Study Title: Combination Latency Reversal with High Dose Disulfiram plus Vorinostat in HIV-infected individuals on ART (DIVA): A Single Arm Clinical Trial

Protocol ID: MSD IIS-55750

Version: 4.0

Date: 17 June 2019

NCT number: NCT03198559

Certain information within this clinical study report has been redacted (ie, specific content is masked irreversibly from view with a black bar) to protect either personally identifiable information or company confidential information.

This may include, but is not limited to, redaction of the following:

- Named persons or organizations associated with the study;
- Proprietary information, which is considered confidential information;
- Other information as needed to protect confidentiality of Merck, personal information, or to otherwise protect the integrity of the clinical study.

PROTOCOL

Combination Latency Reversal with High Dose Disulfiram plus Vorinostat in HIV-infected individuals on ART (DIVA): A Single Arm Clinical Trial

Protocol ID: MSD IIS-55750
Version: #4.0
Date: 17 June 2019

Author/s:
Prof Sharon Lewin, Dr James McMahon and Dr Thomas Rasmussen

Sponsor/s:
The Peter Doherty Institute for infection and Immunity, The University of Melbourne

Statement of Compliance

This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

Approval:

[redacted]

17 June 2019

Sponsor Signature: Professor Sharon Lewin *Date*

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the Doherty Institute with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: MSD-II-55750

Protocol Title: Combination Latency Reversing Therapy with Disulfiram plus Vorinostat in HIV Infected Individuals on Antiretroviral Therapy (ART): A Single Arm Clinical Trial

Protocol Date: 17 June 2019

_____ _____

Investigator Signature ***Date***

_____

Print Name and Title

Site Name _____

Address _____

_____

_____

_____

Phone Number _____

TABLE OF CONTENTS

STUDY SYNOPSIS

Title:	Combination latency reversing therapy with high dose disulfiram plus vorinostat in HIV-infected individuals on antiretroviral therapy (ART): a single arm clinical trial
Short Title:	Combination HIV latency reversal with disulfiram and vorinostat
Design:	Single arm clinical trial
Sponsor	The Peter Doherty Institute for Infection and Immunity
Study Centre:	Alfred Hospital
Study Question:	To assess the feasibility, toxicity and effects of high dose disulfiram and vorinostat in HIV individuals on suppressive ART
Primary Objectives	**Primary efficacy objective:** To determine the effect of 28 days of high dose disulfiram with intermittent administration of 3 days of vorinostat on plasma HIV RNA in HIV infected individuals on suppressive ART **Primary tolerability objective:** To determine the tolerability of 28 days of high dose disulfiram with intermittent administration of 3 days of vorinostat in HIV infected individuals on suppressive ART
Secondary Objective	To determine the effect of 28 days of high dose disulfiram with intermittent administration of 3 days of vorinostat on the frequency of latently infected CD4+ T cells in HIV infected individuals on suppressive ART
Inclusion Criteria:	– Age 18-65 years with documented HIV-1 infection – Receiving combination ART with plasma HIV RNA <50 copies/mL for >3 years – CD4+ T cell count >350/μL at screening – Able to provide informed consent – WOCBP with a negative pregnancy test at screening (visit #1) 1 AND agrees to use one of the following methods of contraception to avoid pregnancy o Complete abstinence from penile-vaginal intercourse from 2 weeks prior to administration of high dose disulfiram, throughout the study, and for at least 2 weeks after discontinuation of all study medications o Double barrier method (male condom/spermicide, male condom/diaphragm, diaphragm/spermicide) o Any intrauterine device (IUD) with published data showing that the expected failure rate is <1% per year o Male partner sterilization ***confirmed prior to the female subject's entry*** into the study, and this male is the sole partner for that subject o Approved hormonal contraception (Where other medications to be used in the study (e.g., efavirenz and darunavir) are known,

	or are likely, to significantly interact with systemic contraceptives, resulting in decreased efficacy of the contraceptive, then alternative methods of non-hormonal contraception are recommended) o Any other method with published data showing that the expected failure rate is < 1% per year. – Women of non-child-bearing potential defined as: o > 12 months of spontaneous amenorrhea and ≥ 45 years of age, or o Documented medical history of one of the following: hysterectomy, bilateral oophorectomy or tubal ligation. – Willing to abstain from alcohol consumption from one day before to 14 days after completing study therapy
Exclusion Criteria:	– 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 metronidazole or any drug formulation that contains alcohol or that might contain alcohol, including the gelatin capsule and liquid formulations of ritonavir, ritonavir/lopinavir, amprenavir and fosamprenavir, and alcohol-containing preparations such as cough syrups, or tonics – Current use of tipranavir or Maraviroc – Current use of zidovudine, stavudine or didanosine (as disulfiram potentially has potent irreversible inhibitory effects on mitochondrial metabolism and hence could exacerbate the toxicity of these drugs) – Concurrent use of rivaroxaban (a CYP3A metabolized medication) as the cytochrome P450 inhibitory effects of disulfiram on rivaroxaban are unknown – Current use of warfarin – Individuals who intend to modify antiretroviral therapy during the study period for any reason – Significant myocardial disease (current myocarditis or reduced left ventricular ejection fraction below the lower limit of normal) or diagnosed coronary artery disease – Significant renal disease (eGFR <50mL/min) – History of psychosis, seizure disorder, abnormal electroencephalogram or brain damage with significant persisting neurological deficit – Prior malignancy active within the previous 3 years except for local curable cancers that have been apparently cured, such as basal or squamous cell skin cancer, superficial bladder cancer or carcinoma in situ of the prostate, cervix or breast – Known hypersensitivity to disulfiram or vorinostat or contraindications to treatment with these agents – Participation in another LRA study or receipt of vorinostat or disulfiram within the previous 12 months before starting the investigational treatment (visit #2) – Any significant acute medical illness requiring hospitalization within preceding 8 weeks

	– Hepatitis B or C co-infection as determined by detection of HBsAg or HCV RNA (Individuals with prior hepatitis infection that is now cleared are eligible for enrolment) – Receipt of immunomodulating agents (excluding immunization) or systemic chemotherapeutic agents within 28 days prior to study entry – Current or recent gastrointestinal disease or surgery that may impact the absorption of the investigational drug – Active substance use that in the opinion of the investigator will prevent adequate compliance with study procedures – Women who are currently pregnant or breastfeeding – WOCBP who are unwilling or unable to use an acceptable method of contraception to avoid pregnancy (as specified in the inclusion criteria) – Unable or unwilling to adhere to protocol procedures – The following laboratory values within 3 weeks before starting the investigational drug (lab tests may be repeated to obtain acceptable values before failure at screening is concluded) o Hepatic transaminases (AST or ALT) ≥3 x upper limit of normal (ULN) o Serum total bilirubin ≥1.5 x ULN o eGFR <50 mL/min o Haemoglobin <10.0 g/dL o Platelet count ≤100 x10^9/L o Absolute neutrophil count ≤1.5x10^9/L o Serum potassium, magnesium, phosphorus outside normal limits o Total calcium (corrected for serum albumin) or ionized calcium ≤lower normal limits
Number of Planned Participants:	15
Investigational product:	Disulfiram (2000mg) tablets daily for 28 days (days 1 – 28) Vorinostat (400mg) tablets once daily on days 8, 9, 10 and days 22, 23, 24
Statistical Methods:	**Sample size:** The primary comparison for study is the level of plasma HIV RNA on day 11 during disulfiram/vorinostat treatment compared to baseline. Based on two previous studies of latency reversing agents (LRAs), where treatment with disulfiram 2000 mg or romidepsin led to increases in plasma HIV RNA, we assume a pre-study mean plasma HIV RNA of 1 copy/mL and a standard deviation of 11 copies/mL. Based on these numbers, the study will require 14 participants to detect an increase in plasma HIV RNA of 10 copies/mL with 80% power at a 0.05 significance level. Targeted enrolment is 15 participants to accommodate for loss to follow-up. **Analyses of primary and secondary endpoints**

	The primary comparisons will be made using paired t-test or signed-rank test as appropriate. By applying the same statistical methods, we will also analyse changes in CA-US HIV RNA following administration of high dose disulfiram and vorinostat. We will use repeated measurement analysis of variance statistics and/or generalized estimating equation statistics to analyse overall changes in primary and secondary endpoints during study intervention by including all data points during study therapy. P-values <0.05 will be considered significant. Assessment of tolerability will include all participants who have received at least one dose of disulfiram. AEs and SAEs will be summarized according to severity assessment showing the number and percentage of participants experiencing at least one event, the number of events, and the causality assessment. No formal statistical comparisons will be made - AE rates are presented for descriptive purpose

1. GLOSSARY OF ABBREVIATIONS & TERMS

Abbreviation	Description
AE	**A**dverse **e**vent
ART	**A**nti**r**etroviral **t**herapy
CA-US HIV RNA	**C**ell **A**ssociated **U**n**s**pliced HIV **RNA**
CD4+ T-cells	White blood cells that are an essential part of the human immune system
CTCL	**C**utaneous **T**-**c**ell **l**ymphoma
DMSO	**D**i**m**ethyl **s**ulf**o**xide
DNA	**d**eoxyribo**n**ucleic **a**cid
ECG	**E**lectro**c**ardio**g**raphy
eCRF	**e**lectronic **c**ase **r**eport **f**orm
eGFR	**e**stimated **g**lomerular **f**iltration **r**ate
HBsAg	**h**epatitis **B** **s**urface **a**nti**g**en
HCV	**H**epatitis **C** **v**irus
HDAC	**H**istone **De**a**c**etylase
HDACi	**H**istone **De**a**c**etylase **i**nhibitor
HIV	**H**uman **I**mmunodeficiency **v**irus
HREC	**H**uman **R**esearch **E**thics **C**ommittee
LRA	**L**atency **R**eversing **A**gents
LTR	HIV **L**ong-**t**erminal **R**epeat, the promotor for HIV transcription
PBMC	**P**eripheral **B**lood **M**ononuclear **C**ell
PCR	**P**olymerase **C**hain **R**eaction
pK	**P**harmaco**k**inetics
qPCR	**q**uantitative **P**olymerase **C**hain **R**eaction
RNA	**R**ibo**n**ucleic **a**cid
SAE	**S**erious **a**dverse **e**vent
SOC	**S**tandard **o**f **c**are

Abbreviation	Description
SUSAR	**S**uspected **U**nexpected **S**erious **A**dverse **R**eaction
TGA	**T**herapeutics **G**oods **A**dministration (Australia)
TILDA	**T**at/rev **I**nduced **L**imiting **D**ilution **A**ssay.
WOCBP	**W**omen **o**f **C**hild **B**earing **P**otential

2. RESEARCHERS

2.1 COORDINATING PRINCIPAL INVESTIGATOR

Professor Sharon R Lewin
Director – The Peter Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital
792 Elizabeth Street
Melbourne VIC 3000
Australia
Email: sharon.lewin@unimelb.edu.au
[redacted]

Consultant infectious diseases physician,
Department of Infectious Diseases, Alfred Hospital and Monash University
50 Commercial Road,
Melbourne VIC 2004
Australia

2.2 ASSOCIATE INVESTIGATORS

Dr James McMahon
Infectious diseases physician
Department of Infectious Diseases, Alfred Hospital and Monash University
50 Commercial Road,
Melbourne VIC 3004
Australia
[redacted]

Dr Thomas Aagaard Rasmussen
Associate Clinical Director of HIV CURE Studies
Doherty Institute
792 Elizabeth Street
Melbourne VIC 3000
Australia
[redacted]

2.3 COLLABORATOR

Associate Professor Sarah Palmer
Deputy Director, Centre for Virus Research
Westmead Millennium Institute for Medical Research
University of Sydney
[redacted]

2.4 CONTACT

[redacted]

3. INTRODUCTION/BACKGROUND INFORMATION

3.1 LAY SUMMARY

Antiretroviral therapy (ART) dramatically reduces HIV replication leading to restoration of immune function and a near normal life expectancy, but treatment is lifelong and there is no cure. The major barrier to a cure is the persistence of long lived CD4+ T-cells that contain a "silenced" form of HIV, called HIV latency.

The purpose of this research is to investigate whether it may be possible to reduce the amount of dormant HIV infection in immune cells, by "turning on" or activating the virus and hence force it out of the latently infected memory T cells. This leads to production of HIV by the cell, which will either die or will be recognised and eliminated by the immune system. As very few T cells are latently infected with HIV, the death of these cells is not expected to affect the function of the immune system and further infection of new cells is expected to be prevented by ART

This study is looking at whether taking a short course of two drugs: high dose disulfiram for 28 continuous days with 2 intermittent 3 day courses of vorinostat (on days 8 – 10 and on days 22-24 of treatment) in addition to regular ART, will activate virus production from latency. The ultimate aim of this research is to identify a potent intervention to reverse latency that could then be combined with other interventions to achieve HIV remission.

Disulfiram, (Antabuse®), is a licensed medication for the treatment of alcohol addiction. Disulfiram inhibits aldehyde dehydrogenase and therefore interferes with the metabolism of alcohol. Alcohol is broken down in the body to a compound called acetaldehyde. This is then normally broken down further by an enzyme in the liver called aldehyde dehydrogenase.

Disulfiram stops this enzyme from working, causing an accumulation of acetaldehyde in the blood stream which causes unpleasant symptoms such as flushing, headaches, nausea and vomiting which are enough to deter people from drinking alcohol. In the laboratory and in a recent clinical trial in HIV-infected individuals on ART, high dose disulfiram was shown to increase production of HIV from latently infected cells and therefore this drug may have a potential role in eliminating HIV that persists on ART.

Vorinostat is an anti-cancer (antineoplastic or cytotoxic) drug, known as a Histone Deacetylase (HDAC) Inhibitor. HDACs are enzymes normally present in cells that affects regulation of gene expression, which is the process by which a gene's information is converted into the structures and functions of a cell. By inhibiting HDAC, vorinostat causes cancer cells to die. Through this mechanism Vorinostat is effective in the treatment of different types of cancer. Vorinostat is approved by the Australian Therapeutic Goods Administration (TGA) for the treatment of subcutaneous T-cell lymphoma (a type of skin cancer). In the laboratory and in several clinical trials in HIV-infected individuals on ART, vorinostat was shown to increase production of HIV from latently infected cells and therefore this drug may have a potential role in eliminating HIV that persists on ART. Both disulfiram and vorinostat are not approved by the TGA for the treatment of HIV.

This study involves study participants to attend the clinic at the Alfred hospital approximately 9 times over a 6 month period to attend specific study visits. These visits will take place at screening, baseline (day 0), days 8, 11, 22, 25, 28, 56 and 196 (month 6).

Study participants will be enrolled after all the inclusion criteria are met. During the course of the study, the investigators will meet regularly to monitor study progress and participant safety by reviewing all information, including side effects.

3.2 INTRODUCTION

One strategy aimed at reducing the frequency of latently infected cells in HIV-infected individuals on antiretroviral therapy (ART) is the use of pharmacological agents to reverse HIV latency, thereby initiating virus-mediated cell lysis or immune-mediated killing[1]. Recent clinical trials of latency reversing agents (LRAs) in HIV infected subjects on ART, including histone deacetylase inhibitors (HDACi)[2-4] and the anti-alcoholism drug disulfiram[5,6], have shown that inducing an increase in CA-US or plasma HIV RNA is possible. Yet, these interventions did not have a demonstrable effect on the frequency of latently infected cells or time to viral rebound after cessation of ART[3], potentially because latency reversal alone didn't trigger an adequate immune response or cell death or that the potency of latency reversal with a single-agent intervention over a very short period of time, lacked sufficient potency, as suggested by recent in vitro studies[7]. It is highly likely that long term remission off ART will require interventions that lead to both a reduction in latently infected cells and an increase in HIV-specific immunity, therefore identifying a strategy to increase viral antigens on the surface of latently infected cells will be a key component of this strategy. Here we propose a single-arm clinical trial of high-dose disulfiram together with intermittent administration of the HDACi vorinostat in HIV-infected individuals on ART as a strategy to increase potency of latency reversal with low risk of increased toxicity

3.3 BACKGROUND INFORMATION

There are multiple factors that lead to the establishment and maintenance of HIV latency[8]. These include the low availability of key transcription factors in resting CD4+ T cells[9] as well as the chromatin environment of the integrated provirus leading to transcriptional silencing or interference[8]. HDACi increase histone acetylation, which promotes initiation of HIV transcription, but additional blocks in RNA splicing, RNA export and low availability of cellular transcription factors may limit the efficiency of virus production[10]. It is plausible that targeting several latency mechanisms will enhance the expression of viral proteins or production of free virions in latently infected resting CD4+ T cells.

When resting CD4+ T cells from HIV-infected individuals on suppressive ART were exposed to combinations of LRAs ex vivo, there was a synergistic increase in HIV production, in some cases approaching that of maximal T cell activation[11]. The most striking synergy was seen with combinations of potent HDACis such as romedepsin and the protein kinase C (PKC) agonist bryostatin-1, but their associated toxicities may not allow for clinical co-administration. Other combinations that are more feasible in the clinical setting, also showed at least additive effects in vitro. For example, in contrast to either drug alone, the combination of HDACi and disulfiram consistently increased levels of intracellular HIV RNA relative to DMSO (dimethyl sulfoxide) controls ex vivo[11]. Synergy analyses indicated that HDACi and disulfiram reverse HIV latency by independent mechanisms[11]. Whereas HDACi reverse HIV latency by promoting histone acetylation and chromatin relaxation[12], disulfiram activates HIV transcription by depletion of phosphatase and tensin homolog (PTEN), which alleviates inhibition of the Akt signalling pathway. Activated Akt phosphorylates hexamethylene bis-acetamide inducible 1 (HEXIM1), which subsequently causes release of positive transcription elongation factor b (p-TEFb) to enhance HIV transcription. P-TEFb is otherwise only present at very low levels in resting CD4+ T cells[13].

Safety and efficacy of disulfiram and vorinostat as LRAs in vivo

Disulfiram and vorinostat have both been shown to increase HIV transcription in vitro[14-16] and in clinical trials[2,6,17]. With its long-standing use to treat alcoholism, favourable tolerability and lack of mutagenic potential, disulfiram can be administered for extended periods. Vorinostat (Zolinza®), is a pan HDAC inhibitor[18] with regulatory approval for the treatment of cutaneous T cell lymphoma. Although less potent than the more recently licensed HDACi panobinostat and romidepsin, vorinostat has the advantage of being orally administered and has an extensive safety profile. In vitro, compared to other HDACi, vorinostat had minimal effects on suppressing HIV-specific T-cell function, in contrast to romidepsin which potently suppressed T-cell function[19]. Finally, we previously showed that vorinostat has acceptable tolerability during daily dosing for 14 days, which led to increases in HIV transcription in ART-suppressed HIV patients in blood and in rectal tissue and no adverse effects on HIV-specific T-cells in vivo[2]. In addition, long term follow up of these participants for two years demonstrated no adverse clinical or virological effects of vorinostat in HIV-infected individuals on ART [Mota, submitted].

Here we propose to administer disulfiram at 2g/day for 28 days with two additional short courses of vorinostat. The rationale here is to give an LRA for a prolonged period alone and in combination. Disulfiram is the only LRA that has an appropriate toxicity profile, which would allow for prolonged administration. Based on our experience with two clinical studies of disulfiram in HIV infection given at standard dose (500mg/day) for 14 days[5] and at high dose

(1 and 2 g/day) for 3 days[6], we believe that administration at 2g/day for 28 days is safe and feasible. Previously, disulfiram was used in the clinical setting at relatively high doses ranging from 1-3 g[20] and up to 6g[21] in individuals with alcoholism, and was generally reported to be safe with the most significant safety concern being fatal disulfiram-induced hepatitis, which is estimated to occur extremely rarely at 1 per 25,000 patients treated/year[20].

In addition, vorinostat will be administered on two occasions for 3 days each. In our study of 14 days of vorinostat, we observed maximal increase in both histone acetylation and US RNA after 8-24 hours of the initial dose and minimal adverse events[2]. With repeated vorinostat dosing, we saw no evidence of a refractory response to vorinostat but a sustained increase in US RNA up to 84 days following repeated administration[2], however, others have reported a potential suppression of the effects of vorinostat after at least 4 courses of 3 days of vorinostat[22]. By administering vorinostat as two separate courses in this study, we will reduce the possibility of a refractory response. Furthermore, in this study we can assess whether addition of vorinostat after 7 days of disulfiram will lead to higher increases in plasma HIV RNA relative to disulfiram alone (first seven days of study) or if potential "priming" is occurring leading to an enhanced response after a second administration of 3 days of vorinostat.

It remains unclear whether the sequence of these two LRAs will have an impact on the potency of latency reversal. Most in vitro studies that have assessed combination latency reversal have administered the two agents concurrently[11,29,30,31]. In this study, we believe the initial administration of disulfiram for 7 days prior to administration of vorinostat will provide additional information of the effects of high dose disulfiram that was not addressed in previous studies[6].

In conclusion, combinations of LRAs that target different pathways that maintain HIV latency may significantly enhance the potency of latency reversal. As disulfiram and vorinostat have already been evaluated alone in HIV-infected individuals on ART, have additive effects on HIV transcription in vitro and ex vivo and have acceptable safety profiles, the combination of these two drugs is ideally suited for the first combination LRA study to be performed in HIV-infected individuals on ART.

4. STUDY OBJECTIVES

4.1 HYPOTHESES

4.1.1 Primary hypothesis:

Administration of 28 days of disulfiram with intermittent administration of 3 days of vorinostat will reverse HIV latency in HIV infected patients on ART as measured by plasma HIV RNA.

4.1.2 Secondary hypothesis:

Latency reversal with 28 days of disulfiram and intermittent administration of 3 days of vorinostat on two occasions will reduce the frequency of latently infected CD4+ T cells through virus- or immune-mediated cell lysis.

4.2 OBJECTIVES

4.2.1 Primary efficacy objective:

To determine the effect of 28 days of disulfiram with intermittent administration of 3 days of vorinostat on two occasions on plasma HIV RNA in HIV infected individuals on suppressive ART.

4.2.2 Primary safety objective:

To determine the tolerability of 28 days of disulfiram with intermittent administration of 3 days of vorinostat on two occasions in HIV infected individuals on suppressive ART.

4.2.3 Secondary objective:

To determine the effect of 28 days of disulfiram with intermittent administration of 3 days of vorinostat on two occasions on the frequency of latently infected CD4+ T cells in HIV infected individuals on suppressive ART.

4.3 OUTCOME MEASURES

4.3.1 Primary endpoint

– Plasma HIV RNA on day 11 relative to baseline

4.3.2 Secondary endpoints

– Tolerability as measured by the incidence and severity of adverse events
– Plasma HIV RNA relative to baseline at additional time points
– HIV transcription measured by cell-associated unspliced HIV RNA (CA-US HIV RNA) in peripheral blood CD4+ T cells relative to baseline
– Cell-associated total and integrated HIV DNA in peripheral blood CD4+ T cells relative to baseline
– The frequency of inducible virus as measured by Tat/rev limiting dilution assay (TILDA) in peripheral blood CD4+ T cells relative to baseline
– Concentrations of vorinostat and disulfiram (including its metabolites) in plasma
– p24 expression in CD4+ T-cells relative to baseline

5. STUDY DESIGN

5.1 STUDY SITES

Study participants will be enrolled at one site:

- The Department of Infectious Diseases, Alfred Hospital, Melbourne

Laboratory support for the virological and immunological assays will be performed at three sites:

[redacted]

[redacted]

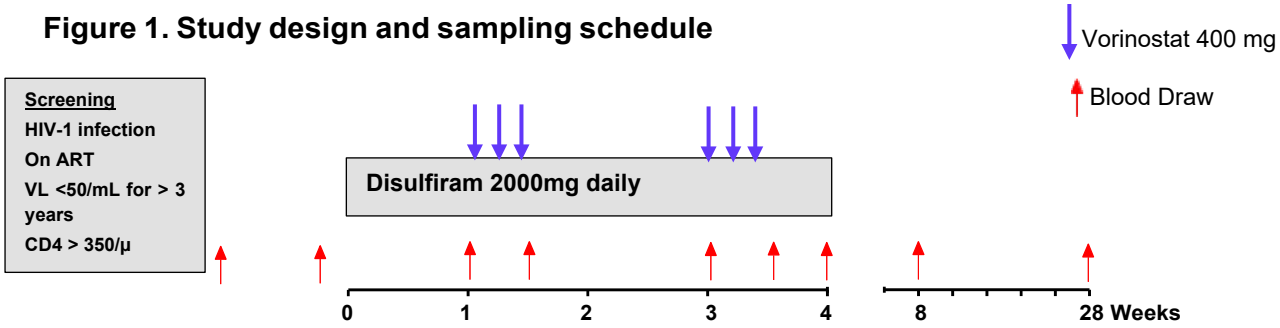
5.2 STUDY DESIGN

This is a single arm, dual site, clinical trial in HIV-infected patients on suppressive ART with a before-after design (figure 1). Targeted enrolment is 15 study participants. All enrolled study participants will receive treatment with

- Disulfiram 2000mg once daily for 28 days (days 1 - 28), plus
- Vorinostat (400mg, once daily) given on days 8, 9 10 and 22, 23 and 24. Total of 6 days.

The duration of study therapy is 28 days.

Figure 1. Study design and sampling schedule



5.3 APPROXIMATE DURATION OF STUDY PARTICIPATION

Study participants will be in the study for up to 203 days. The duration of participation is calculated from the initial screening visit (visit #1) to the last study visit (visit #9) using the first and last day, respectively, of those visit windows.

5.4 APPROXIMATE DURATION OF THE STUDY

The study will be completed in approximately 12 months. The end of the study is defined as last patient enrolled last visit.

5.5 SOURCE DATA

The following documents are defined as source data:

- Patient (medical) charts
- Print outs of biochemical, immunological and virological measurements from Alfred Health Pathology/Clinical Biochemistry/Clinical Immunology laboratories
- Alcohol questionnaire. The study participant will enter answers directly into the electronic questionnaire within the database. There will be no paper copy. The database will date and time stamp when the questionnaire was started. A copy of the completed questionnaire will be made available to the site, by the sponsor as per GCP requirement section 4.9.2.
- Direct results from other laboratory analyses performed during the study (including and not limited to)
    - Cell-associated US HIV-RNA

- Cell associated total and integrated HIV-DNA analyses
- Low-level viremia (single copy assay)
- 2-LTR analysis
- Flow cytometry data
- Soluble biomarker measurements
- p24 expression in PBMC's

– For the following information, site worksheets may contain the following source data:
  - Date of birth, gender, ethnicity, study ID number
  - Anthropometric data and vital signs
  - Brief medical history, including list of medical conditions
  - List of concomitant medications
  - Signs and symptoms noted by a medical examination
  - AE reporting sheet (including SAE and SUSAR)

6. STUDY POPULATION

6.1 RECRUITMENT PROCEDURE

Fifteen (15) HIV-infected individuals on long term ART will be enrolled into the study.

Potential participants will be recruited by a direct approach to clinic patients by study personnel at the treating centre and through advertising the study in regular and social media outlets. [redacted] If the clinician caring for the potential participant is not a study investigator then the research staff (research co-ordinators or investigators) will seek permission from the clinician prior to any contact with the potential participant. If the caring clinician agrees then, the research staff will contact the study participant or meet with them to see if they would be interested in participating in the study.

Any drug which contains, or may contain, alcohol will not be allowed in the study, due to the action of disulfiram. The liquid and/or gelatin capsule formulations of ritonavir and lopinavir/ritonavir contain alcohol. The liquid formulation of ritonavir is 42% alcohol, and the gelatin capsule formulation is 12% alcohol. Notably, the more popular heat-stable tablet formulations of ritonavir and lopinavir/ritonavir do not contain appreciable levels of alcohol and hence will be allowed in this study. Individuals receiving liquid or gelatin forms of ritonavir or lopinavir/ritonavir will be informed that their primary care provider will need to change these drugs to a heat stable tablet formulation prior to screening. Certain formulations of amprenavir contain alcohol and hence this drug will also not be allowed in this study.

The pharmacology of tipranvavir is complex and difficult to predict in the absence of formal pharmacokinetic studies. The toxicity associated with excess exposure to this drug can be significant. We will therefore exclude individuals receiving this drug. Maraviroc is metabolized via the P450 system and has to be dose adjusted when given with ritonavir. Maraviroc may have immunomodulatory effects that could affect the activity of disulfiram. For these reasons, we will exclude individuals receiving maraviroc. There are no expected drug interactions

between disulfiram and most of the other commonly used antiretroviral drugs, including the integrase inhibitors, dolutegravir, elvitegravir and raltegravir.

6.2 INCLUSION CRITERIA

- Age 18 years or older with documented HIV-1 infection (antibody positive or detectable plasma HIV-1 RNA)
- Receiving combination ART with plasma HIV RNA <50 copies/mL for >3 years
- CD4+ T cell count >350/uL at screening
- Able to provide informed consent
- Willing to abstain from alcohol consumption from one day before to 14 days after completing 28 days of disulfiram
- One month post influenza vaccine (from screening visit)
- Women of non-child-bearing potential defined as:
    - > 12 months of spontaneous amenorrhea ***and*** ≥ 45 years of age, or
    - Documented medical history of one of the following: hysterectomy, bilateral oophorectomy or tubal ligation.
- Women of Child Bearing Potential (WOCBP) with a negative pregnancy test at Screening (visit #1) ***and*** agrees to use one of the following methods of contraception to avoid pregnancy:
    - Complete abstinence from penile-vaginal intercourse from 2 weeks prior to administration of IP, throughout the study and for at least 2 weeks after discontinuation of all study medications
    - Double barrier method (male condom/spermicide, male condom/diaphragm, diaphragm/spermicide)
    - Any intrauterine device (IUD) with published data showing that the expected failure rate is <1% per year
    - Male partner sterilization ***confirmed prior to the female subject's entry*** into the study, and this male is the sole partner for that subject
    - Approved hormonal contraception (Where other medications to be used in the study (e.g., efavirenz and darunavir) are known, or are likely, to significantly interact with systemic contraceptives, resulting in decreased efficacy of the contraceptive, then alternative methods of non-hormonal contraception are recommended)
    - Any other method with published data showing that the expected failure rate is <1% per year

Any contraception method must be used consistently, in accordance with the approved product label and for at least 2 weeks after discontinuation of study therapy.

6.3 EXCLUSION CRITERIA

– 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 or recent (in the last 4 days) use of metronidazole or any drug formulation that contains alcohol or that might contain alcohol, including the gelatin capsule and liquid formulations of ritonavir, ritonavir/lopinavir, amprenavir and fosamprenavir,

and alcohol-containing preparations such as cough syrups, tonics etc.

- Current use of tipranavir or Maraviroc
- Current use of zidovudine, stavudine or didanosine (as disulfiram potentially has potent irreversible inhibitory effects on mitochondrial metabolism and hence could exacerbate the toxicity of these drugs)
- Concurrent use of rivaroxaban (a CYP3A metabolized medication) as the cytochrome P450 inhibitory effects of disulfiram on rivaroxaban are unknown
- Current use of warfarin
- Individuals who intend to modify antiretroviral therapy during the study period for any reason
- Significant myocardial disease (current myocarditis or reduced left ventricular ejection fraction below the lower limit of normal) or diagnosed coronary artery disease
- Significant renal disease (eGFR <50mL/min)
- History of psychosis, seizure disorder, abnormal electroencephalogram or brain damage with significant persisting neurological deficit
- Prior malignancy active within the previous 3 years except for local curable cancers that have been apparently cured, such as basal or squamous cell skin cancer, superficial bladder cancer or carcinoma in situ of the prostate, cervix or breast.
- Known hypersensitivity to disulfiram or vorinostat or contraindications to treatment with these agents
- Participation in another LRA study or receipt of vorinostat or disulfiram in the previous 12 months before starting the investigational treatment (visit #2)
- Any significant acute medical illness requiring hospitalization within preceding 8 weeks
- Hepatitis B or C co-infection as determined by detection of HBsAg or HCV RNA (Individuals with prior hepatitis infection that is now cleared are eligible for enrolment)
- Receipt of immunomodulating agents (excluding immunization) or systemic chemotherapeutic agents within 28 days prior to study entry
- Current or recent gastrointestinal disease or surgery that may impact the absorption of the investigational drug
- Active substance use that in the opinion of the investigator will prevent adequate compliance with study procedures
- Women who are currently pregnant or breastfeeding
- Women of Child Bearing Potential (WOCBP) who are unwilling or unable to use an acceptable method of contraception to avoid pregnancy (as specified in the inclusion criteria, section 6.2)
- Unable or unwilling to adhere to protocol procedures
- The following laboratory values within 6 weeks before starting the investigational drug (lab tests may be repeated to obtain acceptable values before failure at screening is concluded)
    - Hepatic transaminases (AST or ALT) ≥3 x upper limit of normal (ULN)
    - Serum total bilirubin ≥1.5 x ULN
    - eGFR <50 mL/min
    - Haemoglobin <10.0 g/dL
    - Platelet count ≤100 x10^9/L

- Absolute neutrophil count ≤1.5x10^9/L
- Serum potassium, magnesium, phosphorus outside normal limits
- Total calcium (corrected for serum albumin) or ionized calcium ≤ lower normal limits

6.4 WITHDRAWAL FROM THE STUDY

Withdrawal from study is defined as any participant included in the study who does not complete the final follow-up visit defined in this protocol.

Reasons why a study participant may be withdrawn from the study include, but are not limited to:

- Participant request (withdrawal of consent)
- Protocol violation
- Adverse events or reactions
- Pregnancy
- Any condition, interaction, or contraindication where continued participation in the study will result in an unacceptable risk for the participant, as assessed by the investigators
- Discontinuation of the study by the Sponsor
- Lost to follow-up

Investigator will contact study participants who fail to return for planned visits and if possible schedule a new visit. Information related to study withdrawal is documented in the eCRF (End of Study) including the reason for withdrawal, date of withdrawal, and whether the participant or investigator made this decision. If withdrawal from the study is due to an adverse event, this will be followed up as detailed under adverse event reporting in this protocol.

Participants withdrawn from the study will resume routine treatment at the Department of Infectious Diseases, Alfred Hospital or their normal site of HIV care following standard treatment guidelines.

6.5 WITHDRAWAL FROM THE INVESTIGATIONAL DRUG(S)

Withdrawal from the investigational drug(s) is defined as any participant who discontinues study treatment but agrees to a modified follow-up until completion of the study. Any participant who withdraws from the investigational drug after having received at least one dose will be asked to return for a follow-up visit 1 week after withdrawal from the study treatment (safety monitoring) and the final follow-up visit as described in this protocol.

Reasons why a study participants may be withdrawn from the investigational drug(s) include, but are not limited to:

- Participant request (withdrawal of consent)
- Adverse events or reactions
- Pregnancy
- Any condition, interaction, or contraindication where continued participation in the study will result in an unacceptable risk for the participant, as assessed by the investigators.

Information related to withdrawal from the investigational drug/placebo is documented in the eCRF (End of Study) including the reason for withdrawal, date of withdrawal, and whether the participant or investigator made this decision. If withdrawal from the investigational drug/placebo is due to an adverse event, this will be followed up as detailed under adverse event report in this protocol.

Participants withdrawn from the investigational drug(s) will resume routine treatment and monitoring at either the Department of Infectious Diseases, Alfred Hospital, Melbourne.

6.6 REPLACEMENTS

A participant is defined as included in the study when he or she has signed consent and completed the screening visit. Any exclusion or withdrawal from the study prior to baseline will be defined as failure at screening.

Participants withdrawn from the study before receipt of investigational drug will be replaced. Participants who withdraw from the study after receipt of the investigational drug(s) will have the potential to be replaced if the investigators assess that the outcome measures of the trial cannot be adequately assessed. Any decision on replacement of such individuals will be by consensus of the principal and associate investigators on a case by case basis.

7. STUDY TREATMENT

7.1 INVESTIGATIONAL DRUGS

The investigational drugs administered in this study are disulfiram and vorinostat.

7.1.1 Disulfiram

Disulfiram (ANTABUSE®), is a sulphydryl (-SH, thiol) group reagent and inhibits enzymes concerned with oxidation of active (-SH group) sites on enzyme protein molecules. The pharmacological action of disulfiram is based on its inhibition of enzymes involved in ethanol catabolism. Normally, ethanol is metabolised to carbon dioxide and water but, in the presence of disulfiram, the enzyme aldehyde dehydrogenase is inhibited and the metabolic chain of reactions stop after the production of acetaldehyde. Although it is accepted that acetaldehyde accumulation produces the disulfiram-ethanol reaction, it is also believed that the reaction may be caused by a toxic quaternary compound.

Disulfiram is TGA approved to act as a deterrent to alcohol consumption and an aid in the overall management of selected chronic alcoholic patients involved in an integrated programme of counselling and psychiatry.

Disulfiram is not approved by the TGA for use in the treatment of HIV infection in adults.

7.1.2 Vorinostat

Vorinostat (Zolinza®), is a pan HDAC inhibitor[18] with TGA approval for the treatment of cutaneous T cell lymphoma. Although less potent than the more recently licensed HDACi panobinostat and romidepsin, vorinostat has the advantage of being orally administered and has an extensive safety profile. In vitro, compared to other HDACi, vorinostat had minimal effects on T-cell function, in contrast to romidepsin which potently suppressed T-cell

function[19]. In previous studies, vorinostat has acceptable tolerability during daily dosing for 14 days, which led to increases in HIV transcription in ART-suppressed HIV patients in blood and in rectal tissue and no adverse effects on HIV-specific T-cells in vivo[2].

Vorinostat has not been approved by the TGA for the treatment of HIV infection in adults.

7.2 Study Dose and Duration of Study Therapy

In this study, all participants will receive the same study regimen – 2000mg disulfiram (4 x 500mg tablets) administered per day continuously for a total of 28 days. Disulfiram is the only LRA currently studied in people with HIV that has an appropriate toxicity profile, which would allow for prolonged administration. By administering an LRA for a prolonged period it is hypothesized that sustained increases in plasma HIV RNA is possible and this may be associated with enhanced depletion of infected HIV cells.

In addition to the disulfiram, all study participants will receive 400 mg vorinostat (4 x 100mg) daily on two occasions for 3 days each: days 8 – 10 and days 22 - 24. By administering vorinostat as two separate courses in this study, we will reduce the possibility of a refractory response as described above.

Both study drugs will be provided free of charge to the study participants.

7.3 Drug Supplies and Administration

7.3.1 Disulfiram

[redacted]

7.3.2 Vorinostat

[redacted]

7.3.2 Drug Administration

The Alfred Hospital Clinical Trials Pharmacy is responsible for receiving, storage and dispensing of the investigational drugs. The clinical trials pharmacist(s) will maintain logs of all investigational drugs received from the manufacturer(s) and also keep accurate logs of investigational drugs dispensed to study participants.

7.4 Drug Storage and Drug Accountability

The Alfred Hospital Clinical Trials Pharmacy is responsible for the secure and correct storage of the investigational drugs. The investigational drug will be clearly marked and stored in a locked medicine storage in the Alfred Clinical Trials Pharmacy at room temperature. Unused drug will be destroyed and disposed of pursuant to the ICH/GCP guidelines and Institutional policies.

7.5 Compliance

The investigational drug disulfiram for the entire study duration will be provided to study participants in the research clinic at the commencement of study therapy day 0 (visit #2). Enough investigational study drug Vorinostat will be provided for 3 days on day 8 and day 22 of the study. At all follow-up visits, participants will be counselled to maintain adherence with study therapy, concomitant therapy and other study procedures. For each participant, any remaining tablets of the investigational drug will be accounted for at the end of study therapy.

7.6 Concomitant medication(s)

Study participants are required to continue their ART regimen while receiving the investigational drug(s). Any changes in anti-retroviral therapy during the study will be recorded in either the hospital/clinic notes or site worksheet. Any other concomitant medical therapy either recorded at study entry or initiated during the study will be recorded in either the hospital/clinic notes or site worksheets.

7.7 Concomitant Therapy Allowed During the Study

Any treatment that is considered necessary for the participant's welfare (including supportive therapy) and which, in the opinion of the investigator, will not interfere with the study therapy, may be given at the discretion of the investigator. Specifically, vaccines that are part of a recommended immunization schedule or used in the event of a disease outbreak are allowed during study therapy.

7.8 Concomitant Therapy prohibited during the study

- Other investigational drugs, agents or medical devices
- Use of use of metronidazole or any drug formulation that contains alcohol or that might contain alcohol, including the gelatin capsule and liquid formulations of ritonavir, ritonavir/lopinavir, amprenavir and fosamprenavir
- Avoid alcohol-containing preparations such as cough syrups, sauces, vinegar, tonics, foods prepared with wine, after shave lotions and alcoholic back rubs.
- Use of tipranavir or Maraviroc
- Use of zidovudine, stavudine or didanosine
- Use of rivaroxaban (a CYP3A metabolized medication) as the cytochrome P450 inhibitory effects of disulfiram on rivaroxaban are unknown
- Use of warfarin

Initiation of any of the above medical products will necessitate withdrawal from the investigational drugs.

8. STUDY PROCEDURES

8.1 SCHEDULE OF ACTIVITIES

A total of 9 visits including one screening and one baseline visit will be performed during the study. All study visits will occur in the morning and the time of visits will always be recorded. A full schedule of activities is provided below:

Table 1: Schedule of assessments

Visit #	**1 screening**	**2 baseline**	**3**	**4**	**5**	**6**	**7 EOT**	**8**	**9 EOS**
Days relative to starting study therapy	**-42 to 0 days pre-study therapy**	**1 pre-study therapy**	**8**	**11**	**22**	**25**	**28**	**56 ± 7**	**196 ± 10**
Study activities									
Informed consent	X								
Medical history (incl/excl criteria)	X	X							
Vital signs (includes weight)	X (+ height for this visit only)	X	X	X	X	X	X	X	X
Clinical review	X (+ physical examination for this visit only	X	X	X	X	X	X		X
Alcohol Questionnaire	X								
Concomitant medication review	X	X	X	X	X	X	X	X	X
Adverse event review	X	X	X	X	X	X	X	X	X
Study drug administration - Disulfiram		X Day 0 - 28							
Study drug administration - Vorinostat			X Days 8 - 10		X Days 22 - 24				
Pathology									
Haematology *(FBE)*	X		X	X	X		X		X
Clinical biochemistry *(renal function, liver enzymes, electrolytes)*	X		X	X	X		X		X
Pregnancy test *(WOCBP only)*	X								

Visit #	1 screening	2 baseline	3	4	5	6	7 EOT	8	9 EOS
Days relative to starting study therapy	**-42 to 0 days pre-study therapy**	**1 pre-study therapy**	**8**	**11**	**22**	**25**	**28**	**56 ± 7**	**196 ± 10**
Hep C Ab and HBsAg	X								
CD4 and CD8 counts	X					X		X	
Plasma HIV RNA (commercial assay)	X*						X		
Study Specific Specimens									
Plasma HIV RNA (SCA)	X	X	X	X	X	X	X	X	X
CA-US HIV RNA	X	X	X	X	X	X	X	X	X
p24 expression	X	X	X	X	X	X	X	X	X
HIV DNA	X	X						X	X
TILDA		X						X	
Plasma vorinostat and disulfiram		X	X	X	X	X	X	X	X
Plasma and PBMC storage	X	X	X	X	X	X	X	X	X
PAX gene tube		X	X	X		X			X
Blood Tubes required									
ACD Tubes (10 mL)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
SST Tube (3.5 mL)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
K2EDTA Tube (4 mL)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
PAXgene tube (3 mL)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Total volume (mL)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

* - only to be performed if the test was not ordered as part of standard of care in the previous 3 months.

8.2 VISIT 1 – SCREENING

This screening visit must occur within the preceding 42 days of initiating study therapy. However, screening lab tests taken as part of standard of care with 84 days of study baseline (visit 2) can be used to assess eligibility.

- Signed informed consent prior to any study related procedures,
- Demographics
- Medical History
- Document HIV history including date of diagnosis, antiretroviral therapy and HIVRNA results
- Complete physical examination, including height and weight. After the participant signs consent, any new events/findings not present in the participant's medical history or is a worsening side effect should be recorded as adverse events in the eCRF.

- Vital signs (heart rate, blood pressure, respirations and temperature).
- A detailed questionnaire regarding past and current alcohol exposure will be obtained (the AUDIT questionnaire will be used, as this is a well validated measurement for quantifying alcohol exposure) [appendix 1].
- Concomitant medication with indication.
- Document current adverse events
- Haematology analysis (haemoglobin, haematocrit, platelet count, white blood cell count including differential count)
- Serum chemistry analysis (sodium, potassium, chloride, creatinine, eGFR, bicarbonate, total protein, albumin, ALT, AST, ALP, total bilirubin), hsCRP
- Women of childbearing potential: serum pregnancy test
- Hepatitis C antibody test and if positive HCV RNA screening, to be performed if not tested within the last 3 months as part of SOC
- Hepatitis B surface antigen test – only to be performed if not tested within the last 3 months as part of SOC
- CD4+ and CD8+ T-cell count
- Plasma HIV RNA (clinical assay) – only to be performed if not part of standard of care tests within the preceding 3 months of anticipated Day 1 of treatment.

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- Cell associated HIV DNA (total and integrated) in CD4+ cells
- p24 expression
- PBMC and plasma storage
- Plasma storage for measuring disulfiram levels (control)
- Serum storage for measuring vorinostat levels (control)

8.3 VISIT 2 - BASELINE

This baseline visit will occur on the day of initiating study therapy ***AND*** prior to the first disulfiram dose.

- Eligibility will be reconfirmed
- Medical history ascertained
- Clinical review including vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit
- 6mL blood test for plasma HIV RNA (clinical assay)

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- Cell associated HIV DNA (total and integrated) in CD4+ cells
- p24 expression
- TILDA assay
- PBMC and plasma storage

- Collection of plasma for disulfiram and vorinostat concentrations
- PAX gene tube for storage and future genetic studies
- Plasma storage for measuring disulfiram pK levels (control)
- Serum storage for measuring vorinostat pK level (control)

8.4 VISIT 3 – DAY 8

This visit and procedures will occur prior to both disulfiram ***AND*** vorinostat dosing.

- Clinical review including vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit
- Haematology and serum chemistry analysis

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- p24 expression
- PBMC and plasma storage
- Collection of plasma for measuring disulfiram pK levels
- Collection of serum for measuring vorinostat pK levels (control in the presence of disulfiram)
- PAX gene tube for storage and future genetic studies

8.5 VISIT 4 – DAY 11

This visit and procedures will occur 24 hours after day 10 vorinostat dosing ***AND*** before day 11 disulfiram dosing.

- Vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit
- Haematology and serum chemistry analysis

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- p24 expression
- PBMC and plasma storage
- Collection of plasma for measuring disulfiram pK levels
- Collection of serum for measuring vorinostat pK levels
- PAX gene tube for storage and future genetic studies

8.6 VISIT 5 – DAY 22

This visit and procedures will occur prior to both disulfiram ***AND*** Vorinostat dosing.

- Clinical review including vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit

- Haematology and serum chemistry analysis

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- p24 expression
- PBMC and plasma storage
- Collection of plasma for measuring disulfiram pK levels
- Collection of serum for measuring vorinostat pK levels

8.7 VISIT 6 – DAY 25

This visit and procedures will occur 24 hours after day 24 vorinostat dosing ***AND*** before day 25 disulfiram dosing.

- Vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit
- CD4+ and CD8+ counts

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- p24 expression
- PBMC and plasma storage
- Collection of plasma for measuring disulfiram pK levels
- Collection of serum for measuring vorinostat pK levels
- PAX gene tube for storage and future genetic studies

8.8 VISIT 7 – END OF TREATMENT

This visit and procedures will occur after the last disulfiram dosing, day 28.

- Clinical review including vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit
- Haematology analysis (haemoglobin, haematocrit, platelet count, white blood cell count including differential count)
- Serum chemistry analysis (sodium, potassium, chloride, creatinine, eGFR, bicarbonate, total protein, albumin, ALT, AST, ALP, total bilirubin), hsCRP
- 6mL blood test for plasma HIV RNA (clinical assay)

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- p24 expression
- PBMC and plasma storage
- Collection of plasma for measuring disulfiram pK levels
- Collection of serum for measuring vorinostat pK levels

8.9 VISIT 8 – SAFETY VISIT

The visit and procedures will occur on day 56 ± 7 days. This is 28 days after the last disulfiram dose.

- Vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit
- CD4+ and CD8+ counts

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- Cell associated HIV DNA (total and integrated) in CD4+ cells
- p24 expression
- TILDA assay
- PBMC and plasma storage
- Collection of plasma for measuring disulfiram pK levels
- Collection of serum for measuring vorinostat pK levels

8.10 VISIT 9 – END OF STUDY

The visit and procedures will occur 6 months (168 days) after the last disulfiram dose.

- Vital signs
- Review of current concomitant medications since last visit
- Review of current adverse events since last visit
- Haematology analysis (haemoglobin, haematocrit, platelet count, white blood cell count including differential count)
- Serum chemistry analysis (sodium, potassium, chloride, creatinine, eGFR, bicarbonate, total protein, albumin, ALT, AST, ALP, total bilirubin), hsCRP
- CD4+ and CD8+ counts

An additional [redacted] of blood will be collected for study specific tests which include:

- Plasma HIV RNA (single copy assay)
- CA-US RNA on CD4+ T cells
- Cell associated HIV DNA (total and integrated) in CD4+ cells
- p24 expression
- PBMC and plasma storage
- Collection of plasma for measuring disulfiram pK levels
- Collection of serum for measuring vorinostat pK levels
- PAX gene tube for storage and future genetic studies

The total study specific blood volume collected for a participant over the 9 visits (approximately 6 months) will be 602.5 mL.

9. EFFECT ASSESSMENTS

9.1 PLASMA HIV RNA

HIV RNA in plasma will be measured by the AmpliPrep/COBAS®TaqMan® HIV-1 test version 2.0 (Roche Diagnostics) on three occasions and on all other visits, will be measured by a real-time PCR assay with single copy sensitivity as previously described[2].

9.2 TOLERABILITY

Tolerability will be assessed by systematically recording incidence and severity of adverse advents. For each adverse event we will assess the causal association with study therapy (see section 10 for more details)

9.3 CELL-ASSOCIATED UNSPLICED HIV RNA

CA-US HIV RNA will be quantified using a semi-nested real time qPCR with a first round amplification of 15 cycles to ensure that the following second round amplification the assay was in the linear range between 1 to 46,000 input copies, as previously described by Pasternak et al [24]. Primers used for 1st and 2nd round amplified gag 25. HIV RNA copy numbers is standardized to cellular equivalents using an 18s RNA real time LUX PCR primer set (Invitrogen). PCR amplification of cDNA for CA-US RNA is performed in quadruplicate with a non-RT control included

9.4 P24 EXPRESSION

Will be measured by using an optimized HIV p24 digital immunoassay on the single-molecule-array platform using frozen PBMC.

9.5 CELL-ASSOCIATED TOTAL AND INTEGRATED HIV DNA

To measure total and integrated HIV DNA we will perform real time qPCR using primers and probes as previously described[2]. The qPCR for HIV DNA will be performed in triplicate. To normalise for cell equivalents in the input DNA, a separate real-time qPCR assay will be used to quantify the CCR5 gene.

9.6 THE FREQUENCY OF INDUCIBLE VIRUS IN CD4+ T CELLS

We will use the recently developed Tat/rev Induced Limiting Dilution Assay (TILDA) to measure the frequency of cells with inducible multiply-spliced (MS) HIV RNA, as these transcripts are usually absent in latently infected cells but induced upon viral reactivation[26]. Briefly, CD4+ T cells will be stimulated with phorbol myristate acetate (PMA) and ionomycin for 12hrs. Dilutions of the stimulated cells are then distributed in a 96 well plate and directly subjected to quantification of MS HIV RNA by qPCR. The frequency of positive cells is calculated using the maximum likelihood method and expressed as a frequency of cells with inducible MS HIV RNA per million CD4+ T-cells.

9.7 PLASMA LEVELS OF VORINOSTAT AND DISULFIRAM

Concentrations of vorinostat and disulfiram and its metabolites in plasma will be measured using liquid chromatography and mass spectrometry.

9.8 NEXT-GENERATION RNA SEQUENCING

We will perform next-generation RNA sequencing and bioinformatic analyses of gene expression activity in samples taken before, during and after study therapy. This will be done by extracting RNA including integrity control, stranded RNA library preparation and using Illumina sequencing with 100 bp single reads with enrichment for polyA tail mRNA.

During the expression analysis samples will undergo quality and adapter trimming, alignment, quantification and normalisation to generate output files that will be subject to bioinformatics analyses.

We will use these data to identify differentially expressed genes and affected gene pathways before study therapy, during treatment with disulfiram (day 0 – day 8), during treatment with disulfiram + vorinostat (day 8 – day 11) and at end of study (months after discontinuation of study therapy).

9.9 QUNATIFICATION OF PLASMA BIOMARKERS OF NEUROINFLAMMATION AND DEGRADATION

Through a collaboration with Magnus Gisslen and Kaj Blennow at the Clinical Neurochemistry Lab, University of Gothenburg, Sweden, we will quantify plasma levels of neurofilament light chain (NFL), p-tau and t-tau. We will assess longitudinal changes in the level of these biomarkers and will also compare against biomarker levels in other cohorts of HIV-infected individuals.

9.10 PLASMA CYTOKINES, CHEMOKINES AND GENE PRODUCTS

We will quantify levels of plasma cytokines and chemokines and selected products of differentially expressed genes. The latter will be based on our bioinformatic analysis of gene expression levels as explained in subsection 9.8. This will be done using commercially available ELISA kits and Luminex multiplexing to quantify a broad panel of cytokines in plasma.

9.11 HISTONE ACETYLATION AND PTEFB AND NFKB ACTIVATION

Through a collaboration with Jonathan Karn at Case Western University, USA, we will assess markers of cellular function relevant to latency reversal. We will apply samples from the study to several newly developed assays, which includes H3K9 histone acetylation, pTEFB activation and NFkB activation.

9.12 EFFECTS OF VORINOSTAT AND DISULFIRAM ON ASTROCYTES

To determine the effect of the transcriptome of vorinostat and or disulfiram in cells from the central nervous system and peripheral blood mononuclear cells we intend to perform RNA

sequencing on primary astrocytes, astrocyte cell lines and PBMCs. These different cells will be exposed to C_{max} drug levels that were observed in the trial. We will determine H3K9 histone acetylation, pTEFB activation and NFkB activation in these cells and determine differential gene expression compared to untreated cells. We will perform bulk RNA sequencing by extracting RNA from 2 million treated cells per condition and perform 3'-end poly A paired ended 2x100bp sequencing using the Illumina Miseq platform. We expect that the epigenetic markers in these different cell types will react differently to these LRAs.

10. PARTICIPANT SAFETY AND WITHDRAWAL

10.1 DEFINITIONS

10.1.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding – see below), symptom, or disease temporally associated with the use of the investigational drug, whether or not causally linked to the investigational drug.

An abnormal laboratory value or test result constitutes an adverse event only if

- It is associated with clinical signs or symptoms
- It leads to a change in study drug dosing or discontinuation from the study
- It requires additional diagnostic testing or medical/surgical intervention
- It is considered to be an adverse event by the investigator

In addition, all cases of drug-drug interaction, pregnancy (with or without outcome), paternal exposure, lactation, overdose, drug abuse and misuse, drug maladministration or accidental exposure and dispensing errors are collected and data based even if no adverse event has been reported.

All adverse events will be reported in accordance with the principles of Good Clinical Practice and the latest requirements of the Medicines for Human Use (Clinical Trials) Regulations.

10.1.2 Serious Adverse Events (SAEs)

A SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization, *unless hospitalization is for:*
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of the study drug

- Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

- Is medically significant, i.e. defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting.

10.1.3 Pregnancy

Any female study participant who becomes pregnant during the study must be immediately withdrawn from study drugs to eliminate further exposure to the embryo/foetus.

Any pregnancy that occurs during study participation is to be reported on a specific pregnancy site worksheet and SAE form. To ensure participant safety, each pregnancy must be reported to Sponsor within one week of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child, which must also be reported to Sponsor using designated data collection forms. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.

Any SAE occurring in association with a pregnancy brought to the investigator's attention after the subject has completed the study and considered by the investigator as possibly related to the investigational product, must be promptly reported to Sponsor.

10.2 RECORDING OF ADVERSE EVENTS

At each contact with the study participant, as indicated in the schedule of activities, information on adverse events is sought by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the CRF. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though they should be grouped under one diagnosis.

All adverse events occurring during the study period - from the time the study participant signs consent (visit 1) to End of study (visit 9) - must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still on-going at the End of Study must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

All AEs must be graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2, November 2014 and recorded in source notes as well as the AE eCRF with the following information:

- The severity grade (see below)
- Its relationship to the study drugs (suspected/not suspected)
- Its duration (start and end dates or if continuing at study visit)
- Whether it constitutes a serious adverse event (SAE)

All adverse events will be graded in the following manner:

- Grade 1 (Mild): Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Grade 2 (Moderate): Events result in a low level of inconvenience or concern. Moderate events may cause some interference with functioning.
- Grade 3 (Severe): Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Grade 4 (Life-threatening): Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death).
- Grade 5 (Death)

10.3 Reporting of Serious Adverse Events

SAEs will be noted in source documentation. Information about all SAEs should be recorded on a Serious Adverse Event Report Form. The investigator must inform the sponsor, principal investigator and HREC, assess the relationship to study drug, complete an SAE Report Form, and send the signed form by fax within 24 hours of notification of the SAE to Merck Global Safety and HREC.

[redacted]

The original copy of the SAE Report Form and the fax confirmation sheet or email confirmation must be maintained at the study site.

10.4 Suspected Unexpected Serious Adverse Reactions (SUSARS)

If the SAE is not previously documented in the product information or package insert (new occurrence) and is thought related to the study drug, it qualifies as a SUSAR. A sponsor associate may require further information from the investigator for Health Authority reporting.

The minimum necessary information to be provided at the time of the initial report includes:

- An identifiable patient
- An identifiable medicinal and/or pharmaceutical product
- An identifiable reporter
- A serious adverse event

10.5 Reporting of SUSARs to local health authority

The study sponsor is responsible for submission of reportable SUSARs to the local Health Authority according to regulatory requirements. The timeline for reporting any suspected adverse reaction that is both serious and unexpected is ***15 calendar days*** after the investigator determines that the suspected adverse reaction qualifies for reporting.

The timeline for reporting any suspected adverse reaction that is both serious and unexpected is ***7 calendar days*** for events that are fatal or life threatening.

11. STATISTICAL METHODS

11.1 SAMPLE SIZE ESTIMATION & JUSTIFICATION

The primary comparison for study is the level of plasma HIV RNA on day 11 during disulfiram/vorinostat treatment compared to baseline. Based on two previous studies of LRAs, where treatment with disulfiram 2000 mg or romidepsin led to increases in plasma HIV RNA [4,6], we assume a pre-study therapy mean of 1 copy/mL and a standard deviation of 11 copies/mL for the increase in plasma HIV RNA. Based on these numbers, the study will require 14 participants to detect an increase in plasma HIV RNA of 10 copies/mL with 80% power at a 0.05 significance level. Targeted enrolment is 15 participants to accommodate for loss to follow-up.

No interim analysis is planned.

11.2 ANALYSES OF PRIMARY AND SECONDARY ENDPOINTS

The primary comparisons will be made using paired t-test or signed-rank test as appropriate. By applying the same statistical methods, we will also analyse whether changes in CA-US HIV RNA and plasma HIV RNA differ following administration of high dose disulfiram and vorinostat. We will use repeated measurement analysis of variance statistics and/or generalized estimating equation statistics to analyse overall changes in primary and secondary endpoints during study intervention by including all data points during study therapy. P-values <0.05 will be considered significant.

The tolerability population will include all participants who have received at least one dose of vorinostat or disulfiram. Adverse events (AEs) and serious adverse events (SAEs) will be summarized according to severity assessment showing the number and percentage of participants experiencing at least one event, the number of events, and the causality assessment. No formal statistical comparisons will be made – AE rates are presented for descriptive purposes.

11.3 GENE EXPRESSION DATA AND ADDITIONAL ANALYSES TO INVESTIGATE NEUROTOXICITY (SECTIONS 9.8 – 9.12)

RNA sequencing data will undergo standard bioinformatic analyses, which is performed by Australian Genome Research Facility (AGRF), Melbourne. This will include quality and adapter trimming, alignment, quantification and normalisation. RNA expression data will be mapped to a reference genome and gene expression levels will be compared across different time points to identify differentially expressed genes. Differential expression of gene pathways will be analysed using Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome platforms.

Other additional analyses related to observed neurotoxicity include plasma levels of cytokines, biomarkers of neuronal injury, levels of histone acetylation, pTEFB and NFkB activation and effects of disulfiram with or without vorinostat on primary astrocytes, astrocyte cell lines and PBMCs. These data will be summarised using descriptive statistics. Where feasible different time points or conditions will be compared using paired t-test or signed-rank test.

12. STORAGE OF BLOOD SAMPLES

Blood samples will be collected for PBMC and plasma storage at each visit as indicated in section 8 of this protocol. Samples will be stored at the Doherty Institute, University of Melbourne (Lewin Laboratory) and will only be accessible to research staff following approval by the Protocol Steering Committee, which will include Professor Sharon Lewin, Dr James McMahon, Dr Tina Schmidt and a community representative. Stored samples may be used for future analyses conditioned on adherence to local regulations and ethical guidelines and approval of human research ethics committee.

13. ETHICAL CONSIDERATIONS

13.1 STUDY APPROVAL

Sponsor must obtain prospective approval before study initiation of the study protocol, informed consent documents and other relevant documents from the HREC and the Therapeutic Goods Administration (TGA) through the Clinical Trial Notification (CTN) scheme.

13.2 ETHICAL CONDUCT OF THE STUDY

The Sponsor and principal investigator is responsible for planning and conducting the study in accordance with The Helsinki Declaration (1996 version), guidelines for Good Clinical Practice (GCP; International Conference on Harmonization 1996), and Australian ethical guidelines and law.

13.3 HREC REVIEW AND INFORMED CONSENT

This protocol, the informed consent document and any subsequent modifications will be reviewed and approved by the HREC responsible for oversight of the study. A signed consent form will be obtained from each study participant prior to any study procedures. The consent form will describe the purpose of the study, the procedures to be followed, and the potential risks and benefits of participation. A copy of the consent form will be given to the study participant, parent, or legal guardian. During the informed consent process, research staff will ensure that potential participants understand the purpose of the study, the study intervention, study procedures, any risks associated with participation and that participation is voluntary. Any questions posed during the informed consent process or subsequently during the study will be carefully addressed. Potential participants will be given as much time as they need to read and understand the consent form.

13.4 HARMS, RISKS AND INCONVENIENCES

The potential harms, risks and/or inconveniences associated with participation in this study will be that of exposure to the study drugs disulfiram and vorinostat, the risk of phlebotomy, the risk of pregnancy in women of childbearing potential and the inconveniences associated with attending study visits. To monitor the safety and ensure the well-being of study participants, monitoring and handling of adverse events will be done at study visits as detailed in section 8 and 10 of this protocol. In the event of adverse effects, participants will have

medical care available through the Alfred Hospital. Any medical testing and evaluation undertaken by study investigators to follow up abnormal findings will also be at no cost to participants.

13.4.1 Disulfiram

Undesirable effects of disulfiram are reported in individuals that have not abstained from alcohol, which results in a disulfiram-ethanol toxic reaction, as specified in the product information and includes an intense cutaneous flushing from the head downwards, involving the face, sclera, upper limbs, and chest. The cutaneous flushing is caused by vasodilation and is accompanied by a sensation of heat and sweating, palpitations, with tachycardia, dyspnoea, hyperventilation and the patient develops a pounding headache. There is a feeling of constriction and irritation of the throat and trachea, resulting in spasms of coughing. Chest pains may occur simulating coronary spasm. Restlessness or a sense of uneasiness and fear of dying may develop. These symptoms are accompanied by a steep rise in blood pressure, followed by hypotension if vasodilation is significant. Flushing is then replaced by pallor, weakness, vertigo, and nausea develops that turns into violent vomiting with abdominal cramps. Other symptoms reported include thirst, dizziness, blurred vision, numbness of hands and feet, and insomnia. Severe reactions may affect the heart, and there may be convulsions, loss of consciousness, and death from cardio-respiratory failure.

The intensity of the reaction varies with the individual and the duration of the reaction continues as long as there is ethanol in the blood. Confusion, drowsiness and sleep usually follow. Frequently, there are transient ECG changes, such as flattening of T waves, depression of S-T segment, and Q-T prolongation in a pattern suggestive of right ventricular strain.

13.4.2 Vorinostat

The most common drug-related adverse reactions, which have been reported in cancer patients can be classified into 4 symptom complexes.

1. Gastrointestinal symptoms. Anorexia, nausea, vomiting, diarrhoea, weight loss and constipation have been reported. Antiemetic and antidiarrheal medications have been used with good effect. Fluid and electrolyte replacement may be required. Patients should be encouraged to drink at least 2L of fluid each day, and aggressive management of nausea and diarrhoea is strongly encouraged, to avoid renal impairment secondary to dehydration.

2. Constitutional symptoms. Fatigue has been reported commonly with use of vorinostat and occurred more commonly at doses of vorinostat greater than 400mg per day. Chills and fever have also been reported.

3. Hematologic abnormalities. Dose-related anaemia and thrombocytopenia can occur with vorinostat and are more common at doses of more than 400mg per day. Neutropenia is uncommon, but has been reported. Monitoring of blood cell counts should be performed every 2 weeks during the first 2 months of therapy and monthly thereafter. If platelet counts and/or haemoglobin are reduced during treatment with vorinostat, the dose should be modified or therapy discontinued.

4. Taste disorders. Dysgeusia and dry mouth have been reported with use of vorinostat.

The most common serious adverse events, regardless of causality, in the 86 CTCL patients in two clinical studies were pulmonary embolism reported in 4.7% (4/86) of patients, squamous

cell carcinoma reported in 3.5% (3/86) of patients and anaemia reported in 2.3% (2/86) of patients. Patients on vorinostat should therefore be monitored for pertinent signs or symptoms of deep vein thrombosis or pulmonary embolism.

Of the CTCL patients who received the 400-mg once daily dose, 9.3% (8/86) of patients discontinued vorinostat due to adverse events and 10.5% (9/86) of patients required a dose modification due to adverse events. The median time to the first adverse event resulting in dose reduction was 42 days (range 17 to 263 days).

Laboratory abnormalities were reported in the 86 patients who received the 400-mg dose and one patient who received a 350-mg dose.

Increased serum glucose was detected by laboratory safety tests in 69% (60/87) of CTCL patients, but was severe (Grade 3) in only 5 of these. Hyperglycaemia was reported as a drug-related adverse experience in 4.7% (4/86) of CTCL patients who received the 400–mg once daily dose. Serum glucose should be monitored, especially in diabetic or potentially diabetic patients. Adjustment of diet and/or therapy for increased glucose may be necessary. Blood glucose monitoring of diabetic patients is recommended.

Transient increases in serum creatinine were detected in 47.1% (41/87) of CTCL patients; in most cases these increases were non-severe, however, Grade III (severe) cases have been observed. Based on reports of dehydration as a serious drug-related adverse experience in clinical trials, patients were instructed to drink at least 2 L/day of fluids for adequate hydration. After these precautions were implemented, the incidence of dehydration decreased.

Proteinuria was detected as a laboratory abnormality (51.4%) in 38 of 74 patients tested. The clinical significance of this finding is unknown.

A phase 1 study has been performed of 25 patients given a single supratherapeutic dose of vorinostat 800mg. Of the 24 patients included in analysis, no patient had a significant change in QTc over 24 hours of monitoring. In other studies three of 86 CTCL patients exposed to 400mg once daily had Grade 1-2 QTc prolongation. In a retrospective analysis of three Phase I trials and two Phase II trials, 4 of 116 evaluable patients had grade 2 QTc prolongation, and 1/116 had grade 3 QTc prolongation. Of 49 non-CTCL patients from 3 clinical trials 1 had grade 2 prolongation and 2 had grade 3 QTc prolongation. Baseline and periodic ECGs should be performed during treatment. Vorinostat should be administered with particular caution in patients with congenital long QT syndrome, and patients taking anti-arrhythmic medicines or other medicinal products that lead to QT prolongation. Hypokalemia or hypomagnesemia should be corrected prior to administration of vorinostat, and consideration should be given to monitoring potassium and magnesium in symptomatic patients (e.g., patients with nausea, vomiting, diarrhoea, fluid imbalance or cardiac symptoms).

13.4.3 Reproductive risks

Safe use of either disulfiram or vorinostat in human pregnancy or during lactation has not been established.

Available reproductive toxicity studies in animals do not necessitate that females of childbearing potential are excluded from clinical trials of disulfiram or vorinostat, but use of effective contraception for both males and females are required for participation in this study. In addition, for female participants, a negative pregnancy tests is required during screening.

13.4.4 Phlebotomy

Phlebotomy may cause some discomfort, bleeding or bruising where the needle enters the skin, and rarely, fainting or infection may occur.

13.5 Risk/benefit Analysis

Even though the implementation of combination ART to treat HIV infection has had a tremendous impact on the epidemic by restoring the health of well-treated individuals, HIV infection remains a major public health challenge world-wide. Moreover, as ART discontinuation invariably results in viral rebound to pre-treatment levels, the development of a cure for HIV continues to be a fundamental goal in infectious disease research. Although the initial studies of LRAs showed no decline in latently infected cells, it is likely that an effective intervention to reverse latency will be needed in combination with interventions that eliminate latently infected cells. Identification of safe interventions that can increase potency of latency reversal remains a high scientific priority.

The design of the study with a relatively short administration of the well-studied and safe drug disulfiram, together with a short period of administration of vorinostat to background ART is aimed at increasing the potency of latency reversal. As such, it is unlikely that participation is associated with any sustained significant clinical benefit for the participants. Hence, any benefits accrued by completing this study will primarily be for society rather than the individual study participant.

The harms, risks and inconveniences associated with study participation are outlined above and include known and unknown adverse effects of both disulfiram and vorinostat, reproductive risks and phlebotomy. These risks have been minimized by choosing a relatively short period for the study intervention, by excluding candidates with any condition that would increase the risk of adverse effects and by the proper conduct and monitoring of the study. Given these considerations, we believe that this study is ethically and scientifically justified.

14. Study Monitoring and Quality Assurance

14.1 The responsibilities of sponsor and principal investigator

The sponsor is responsible for establishing and maintaining a quality assurance system that guarantees the quality of the study in all aspects. The principal investigator can appoint qualified staff co-investigators that may assist in the conduct of the study in accordance with the study protocol. All co-investigators must be appointed and recorded on the study personnel list in due time before any study related procedures are carried out and must be supplied with the study protocol and all necessary information. Co-investigators are supervised by the principal investigator and act under her responsibility.

The sponsor must notify the regulatory authorities and the HREC about the completion of the study in accordance with local regulation. If the study is prematurely terminated this must be notified and the reason for this must be clarified.

14.2 STUDY MONITORING/AUDIT

The study will be monitored by an internal staff member of the Department of Infectious Diseases, Alfred Hospital and/or Melbourne University, as per department protocol. The study monitor will visit the clinical research site to monitor and assess the conduct of the study. Adherence to protocol and regulatory requirements will be monitored as well as the handling of irregularities if such occur. Study monitoring also includes review of drug storage and management. During monitoring visits all, but not necessarily limited to, of the following issues will be discussed and assessed: informed consent, participant recruitment, follow-up, documentation, recording and reporting of AE and SAE, compliance, data quality, and data handling. The study investigator will make study documents (e.g., consent forms, drug distribution forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the study monitor and/or HREC if required.

15. DATA HANDLING & RECORD KEEPING

15.1 HANDLING OF PERSONAL INFORMATION

Study investigators and associated research staff must handle and keep all study material and documents as confidential information and take steps to prevent wrongful or premature destruction of these. All personal data are protected in accordance with relevant Australian law. Blood samples and person-specific documents will not contain information that directly identifies the study participant, but will be supplied with a study ID unique for each participant.

To enable evaluations and/or audits from regulatory authorities or HREC, the principal investigator will keep study records including the identity of all study participants, e.g. CRFs, signed informed consent documents, AE/SAE forms, source documents and study correspondence. As this is a CTN drug trial, records will be maintained by the principal investigator according local regulation which is a minimum of 15 years post study closure.

15.2 DATA MANAGEMENT

All clinical data for this study will be managed by the Data Management Unit at the clinical research unit for the Department of Infectious Diseases at Alfred Health. The study database will be managed by the sponsor.

All trial data required for the monitoring and analysis of the study will be recorded on the eCRFs provided. All required data entry fields must be completed. Data corrections will be done according to the instructions provided. The investigator will be asked to confirm the accuracy of completed CRFs by signing key CRFs as indicated.

15.3 STORAGE OF BIOLOGICAL MATERIAL AND GENETIC TESTING

Blood samples will be collected for PBMC and plasma storage at each visit as indicated in section 8 of this protocol. Stored samples may be used for future analyses related to HIV cure research, including genetic testing, conditioned on adherence to local regulations and ethical guidelines. Samples will be stored at the Doherty Institute, University of Melbourne (Lewin

Laboratory) and will only be accessible to research staff following approval by the Protocol Committee.

16. FINANCES

[redacted] Merck will also supply vorinostat for use in this study.

Study participants will be offered reimbursement for costs attending study visits.

17. PUBLICATION OF THE STUDY

17.1 PUBLIC REGISTRATION OF STUDY

The study will be registered at www.clinicaltrials.gov prior to study start; a brief outline of the study will be available according to current guidelines.

17.2 PUBLICATION OF THE STUDY

Research findings from this study will be published in a timely manner in international peer-reviewed journals. The principal investigator, in collaboration with the protocol steering committee will have overall responsibility for the content of such publications.

18. REFERENCES

1. Deeks, S. G. HIV: Shock and kill. *Nature* **487**, 439-440, doi:10.1038/487439a (2012).
2. Elliott, J. H. *et al.* Activation of HIV Transcription with Short-Course Vorinostat in HIV-Infected Patients on Suppressive Antiretroviral Therapy. *PLoS pathogens* **10**, e1004473, doi:10.1371/journal.ppat.1004473 (2014).
3. Rasmussen, T. A. *et al.* Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV* **1**, e13-21, doi:10.1016/S2352-3018(14)70014-1 (2014).
4. Sogaard, O. S. *et al.* The Depsipeptide Romidepsin Reverses HIV-1 Latency In Vivo. *PLoS pathogens* **11**, e1005142, doi:10.1371/journal.ppat.1005142 (2015).
5. Spivak, A. M. *et al.* A pilot study assessing the safety and latency-reversing activity of disulfiram in HIV-1-infected adults on antiretroviral therapy. *Clin Infect Dis* **58**, 883-890, doi:10.1093/cid/cit813 (2014).

6 Elliott, J. H. *et al.* Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV* **2**, e520-529, doi:10.1016/S2352-3018(15)00226-X (2015).

7 Bullen, C. K., Laird, G. M., Durand, C. M., Siliciano, J. D. & Siliciano, R. F. New ex vivo approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. *Nat Med* **20**, 425-429, doi:10.1038/nm.3489 (2014).

8 Kumar, A., Darcis, G., Van Lint, C. & Herbein, G. Epigenetic control of HIV-1 post integration latency: implications for therapy. *Clin Epigenetics* **7**, 103, doi:10.1186/s13148-015-0137-6 (2015).

9 Budhiraja, S., Famiglietti, M., Bosque, A., Planelles, V. & Rice, A. P. Cyclin T1 and CDK9 T-loop phosphorylation are downregulated during establishment of HIV-1 latency in primary resting memory CD4+ T cells. *J Virol* **87**, 1211-1220, doi:10.1128/JVI.02413-12 (2013).

10. Mota, T. M. *et al.* in *International Aids Society (IAS) Towards an HIV Cure Symposium 2015* (Vancouver, Canada, 2015).

11 Laird, G. M. *et al.* Ex vivo analysis identifies effective HIV-1 latency-reversing drug combinations. *The Journal of clinical investigation* **125**, 1901-1912, doi:10.1172/JCI80142 (2015).

12 Van Lint, C., Emiliani, S., Ott, M. & Verdin, E. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *Embo J* **15**, 1112-1120 (1996).

13 Bartholomeeusen, K., Fujinaga, K., Xiang, Y. & Peterlin, B. M. Histone deacetylase inhibitors (HDACis) that release the positive transcription elongation factor b (P-TEFb) from its inhibitory complex also activate HIV transcription. *J Biol Chem* **288**, 14400-14407, doi:10.1074/jbc.M113.464834 (2013).

14. Xing, S. *et al.* Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary CD4+ T cell model without inducing global T cell activation. *J Virol* **85**, 6060-6064, doi:10.1128/JVI.02033-10 (2011).
15. Archin, N. M. *et al.* Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. *AIDS Res.Hum.Retroviruses* **25**, 207-212 (2009).

16 Wightman, F., Ellenberg, P., Churchill, M. & Lewin, S. R. HDAC inhibitors in HIV. *Immunol Cell Biol* **90**, 47-54, doi:10.1038/icb.2011.95 (2012).

17. Archin, N. M. *et al.* Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* **487**, 482-485, doi:10.1038/nature11286 (2012).
18. Falkenberg, K. J. & Johnstone, R. W. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. *Nat Rev Drug Discov* **13**, 673-691, doi:10.1038/nrd4360 (2014).
19. Jones, R. B. *et al.* Histone Deacetylase Inhibitors Impair the Elimination of HIV-Infected Cells by Cytotoxic T-Lymphocytes. *PLoS pathogens* **10**, e1004287, doi:10.1371/journal.ppat.1004287 (2014).
20. Fuller, R. K. & Gordis, E. Does disulfiram have a role in alcoholism treatment today? *Addiction* **99**, 21-24 (2004).
21. Talbott, G. D. & Gander, O. Antabuse, 1973. *Md State Med J* **22**, 60-63 (1973).
22. Archin, N. M. *et al.* HIV-1 expression within resting CD4+ T cells after multiple doses of vorinostat. *J Infect Dis* **210**, 728-735, doi:10.1093/infdis/jiu155 (2014).
23. Palmer, S. *et al.* New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J.Clin.Microbiol.* **41**, 4531-4536 (2003).
24. Pasternak, A. O. *et al.* Cellular levels of HIV unspliced RNA from patients on combination antiretroviral therapy with undetectable plasma viremia predict the therapy outcome. *PLoS One* **4**, e8490, doi:10.1371/journal.pone.0008490 (2009).
25. Lewin, S. R. *et al.* Use of real-time PCR and molecular beacons to detect virus replication in human immunodeficiency virus type 1-infected individuals on prolonged effective antiretroviral therapy. *J Virol* **73**, 6099-6103 (1999).
26. Procopio, F. A. *et al.* A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV-infected Individuals. *EBioMedicine* **2**, 872-881, doi:10.1016/j.ebiom.2015.06.019 (2015).
27. Vandergeeten C*, et al.* Cross-Clade Ultrasensitive PCR-Based Assays To Measure HIV Persistence in Large-Cohort Studies. *J Virol* 88(21):12385-12396 (2014).
28. Palmer S*, et al.* Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc.Natl.Acad.Sci.U.S.A* 105(10):3879-3884 (2008).
29. Bouchat S, *et al*. Sequential treatment with 5-aza-2'-deoxycytidine and deacetylase inhibitors reactivates HIV-1. *EMBO Mol Med* 8:117-138 (2016)
30. Bouchat S, *et al*. Histone methyltranserase inhibitors induce HIV-1 recovery in resting CD4+ T cells from HIV-1 infected HAART-treated patients. *AIDS* 26(12):1473-1482 (2012).
31. Tripathy MK, *et al*. H3K27 Demethylation at the proviral promoter sensitizes latent HIV to the effects of Vorinostat in Ex vivo cultures of resting CD4+ T cells. J Virol 89(16):8392-8405 (2105)

APPENDIX 1 – AUDIT QUESTIONNAIRE

This questionnaire contains some questions about your use of alcoholic beverages during the past year.

As part of the screening process for this research project, it is important that we know this information. The information will assist in giving you an idea whether participation in this study is feasible. By participating in this study, your body will not be able to breakdown alcohol normally, as the study drug Disulfiram interferes with the process.

Therefore, we ask you to complete this questionnaire that asks about your use of alcoholic beverages during the past year. Please answer as accurately and honestly as possible.

Field Title	Field Type	Field options/instructions
Date of questionnaire	Date (DD/MM/YYYY) and time (HH:MM) 24 hr format	Automatically date and time stamped by REDCap database
1. How often do you have a drink containing alcohol?	- Never - Monthly or less - 2 - 4 times a month - 3 - 4 times a week - 4 or more times a week	Dropdown field – only 1 option can be selected If Never is chosen, then participants skip to question 9
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	- 1 or 2 - 3 or 4 - 5 or 6 - 7, 8 or 0 - 10 or more	Dropdown field – only 1 option can be selected
3. How often do you have six or more drinks on one occasion?	- Never - Monthly or less - 2 - 4 times a month - 3 – 4 times a week - 4 or more times a week	Dropdown field – only 1 option can be selected
4. How often during the last year have you found that you were not able to stop drinking once you had started?	- Never - Monthly or less - 2 - 4 times a month - 3 - 4 times a week - 4 or more times a week	Dropdown field – only 1 option can be selected
5. How often during the last year have you failed to do what is normally expected from you because of drinking?	- Never - Monthly or less - 2 - 4 times a month - 3 – 4 times a week - 4 or more times a week	Dropdown field – only 1 option can be selected
6. How often during the last year have you been unable to remember what happened the night before because you had been drinking?	- Never - Monthly or less - 2 to 4 times a month - 2 to 3 times a week - 4 or more times a week	Dropdown field – only 1 option can be selected

7. How often during the last year have you needed an alcoholic drink first thing in the morning to get yourself going after a night of heavy drinking?	- Never - Monthly or less - 2 to 4 times a month - 2 to 3 times a week - 4 or more times per week	Dropdown field – only 1 option can be selected
8. How often during the last year have you had a feeling of guilt or remorse after drinking?	- Never - Monthly or less - 2 to 4 times a month - 2 to 3 times a week - 4 or more times per week	Dropdown field – only 1 option can be selected
9. Have you or someone else been injured as a result of you drinking?	- Yes - No	Dropdown field – only 1 option can be selected
10. Has a relative, friend, or another health professional expressed concern about your drinking or suggested you cut down?	- Yes - No	Dropdown field – only 1 option can be selected
11. Participation in this research study will require you not to drink alcohol from 1 day before commencing study treatment to 2 weeks after your last study drug – a total of approximately 6.5 weeks. Are you happy not to drink any alcohol during this period?	- Yes - No	Dropdown field – only 1 option can be selected