not be excluded from the study if receiving this standard of care. Azithromycin administered for treatment of presumed or confirmed infection will be permitted.

After study day 31, use of anti-COVID-19 treatments as salvage therapy for those with persistent severe COVID-19 are permitted but should be recorded.

5.4 Study procedures

5.4.1. Figure 1. SCHEDULE OF EVALUATIONS:

	Screen/ Baseline	Day 1 Clinic	Day 2 Clinic	Day 3 Phone	Day 4 Phone	**Day 5 Pre dose**	**Day 5 2-hour Post dose**	**Day 5 4-hour Post dose**	Day 7 Phone	Day 9 Phone	Day 15 Clinic	Day 31 Clinic
Informed Consent	X											
Release of information	X											
Questionnaire		X					**X**				X	X
Symptom diary/ AE check	X	X	X	X	X		**X**		X	X	X	X
Vital signs (BP, HR, R, Temp, SP02)	X	X[a]				**X[a]**	**X[b]**	**X[b]**			X	X
Targeted Physical Exam	X	X				**X**					X	X
SARS-COV-2 PCR TEST	X											
Saliva		X				**X[a]**					X	X
Complete blood count with differential	X		X[b]			**X[a]**					X	X
Comprehensive Metabolic Panel	X		X[b]			**X[a]**					X	X
Erythrocyte Sedimentation Rate (ESR)	X		X[b]			**X[a]**					X	X
C-Reactive Protein (Inflammation)	X		X[b]			**X[a]**					X	X
D-Dimer	X		X[b]			**X[a]**					X	X
Lactate Dehydrogenase (LDH)	X		X[b]			**X[a]**					X	X

Ferritin	X		X[b]			**X[a]**					X	X
Urine Pregnancy test (if WOCBP)	X					**X[a]**						
CD4+/CD8+ T cell counts (if HIV positive)	X											X
HIV RNA-standard Assay (if HIV positive)	X											X
PK Samples			X[b]			**X[a]**	**X[b]**	**X[b]**			X	X
PBMC		X				**X[a]**	**X[b]**	**X[b]**			X	X
Plasma		X				**X[a]**	**X[b]**	**X[b]**			X	X
Serum		X				**X[a]**	**X[b]**	**X[b]**			X	X
NP Swab		X	X[b]			**X[a]**					X	X
Study Drug/Placebo Administration (Given after blood collection on clinic visit days) • **Cohort 1 = (N=20) 1000mg/day; (N=10 placebo)** • **Cohort 2 = (N=20) 2000mg/day; (N=10 placebo)**		X	X	X	X	**X**						

a=predose
b=post-dose

The frequent time points are to detect both drug levels and human immune response. Red Cross limits are 500 mL per two months, which we do not exceed in this study. There is no stated guideline for blood volumes for this patient population, so we are utilizing the guidelines from interventional HIV cure trials, when often, serial blood sampling are completed in a single day. The total volume collected at each visit is as follows:

Day 0 Screen/Baseline:	15 mL
Day 1: Pre-Dose	65 mL
Day 2: Post Dose	19 mL
Day 5 Hour -0.5 pre-dose:	59 mL
Day 5 Hour 2 post dose:	45 mL
Day 5 Hour 4 post dose:	45 mL
Day 15:	79 mL
Day 31:	79 mL

The total volume collected during the study is 406 mL and does not exceed the Red Cross limit of 500 mL. Safety labs collected during these visits will be monitored, and volume will be decreased at the discretion of the PI if needed. The blood volumes in this study were carefully calculated to stay within Red Cross Guidelines. This is the approach that is taken in HIV interventional trials, and indeed, the PK sampling days can be tricky. Even for observational (non-interventional trials), there are large volume protocols with separate Informed Consent, for example, the HIV SCOPE cohort that allows a much larger blood draw in a single day for the type of research assays being performed for DISCO. Indeed, most of the whole blood is processed and cryopreserved for several years to be applied towards future assays that have yet to be discovered at this time. In a recent project, 202 HIV+ samples were pulled, that were collected in the 1990s from the SCOPE cohort to be applied to advanced host whole genome sequencing assays that we now have available today.

This trial is not only looking at clinical outcomes. The mechanism by which disulfiram acts is not well understood but based on Dr. Lieberman's recent data in Nature Immunology- this drug may have important implications not just for SARS-CoV-2, but other infectious pathogens that trigger an aberrant inflammatory response. If we are going to ask these study participants to take a study drug, we would like to understand HOW it is working, which may point to upstream novel drug targets that may be even more effective down the line in treating COVID-19. Each research assay requires approximately 10 cc of whole blood (Ficolled into PBMCs, peripheral blood mononuclear cells). Some assays require 2 aliquots.

Red Cross Guidelines recommend staying with 480 cc over a 56-day period. We have tried to stay within that in order to draw the minimum samples needed to study host gene expression, host immune cell, PK changes after study drug; this includes changes in genes that are expressed in the hours after taking disulfiram. Although we are drawing this amount of blood in a 31-day period of time, the rationale for this is that in order to study an ACUTE viral illness, the first month of the host response is critical. Drawing blood later after the acute illness has passed makes those latter timepoint blood collections less informative. Moreover, even if the patient continues in clinical care, you can see from the attached blood volumes chart that the clinical lab draws make up a small proportion of the blood draws for these types of translational immunologic studies.

Prior to the DISCO trial, we conducted a pilot study titled COVID-19 Host Immune Response Pathogenesis study (CHIRP) and the amount of blood drawn during the study in 3 weeks was 486 mL. There was no adverse event observed during the study. DISCO trial is designed to enroll patients with mild cases of COVID infection and further understand the immunogenicity of COVID-19 infection.

5.5 Study procedures by day

5.5.1 Screen and Baseline (Day -3 to Day 1)

1. Informed consent (obtained before performing study related activities)
2. Questionnaire Evaluation to include the COVID-19 Index Initial General questionnaire, COVID-19 Index Initial Substance Use, Reproductive Health survey, and Mental Health Supplemental Form
   - Demographics: Age, sex, race
   - Medical history: acute and chronic medical conditions that are risk factors for COVID – e.g., diabetes, cardiovascular disease, chronic obstructive pulmonary disease.
   - Transmission history: timing of exposure to COVID-19 source patient
   - Medications, allergies
   - Any medical condition arising after consent to be recorded as AE
   - Prior COVID-19 testing history (SARS-CoV-2 RT-PCR or antibody): positive test PCR required within 7 days of screening must be documented
   - COVID-19 symptom screen: 5-point ordinal adapted somatic symptom scale (SSS-8)[3]
3. Targeted physical exam and vital signs (temperature, heart rate, blood pressure, respiratory rate, oxygen saturation by pulse oximetry)
4. Clinical lab collection (run in real-time)
   a. Screening labs: **complete** blood count with differential, urea, electrolytes, creatinine, calcium, magnesium, phosphate, liver function tests
   b. Clinical labs associated with severe COVID: erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hs-CRP), D-dimer, lactate dehydrogenase (LDH), and ferritin.
   c. Clinical labs to confirm COVID+ status: SARS-CoV-2 NP swab PCR assay
   d. Females of childbearing potential: plasma beta-hCG urine pregnancy test
   e. HIV+ participants: a CD4+/CD8+ T cell count and standard plasma HIV RNA assay.
5. Samples collected for research (run in batch at end of study enrollment)
   a. Saliva for host DNA sequencing and potential future SARS-CoV-2 quantification (RNA levels and anti-SARS-CoV-2 IgA)
6. Randomization of those found to be eligible

5.5.2 Study Drug/ Placebo Administration (Day 1)

5.5.2.1 Pre-Dose (Between Hour -0.5 and 0)

1. Questionnaire Evaluation to include the Index Follow up Questionnaire
2. Targeted physical exam and vital signs
3. Clinical lab collection

4. Samples collected for research (storage)
    a. Blood: PBMC (CyTOF, single cell sequencing), plasma (plasma SARS-CoV-2 viral load), serum (SARS-CoV-2 serology)
    b. Nasopharyngeal swab for SARS-CoV-2 viral load
    c. Saliva for host DNA sequencing and potential future SARS-CoV-2 quantification (RNA levels and anti-SARS-CoV-2 IgA)

5.5.2.2 Dose (Hour 0)

Study staff to dispense the first dose of disulfiram or placebo to participant in person. The subsequent doses will be self-administered at home and the time noted as below.

5.5.3 Study Drug/ Placebo Administration (Day 2)

Participant will come to the clinic for clinical laboratory to include complete blood count with differential, urea, electrolytes, creatinine, calcium, magnesium, phosphate, liver function tests, erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hs-CRP), D-dimer, lactate dehydrogenase (LDH), and ferritin, plasma PK testing, and Nasopharyngeal swab for SARS-CoV-2 viral load. Saliva for host DNA sequencing and potential future SARS-CoV-2 quantification (RNA levels and anti-SARS-CoV-2 IgA) will be collected.

The participant will be asked to hold the day 2 study medication until after the blood draw. After the blood has been collected, the participant will be asked to take their day 2 dose of study medication. The time of pre-dose blood draw and the study medication administration date and time will be recorded.

The subject will complete a daily symptom check using the COVID-19 symptom severity questions adapted from the Somatic Symptom Scale (SSS-8) (table 3) while at the clinic and will be assessed for any adverse events.

5.5.4 Study Drug/ Placebo Administration (Day 3)

Participant will self-administer the third dose of disulfiram or placebo at home. The participant will note the time of self-administration and will complete a daily symptom diary. Study coordinator will call patient to verify their symptoms, confirm self-administered dose, and evaluate for any adverse events. If any AEs are reported, an AE form will be completed by the study coordinator, and the PI will be notified for any Grade 3 or above AE.

5.5.5 Study Drug/ Placebo Administration (Day 4)

Participant will self-administer the fourth dose of disulfiram or placebo at home. The participant will note the time of self-administration and will complete a daily symptom diary. Study coordinator will call patient to verify their symptoms, confirm self-administered dose, and evaluate for any adverse events. If any AEs are reported, an AE form will be completed by the study coordinator, and the PI will be notified for any Grade 3 or above AE.

5.5.6 Study Drug/ Placebo Administration (Day 5)

5.5.4.1 Pre-Dose (Hour -0.5 to Hour 0)

1. Questionnaire Evaluation
2. Targeted physical exam and vital signs
3. Clinical lab collection to include;

   complete blood count with differential, urea, electrolytes, creatinine, calcium, magnesium, phosphate, liver function tests, erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hs-CRP), D-dimer, lactate dehydrogenase (LDH), and ferritin.
4. Samples collected for research (storage)
   a. Blood: PBMC (CyTOF, single cell sequencing), plasma (plasma SARS-CoV-2 viral load), serum (SARS-CoV-2 serology)
   b. Nasopharyngeal swab for SARS-CoV-2 viral load
   c. Saliva for host DNA sequencing and potential future SARS-CoV-2 quantification (RNA levels and anti-SARS-CoV-2 IgA)

5.5.4.2 Dose (Hour 0)

Study staff to dispense the fifth (last) dose of disulfiram or placebo to the participant.

5.5.4.3 Post-Dose (Hour 2)

1. Samples collected for research (storage)
   a. Blood: PBMC (CyTOF, single cell sequencing), plasma (PK, plasma SARS-CoV-2 viral load), serum (SARS-CoV-2 serology)

5.5.4.4 Post-Dose (Hour 4)

1. Samples collected for research (storage)
   a. Blood: PBMC (CyTOF, single cell sequencing), plasma (PK, plasma SARS-CoV-2 viral load), serum (SARS-CoV-2 serology)

5.5.7 Symptom/Adverse Event Telephone Follow-Up (Day 7)

Participant will self-administer a daily symptom/adverse event diary. Study coordinator will call patient to verify their symptoms and evaluate for any adverse events. If any AEs are reported, an AE form will be completed by the study coordinator, and the PI will be notified for any Grade 3 or above AE.

5.5.8 Symptom/Adverse Event Telephone Follow-Up (Day 9)

Participant will self-administer a daily symptom/adverse event diary. Study coordinator will call patient to verify their symptoms and evaluate for any adverse events. If any AEs are reported, an AE form will be completed by the study coordinator, and the PI will be notified for any Grade 3 or above AE.

5.5.9 Symptom/Adverse Event Telephone Follow-Up (Day 11)

Participant will self-administer a daily symptom/adverse event diary. Study coordinator will call patient to verify their symptoms and evaluate for any adverse events. If any AEs are reported, an AE form will be completed by the study coordinator, and the PI will be notified for any Grade 3 or above AE.

5.5.10 Follow-Up (Day 15)

1. Questionnaire Evaluation
2. Targeted physical exam and vital signs
3. Clinical lab collection
    a. Complete blood count with differential, urea, electrolytes, creatinine, calcium, magnesium, phosphate, liver function tests, erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hs-CRP), D-dimer, lactate dehydrogenase (LDH), and ferritin.
4. Samples collected for research (storage)
    a. Blood: PBMC (CyTOF, single cell sequencing), plasma (plasma SARS-CoV-2 viral load), serum (SARS-CoV-2 serology)
    b. Nasopharyngeal swab for SARS-CoV-2 viral load
    c. Saliva for host DNA sequencing and potential future SARS-CoV-2 quantification (RNA levels and anti-SARS-CoV-2 IgA)

5.5.11 Follow-Up (Day 31)

5. Questionnaire Evaluation
6. Targeted physical exam and vital signs
7. Clinical lab collection
    a. Complete blood count with differential, urea, electrolytes, creatinine, calcium, magnesium, phosphate, liver function tests, erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hs-CRP), D-dimer, lactate dehydrogenase (LDH), and ferritin.
8. Samples collected for research (storage)
    a. Blood: PBMC (CyTOF, single cell sequencing), plasma (plasma SARS-CoV-2 viral load), serum (SARS-CoV-2 serology)
    b. Nasopharyngeal swab for SARS-CoV-2 viral load
    c. Saliva for host DNA sequencing and potential future SARS-CoV-2 quantification (RNA levels and anti-SARS-CoV-2 IgA)
    d. HIV+ participants: a CD4+/CD8+ T cell count and standard plasma HIV RNA assay.
9. Samples collected for research (storage)
    a. Blood: PBMC (CyTOF, single cell sequencing), plasma (plasma SARS-CoV-2 viral load), serum (SARS-CoV-2 serology)
    b. Nasopharyngeal swab for SARS-CoV-2 viral load

c. Saliva for host DNA sequencing and potential future SARS-CoV-2 quantification (RNA levels and anti-SARS-CoV-2 IgA)

5.6 Table 3. COVID-19 symptom severity questions adapted from the Somatic Symptom Scale (SSS-8). 3

Symptom	New occurrence or worsening of pre-existing symptom during illness	When did it start? (MMM/DD/YY) If day cannot be estimated within 3 days of true onset, include month and year and mark day not known.	Skip if start date known. Was it present in the first 3 days of illness?	Are you still experiencing it, meaning in the past 2 days? For recovered phase, add: By this we mean that your initial symptoms you had when you first developed COVID-19 have not gone away.	Skip if still experiencing the symptom. How many days did it last? This is defined as start date to last date experienced.	When the symptom was at its worst, how much did it bother you? Please answer on a scale from 1 to 5, where 1 is NONE and 5 is VERY MUCH.
Feeling feverish	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Measured a temperature of >100.4ºF or 38ºC	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Chills, feeling unusually cold	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Fatigue or feeling tired	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Cough	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Shortness of breath or difficulty breathing	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Chest pain	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Runny nose or congestion	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4

Symptom	New occurrence or worsening of pre-existing symptom during illness	When did it start? (MMM/DD/YY) If day cannot be estimated within 3 days of true onset, include month and year and mark day not known.	Skip if start date known. Was it present in the first 3 days of illness?	Are you still experiencing it, meaning in the past 2 days? For recovered phase, add: By this we mean that your initial symptoms you had when you first developed COVID-19 have not gone away.	Skip if still experiencing the symptom. How many days did it last? This is defined as start date to last date experienced.	When the symptom was at its worst, how much did it bother you? Please answer on a scale from 1 to 5, where 1 is NONE and 5 is VERY MUCH.
Sore throat	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Muscle aches	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Loss of appetite	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Nausea	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Vomiting	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Abdominal pain	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Diarrhea	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Trouble with smell or taste	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4

Symptom	New occurrence or worsening of pre-existing symptom during illness	When did it start? (MMM/DD/YY) If day cannot be estimated within 3 days of true onset, include month and year and mark day not known.	Skip if start date known. Was it present in the first 3 days of illness?	Are you still experiencing it, meaning in the past 2 days? For recovered phase, add: By this we mean that your initial symptoms you had when you first developed COVID-19 have not gone away.	Skip if still experiencing the symptom. How many days did it last? This is defined as start date to last date experienced.	When the symptom was at its worst, how much did it bother you? Please answer on a scale from 1 to 5, where 1 is NONE and 5 is VERY MUCH.
New spots or rash on your skin	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Trouble concentrating, trouble with your thinking, or trouble with your memory	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Headache	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Trouble with vision (e.g. double vision, blurry vision, or other visual issues)	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Dizziness, feeling like you are spinning, or the world is spinning around you	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Trouble with balance or feeling unsteady	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Numbness, tingling, or "pins and needles" in your arms or legs	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Difficulty falling or staying asleep	○ Yes ○ No	__ __ __/__ __/__ __	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4

		○ Day not known				
Symptom	**New occurrence or worsening of pre-existing symptom during illness**	**When did it start? (MMM/DD/YY)** If day cannot be estimated within 3 days of true onset, include month and year and mark day not known.	Skip if start date known. **Was it present in the first 3 days of illness?**	**Are you still experiencing it, meaning in the past 2 days?** For recovered phase, add: **By this we mean that your initial symptoms you had when you first developed COVID-19 have not gone away.**	Skip if still experiencing the symptom. **How many days did it last?** This is defined as start date to last date experienced.	**When the symptom was at its worst, how much did it bother you? Please answer on a scale from 1 to 5, where 1 is NONE and 5 is VERY MUCH.**
Irritability, agitation, or difficulty controlling your emotions	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Decreased interest in activities or socializing with others	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4

Use the following instruction table to determine what to ask next based on previous responses.

Reported symptoms in Q19?	**Symptoms uncovered in table above?**	**Question to ask**
Yes	Yes or No	Were there other symptoms that you experienced? ○ Yes → ***Go to 23*** ○ No → ***SKIP to 24***
No	Yes	I'm glad we went through this list. Do any other symptoms come to mind? ○ Yes → ***Go to 23*** ○ No → ***SKIP to 24***
No	No	→ ***SKIP to 26***

1. What other symptoms did you experience? Follow the instructions from question 22 for completing each row of the table.

Other: Specify _____	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4
Other: Specify _____	○ Yes ○ No	__ __ __/__ __/__ __ ○ Day not known	○ Yes ○ DK ○ No	○ Yes ○ No	___ ___ ○ Don't know	○ 1 ○ 3 ○ 5 ○ 2 ○ 4

5.7 Table 4. Adverse Event Log form.

DISCO PIDXXXX-04-2021	**Adverse Event Log** Subject ID______________________ Initials ______

AE #	Adverse Event *(diagnosis or symptom)*	Dates	Severity	Considered an SAE	Relationship to Investigational drug	Action taken with study drug	Outcome
	______________ ______________ INVESTIGATOR SIGNATURE	**Start Date** Time: **Stop Date** Time: □ continuing	□ mild □ moderate □ severe	□ yes □ no If yes, related to study / protocol procedures □ yes □ no	□ Definitely Related □ Probably related □ Possibly Related □ Unlikely Related □ Not related	□ none □ discontinued □ dose adjusted □ interrupted □ unknown □ N/A	□ ongoing □ ongoing and stable □ resolved □ resolved with sequelae □ unknown □ death
	______________ ______________ INVESTIGATOR SIGNATURE	**Start Date** Time: **Stop Date** Time: □ continuing	□ mild □ moderate □ severe	□ yes □ no If yes, related to study / protocol procedures □ yes □ no	□ Definitely Related □ Probably related □ Possibly Related □ Unlikely Related □ Not related	□ none □ discontinued □ dose adjusted □ interrupted □ unknown □ N/A	□ ongoing □ ongoing and stable □ resolved □ resolved with sequelae □ unknown □ death
	______________ ______________ INVESTIGATOR SIGNATURE	**Start Date** Time: **Stop Date** Time: □ continuing	□ mild □ moderate □ severe	□ yes □ no If yes, related to study / protocol procedures □ yes □ no	□ Definitely Related □ Probably related □ Possibly Related □ Unlikely Related □ Not related	□ none □ discontinued □ dose adjusted □ interrupted □ unknown □ N/A	□ ongoing □ ongoing and stable □ resolved □ resolved with sequelae □ unknown □ death

I attest that the above information has been reviewed and is true and accurate

______________________________ ______________
Principal Investigator Signature Date

Adverse Event Log Page ____ of ____

6 STATISTICAL PLAN

6.1 Sample Size and Power Considerations

Based on a design with 20 patients per disulfiram treatment group within each dosing cohort (1000 mg, 2000 mg) and a null hypothesis that there will be a differential change in plasma inflammatory cytokine levels (e.g., IL-6) in both groups (disulfiram treatment and placebo compared to baseline, we use reported ranges of plasma IL-6 levels in COVID-19 disease[119] to estimate sample size and power. Based on a mean plasma IL-6 level of 36.7pg/mL (95% CI 21.6-62.3 pg/mL), we estimate that the study will have approximately 80% power to detect a 35% difference in plasma IL-6 levels between the disulfiram versus placebo groups. These measurements will be assessed at day 5, 15 and 31 at the 0.05 significance level using a log-likelihood ratio test and non-linear mixed effect regression modeling. This estimate is based on the assumptions that plasma IL-6 levels are log normally distributed and that the standard deviation of the pre- and post-dose levels is is 12 ng/mL (derived from ref: DE Leisman et al. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes). The adjustments will be made for pre-treatment plasma inflammatory cytokine (e.g., IL-6) levels. Safety endpoints will include the total number of Grade 2 or higher events; the number of Grade 2 or higher events possibly, probably or definitely related to study drug; the number of serious adverse events; and the number and cause for treatment or study discontinuation.

6.2 Statistical Analysis

All main analyses will be conducted on an intention-to-treat basis. Models will assess the relationship between the timing of disulfiram treatment initiation relative to symptom onset and study outcomes for key efficacy/activity endpoints. The model will be fitted using generalized estimating equations (GEE) to handle the repeated measurements, where the comparison between randomized arms will use a two-sided Wald test. Levels of plasma inflammatory biomarker levels will be compared between arms using non-parametric Wilcoxon rank-sum tests (e.g., separately on days 5 and 15) considering results below the assay limit as the lowest rank. For the analysis of the SARS-CoV-2 viral loads, for the comparison of the proportion of participants with detectable SARS-CoV-2 RNA, we will use binary regression, with adjustment for risk stratum and pre-treatment SARS-CoV-2 RNA level.

For longitudinal analyses, we will perform both statistical summaries at each time point and comprehensive regression modeling. For the regression model, a full non-linear mixed effects (NMLE) model will be developed for disulfiram including all 60 participants across the 5 dosing days. The pharmacokinetics of disulfiram and the measured metabolites will be described by compartmental models parameterized in terms of clearance from the central compartment, apparent central and peripheral Vd and intercompartmental clearance. This will be followed by a full covariate screening process and then inclusion into the pharmacokinetic model. This may include covariates like drug interactions due to other therapy, weight, sex and other biologically plausible covariates collected during the study. From the final model, we will provide the $AUC_{0\text{-}72}$, C_{min}, and C_{max} for each patient.

For the primary pharmacodynamics response (plasma inflammatory biomarker levels – e.g., IL-6) the PK and PD data will combined and analyzed simultaneously by nonlinear mixed-effects modelling (NONMEM version 7.1, Globomax LLC, Hanover, PA). Data will be analyzed using the first-order conditional estimation method with interaction, and ADVAN6 will be used to solve the differential

equations. Both empiric and mechanistic based pharmacodynamics models will be evaluated to describe the concentration effect relationship with the primary pharmacodynamics response.

Model selection will be based on visual inspection of diagnostic scatter plots, the objective function value (OBJ) computed by NONMEM and biological plausibility of parameter estimates. All final models will be evaluated by performing a visual predictive check (VPC). For this, 1000 data sets will be simulated from the final parameter estimates using the original data as a template. This approach has been successfully applied for the development of animal and human models. From the final model, Monte-Carlo simulations will be undertaken to evaluate if different dosing schedules (e.g. larger doses less often or a single dose) can optimize the dose-concentration-effect relationship.

As this is an exploratory study, we will collect all possible trial outcomes, including those for participants who discontinue treatment. We will attempt to minimize the amount of missing data as much as possible to avoid bias in the interpretability of the findings. However, in the event that missing data is present we will follow standardized methods for handling these data. For time dependent covariates, if one covariate value of a participant is available, this will be carried forward unless the first value of the covariate is missing, then it will be carried backward to occasions with missing values of the respective covariate. The population median value at each time point will be used for missing data from a continuous variable (e.g. age and weight) for a particular participant. If a covariate, which can be classified as a categorical variable (e.g. sex and race), is recorded missing, the observation will be set to the mode. If either race or sex is found significant and there is missing data of the covariate in question in more than 10% of the population, then a sensitivity analysis will be performed, re-estimating the model with and without the covariate only in the participants that have information of the covariate in question. If a large proportion of participants (>15%) have a missing covariate this may be imputed from other covariates. The imputation will be based on a mixed effect linear regression model developed for the highly correlated covariates. Multiple imputation approach will be used to account for uncertainty in missing values. If any value is missing at baseline, screening values may be used for imputation, if available.

From the time to treatment initiation to clinical recovery or improvement, all participants will be censored at the end of follow-up, not at the time of event (e.g., for mortality). Sensitivity analyses will be performed using imputation of missing data to differentiate discontinuation of participants from treatment versus study evaluations.

Duration of fever, duration of symptoms and duration of time to self-reported return to usual health will be summarized with descriptive statistics. Participant-specific durations of fever and symptoms will be compared between study arms using a two-sided stratified Wilcoxon test with a 5% Type I error rate. Total symptom score will be calculated on each day and the participant-specific area under the curve (AUC) over time will also be compared using a two-sided stratified Wilcoxon test. Participants who do not have complete diary cards due to hospitalization or death will be ranked in these analyses as having poorer outcomes than participants who survived without hospitalization.

6.2.1 Primary endpoint

- Changes in plasma inflammatory biomarker levels[2] (e.g., IL-6, IL-1β) at days 5, 15, and 31.

6.2.2 Secondary endpoint(s):

- Safety and tolerability (frequency of Grade 2 or higher adverse events) at days 2, 3, 4, 5, 7, 9, 11, 15, and 31.
- Change in SARS-CoV-2 PCR quantitative viral load[4] at days 5, 15, and 31.
- Change in COVID-19 clinical stage (five-point symptom severity scale[3]) at days 5, 15, and 31.
- The proportion of detectable versus not detectable levels of SARS-CoV-2 RNA from site-collected NP swabs at entry, day 5, and day 15 among a subset of participants.

6.2.3 Other secondary endpoints

- SARS-CoV-2 specific T- and B- cell responses[5]
- High sensitivity SARS-CoV-2 antibody titer (IgM, IgE, IgA, IgG1, IgG2, IgG3, IgG4)
- Changes in host gene and protein expression from matched blood and respiratory (NP) samples at the single cell level (B, T, natural killer, and myeloid cells).
- Changes in T- and B-cell receptor (TCR/BCR) repertoire from matched blood and respiratory (nasal/oropharyngeal) samples.
- Pyroptosis (aberrant proinflammatory cell death) assays.[6]

6.2.4 Analysis of AE data

Analysis of AE data will primarily be descriptive based on MedDRA coding of events. Rates of AE will be compared between randomized arms using Fisher's Exact Test.

6.2.5 Analysis of plasma inflammatory biomarkers and SARS-CoV-2 antibody levels

Analysis of titers will primarily be descriptive, comparing the geometric mean titers at days 0, 1, 5, 15, and 31 between the randomized arms. Furthermore, it is of interest to describe the entire distributions of plasma inflammatory cytokine levels or anti-SARS-CoV-2 antibody titers by randomized arms and contrast these distributions. Therefore, we will use quantile regression in order to describe whether there is a shift or change in the titer distribution between randomized arms. Given that repeated measures of titers will be obtained, we will account for the correlation in measures within individuals using a cluster bootstrap in order to properly estimate the p-value and 95% confidence intervals. Similar analysis will also be applied to lymphocyte counts on days 0, 1, 5, 15, and 31, hematological measurements (D-dimer, ferritin, LDH, hsCRP) on days 0, 1, 5, 15, and 31, and other lab measures showing right-skewed distributions

7 HUMAN PARTICIPANTS PROTECTIONS

7.1 Risks and Benefits

Human participants' involvement and characteristics. COVID-infected adults 18 years of age and older who are enrolled in the prospective COVID-19 Host Immune Response Pathogenesis (CHIRP) cohort for Aims 1 & 2, and acute COVID-positive individuals 18 years of age and older who completed a Phase 2a trial of disulfiram (IND 151837, NCT04485130) from the UCSF CHIRP cohort.

Sources of materials. Research materials include questionnaire and biospecimen data (serum, plasma, PBMCs, nasopharyngeal swabs, saliva) stored in the UCSF Core Immunology Lab and UCSF AIDS Specimen Bank CHIRP repository will be used to perform new assays as part of the experiments described in this proposal. Data will be identified by participants pre-existing de-identified study number.

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number so that participant confidentiality can be maintained. All records will be kept locked. All computer entry and networking programs will be performed with coded numbers only.

There are no direct benefits to the study participants. However, there are some potential secondary benefits. However, the results from this study may eventually benefit other SARS-Cov-2-infected individuals in the future. This study may provide data to help identify novel therapeutic strategies for treating COVID-19. Participants will be reimbursed to cover costs related to the study (travel, loss of time). The knowledge gained from the proposed study has the potential to impact clinical care and research efforts with regard to individuals infected with SARS-CoV-2.

7.1.1 Potential risks

Confidentiality: Participation in research may involve loss of privacy. Participant's records will be handled as confidentially as possible. All research records will be coded with a four-digit study ID code. Only the study investigators and their staff will have access to study records and test results. Study charts will be kept in a locked file cabinet in a locked office. Electronic data will be protected with a password and kept on a secure network on the UCSF Fresno Network server.. All collaborators will receive specimens only identified by the four-digit study ID. No individual identities will be used in any reports or publications resulting from this study.

Disulfiram: Disulfiram is an irreversible inhibitor of sulfhydryl-containing enzymes resulting in inhibition of ALDH and in the accumulation of acetaldehyde after ingestion of ethanol. It is FDA-approved as a pharmacotherapy for alcoholism. Side effects reported with disulfiram include drowsiness, peripheral neuropathy, hepatotoxicity, seizures, optic neuritis, hypertension, and metallic or garlic-like taste in the mouth. Adverse events associated with disulfiram administration are relatively infrequent occurrences with the proposed FDA approved doses, but we will mitigate against such events by thorough medical history-taking and examination as well as close clinical monitoring during the testing and by the brief duration of administration (up to 5 days total). We will repeat liver function tests at the conclusion of the

studies. Disulfiram produces an aversive reaction to ethanol due to the rapid accumulation of acetaldehyde in the blood. This reaction is characterized by flushing, diaphoresis, palpitations, nausea, vomiting, and sometimes, hypotension. This may last for up to 5 hours and in some cases the reaction has been complicated by myocardial infarct, heart failure, and death. Such reactions will be minimized through education and close clinical monitoring of participants. Participants will also be instructed to abstain from alcohol during disulfiram administration and for two weeks after finishing these studies. They will also be instructed to avoid inadvertent alcohol exposure through various alcohol containing products. Participants are given an Emergency Card that lists disulfiram as a current medication and provides contact information for study investigators. Participants have access to study investigators 24 h/d, 7 d/week for any medical issues or questions that may arise.

Risk of Pregnancy for Women of Childbearing Potential: Women of childbearing potential are not excluded from these studies. They must have a plan for avoiding pregnancy while on study protocol and this is discussed with each woman during the assessment process. We also test women for pregnancy in the medical workup for study entry. A woman would be discharged from the study if the test were to be positive and referred to standard prenatal care. The effects of disulfiram on an unborn baby are unknown. All participants will be counseled to use two forms of birth control during the study. Females of childbearing potential must have a negative pregnancy test at screening and agree to use a double-barrier method of contraception throughout the study period.

Safety measures

Recruitment and informed consent: Informed consent for phlebotomy will be obtained as part of the consent for enrollment into the disulfiram trial. This study has been reviewed by Community Medical Centers Institutional Review Board. All participants will be informed of the discomforts, risks, and benefits of blood sampling
Monitoring: Participants will be monitored carefully throughout the study. At each visit, participants will be assessed for any new symptoms and vital signs will be obtained by a study coordinator.

Protection against risks: Research records will be confidential to the extent permitted by law. Participants will be identified by a code, and personal information from study records will not be released without the participant's permission. Study participants will not be identified in any publication about this study.

7.2 Definitions

Adverse Event (AE): Any untoward medical occurrence in a clinical investigation participant who has received a study intervention and that does not necessarily have to have a causal relationship with the study product. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of the study product, whether or not considered related to the study product.

Serious Adverse Event (SAE): Any adverse event that results in any of the following outcomes:

- Death
- Life-threatening (immediate risk of death)
- Prolongation of existing hospitalization

- Persistent or significant disability or incapacity
- Important medical events that may not result in death, be life threatening, or require intervention or escalation of care may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

Unexpected Adverse Event (UAE): An adverse reaction, the nature or severity of which is not consistent with the investigator's brochure.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): An adverse reaction, the nature of which is not consistent with the investigator's brochure with severity as defined by SAE above.

Unanticipated Problem (UP): Unanticipated Problem that is not an Adverse Event (e.g. breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug).

Protocol Deviation: Deviation from the IRB-approved study procedures. Designated serious and non-serious

Serious Protocol Deviation: Protocol deviation that is also an SAE and/or compromises the safety, welfare or rights of participants or others

7.3 Safety Reporting Requirements

7.3.1 Reporting Interval

All AEs and SAEs will be documented from the first administration of study product. All AEs and SAEs will be followed until resolution even if AEs extend beyond the study-reporting period. Resolution of an adverse event is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic. At any time after completion of the study, if the investigator becomes aware of a SAE that is suspected to be related to study product.

7.3.2 Investigator's Assessment of Adverse Events

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose adverse event information, provide a medical evaluation of adverse events, and classify adverse events based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

Laboratory abnormalities will be reported as AEs if there is a 2 standard deviation increase above baseline.

7.3.3 Assessment of Seriousness

Event seriousness will be determined according to the protocol definition of an SAE.

Event severity will be graded using the DAIDS toxicity table
https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

7.3.4 Assessment of Association

The association assessment categories that will be used for this study are:

- **Associated –** The event is temporally related to the administration of the study product and no other etiology explains the event.
- **Not Associated –** The event is temporally independent of the study product and/or the event appears to be explained by another etiology.

The investigator must provide an assessment of association or relationship of AEs to the study product based on:

- Temporal relationship of the event to the administration of study product
- Whether an alternative etiology has been identified
- Biological plausibility
- Existing therapy and/or concomitant medications.

7.4 Safety Oversight

7.4.1 Monitoring Plan

All AE and SAE will be reviewed by protocol team weekly, or more often if needed.

We will develop an independent Safety Monitoring Committee (SMC) or Data Safety Monitoring Board (DSBM) prior to the initiation of of the study. We expect to recruit independent experts in **infectious disease medicine, pulmonary medicine, emergency medicine and biostatistics without conflicts of interest.**. Primary safety endpoints will be developed in collaboration with the study sponsor and reviewed with the SMC/DSMB before the study is initiated. The SMC/DSMB will first meet after the first 5 participants have completed 16 weeks of observation. The SMC or DSMB will meet quarterly during the study and at approximately 2 to 4 weeks after the last person's follow-up are completed. The members of the SMC will be independent of the study team and free of any conflict of interest with the investigation and the sponsors.In order to monitor study participants for potential adverse events, the study coordinator will perform telephone symptom/adverse event visits on Days 2, 3, 4, 7, 9, and 11, in addition to the in-person monitoring on Days 5, 15, and 31 (see Table 1 Schedule of Events). This is particularly important for Days 6-14 during which there are no scheduled in-person study visits after completion of the study treatment. Study staff will call the participant on these days to verify their symptoms and evaluate for any adverse events that need to be reported to the study investigators.

7.5 Study compliance with clinical trial requirements

Study will be conducted in accordance with GCP and ICH. Specifically, regular QC will be conducted to document the following:

- There is documentation of the informed consent process and signed informed consent documents for each participant
- There is compliance with recording requirements for data points
- All SAEs are reported as required
- Individual participant study records and source documents align
- Investigators are in compliance with the protocol
- Regulatory requirements as per Office for Human Research Protections (OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed.

8 STUDY MODIFICATION

8.1 Criteria for Discontinuation of the Study

Two cohorts of participants (N=30 each) will be sequentially enrolled and receive disulfiram daily for five days at doses of 1000mg or 2000mg. The independent Safety Monitoring Committee will review the safety and tolerability data after the first cohort has completed dosing and determine whether it is safe to proceed with enrolling the final cohort using the below criteria.

In addition, the study enrollment and dosing will be stopped and an ad hoc review by the SMC will be performed if any of the specific following events occur or, if in the judgment of the study physician, participant safety is at risk of being compromised:

a. Death within one hour of drug administration

b. Occurrence of a life-threatening allergic/hypersensitivity reaction (anaphylaxis), manifested by bronchospasm with or without urticaria or angioedema requiring hemodynamic support with pressor medications or mechanical ventilation, TRALI, TACO.

c. One participant with an SAE associated with study product.

d. Two participants with a Grade 3 or higher lab toxicity for the same parameter associated with study product. (Grading will be assessed using DAIDS Toxicity Table https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf)

e. An overall pattern of symptomatic, clinical, or laboratory events that the SMC considers associated with study product and that may appear minor in terms of individual events but that collectively may represent a serious potential concern for safety.

f. Any other event(s) which is considered to be a serious adverse event in the good clinical judgment of the responsible physician. This will be appropriately documented.

8.2 Individual Criteria for Discontinuation

A participant will be closely monitored if AST or ALT > 3 x ULN, and a participant should be removed from disulfiram treatment if AST or ALT > 5 x ULN (DAIDS Grade 3 toxicity).

A participant whose treatment is terminated should remain in the study for appropriate follow up assessments as outlined in section 6.2.

9 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

9.1 Ethical Standard

UCSF is committed to the integrity and quality of the clinical studies it coordinates and implements. UCSF will ensure that the legal and ethical obligations associated with the conduct of clinical research involving human participants are met. The information provided in this section relates to all UCSF sites participating in this research study

This assurance commits a research facility to conduct all human participants' research in accordance with the ethical principles in The Belmont Report and any other ethical standards recognized by OHRP. Finally, per OHRP regulations, the research facility will ensure that the mandatory renewal of this assurance occurs at the times specified in the regulations.

9.2 Institutional Review Board

At UCSF main campus, this study will be reviewed by Advarra, under an intent to relay agreement via the UCSF IRB. At UCSF Fresno, this study will be reviewed by Community Medical Centers Institutional Review Board.

9.3 Informed Consent Process

The informed consent process will be initiated before a volunteer agrees to participate in the study and should continue throughout the individual's study participation. The California Subject's Bill of Rights will be provided to the potential participant prior to the informed consent. Informed consent will be obtained in accordance with site SOP for COVID consenting and in compliance with current FDA guidance for consenting in setting of COVID pandemic https://www.fda.gov/media/136238/download. The method of consent includes both in person and telephone/electronic consent. A copy of the signed informed consent document will be given to the participant for their records prior to any study related procedures being completed. The consent will explain that participants may withdraw consent at any time throughout the course of the trial. Extensive explanation and discussion of risks and possible benefits of this investigation will be provided to the participants in understandable language. Adequate time will be provided to ensure that the participant has time to consider and discuss participation in the protocol. The consent will describe in detail the study interventions/products/procedures and risks/benefits associated with participation in the study. The rights and welfare of the participants will be protected by emphasizing that their access to and the quality of medical care will not be adversely affected if they decline to participate in this study.

9.4 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsors and their agents. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. The results of the research study may be published, but participants' names or identifiers will not be revealed. Records will remain confidential. To maintain confidentiality, the PI will be responsible for keeping records in a locked area and results of tests coded to prevent association with participants' names. Data entered into computerized files will be accessible only by authorized personnel directly involved with the study and

will be coded. Participants' records will be available to the FDA, investigators at the site involved with the study, and the IRB.

9.5 Future Use of Stored Specimens

Participants will be asked for consent to use their samples for future testing before the sample is obtained. The confidentiality of the participant will be maintained. There will be no plans to re-contact them for consent or to inform them of results. The risk of collection of the sample will be the small risk of bruising or fainting associated with phlebotomy however these samples will be taken at the same time as other protocol required samples. These samples will be used to answer questions that may arise while the study is underway or after it is completed. If for instance, there were unanticipated AEs, blood samples could be used to run tests that might help determine the reason for the AEs.

9.6 Data management and monitoring

Whole blood for local safety labs will be collected and processed by Community Medical Center laboratory. This will include complete blood count with differential, comprehensive metabolic panel, western sedimentation rate, C-reactive protein, D-Dimer, Ferritin, and urine pregnancy test. If subject is known to be HIV positive, CD4/CD8 T cell count and HIV RNA standard assay will be completed. Local labs will be reviewed by the Investigator to monitor safety. Any abnormal values will be assessed for clinical significance by the Investigator. Any clinically significant laboratory results will be reported as an adverse event and assessed for causality.

Research labs that include nasopharyngeal (NP) swab for PCR + IgG, IgM, IgA, cytokine quantification; Saliva for host genomics will be collected, labeled with the subject ID, visit number and collection date. Saliva and NP swab will be frozen and stored in the -80 freezer located in the UCSF Fresno Clinical Research Center until requested by UCSF Core Immunology Lab (CIL). The freezer is locked and requires badge access. All blood collected for research only purposes will be sent to UCSF CIL laboratory by courier on the same day as collection.

9.6.1 Source Documents

The primary source documents for this study will be the participants' medical records. If the investigators maintain separate research records, both the medical record and the research records will be considered the source documents for the purposes of auditing the study. The investigator will retain a copy of source documents. The investigator will permit monitoring and auditing of these data and will allow the IRB and regulatory authorities access to the original source documents. The investigator is responsible for ensuring that the data collected are complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected and entered into the study database and must be signed and dated by the person recording and/or reviewing the data. All data submitted should be reviewed by the site investigator and signed as required with written or electronic signature, as appropriate. Data entered into the study database will be collected directly from participants during study visits or will be abstracted from participants' medical records. The participants' medical records must record their participation in the

clinical trial and what medications (with doses and frequency) or other medical interventions or treatments were administered, as well as any AEs experienced during the trial.

9.6.2 Data Management Plan

Study data will be collected at the study site(s) and entered into the study database. Data entry is to be completed on an ongoing basis during the study.

9.6.3 Data Capture Methods

Clinical data will be entered into a 21 CFR 11-compliant Internet Data Entry System (IDES). The data system includes password protection and internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

9.6.4 Study Record Retention

The PI is responsible for retaining all essential documents listed in the ICH GCP Guidelines. The FDA requires study records to be retained for up to 2 years after marketing approval or disapproval (21 CFR 312.62), or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agent for a specific indication. These records are also to be maintained in compliance with IRB/IEC, state, and federal medical records retention requirements, whichever is longest. All stored records are to be kept confidential to the extent provided by federal, state, and local law.

No study document should be destroyed. Should the investigator wish to assign the study records to another party and/or move them to another location, the site investigator must provide written notification of such intent to sponsor with the name of the person who will accept responsibility for the transferred records and/or their new location. The sponsor must be notified in writing and written permission must be received by the site prior to destruction or relocation of research records.

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