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Short-term Disulfiram to Reverse Latent HIV Infection: A Phase 2 Dose Escalation Study

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Abstract

Background—Disulfiram activates HIV transcription in a primary T-cell model of HIV latency and in a pilot clinical study increased plasma HIV RNA in individuals with adequate diulfiram exposure.

Methods—We conducted a prospective dose escalation study in order to optimise disulfiram exposure. Thirty people with HIV on suppressive antiretroviral therapy (ART) were enrolled, allocated sequentially to one of three dosing cohorts and received disulfiram daily for three days at a dose of 500mg, 1000mg or 2000mg. The primary endpoint was cell-associated unspliced (CA-US) HIV RNA in CD4+ T-cells. The study is registered with ClinicalTrials.gov, number NCT01944371.

Findings—The estimated fold increases in CA-US HIV RNA during and post-disulfiram for each cohort were: 500mg: 1·7 (95% confidence interval 1·3 – 2·2) and 2·1 (1·5 – 2·9); 1000mg: 1·9 (1·6 – 2·4) and 2·5 (1·9 – 3·3); and 2000mg: 1·6 (1·2 – 2·1) and 2·1 (1·5 – 3·1) respectively ($p<0·003$ for all). Disulfiram was well tolerated at all doses.

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Designed the study: SGD, SRL, JHE; conducted the experiments: JHE, JHMcM, CCC, NB, JR, RH, AS, MP, RJG, JF, SGD, SRL; analysed the data: WH, PB, SAL, RS, CC, SRL; wrote the manuscript: JHE, SRL

Interpretation—Short-term administration of disulfiram resulted in increases in CA-US HIV RNA at all doses, consistent with activating HIV latency. Disulfiram may be suited for future studies of combination and prolonged therapy to activate latent HIV.

Introduction

Combination antiretroviral therapy (ART) has profound health benefits for people with HIV[1], but is not able to cure the infection[2]. This is primarily due to the ability of HIV to establish latency, particularly in long-lived memory CD4+ T-cells[3]. Finding a safe and scalable cure for HIV is a public health priority, as it would reduce the personal and societal costs and harms associated with HIV infection and its treatment[4]. A major strategy under investigation is the elimination of latently infected cells by inducing production of virus from these cells and thus making the cell susceptible to virus-induced cytolysis or killing by HIV-specific T-cells ("shock and kill")[5]. The administration of histone deacetylase inhibitors (HDACi) to people with HIV receiving ART to activate latent HIV infection showed an increase in transcription, as measured by cell associated unspliced (CA-US) HIV RNA, but no change in the number of infected cells, measured as HIV DNA. However, these drugs have significant adverse effects and therefore prolonged administration is not feasible[6–8].

Disulfiram has been used for the treatment of alcohol dependence for more than sixty years[9] and was recently identified to activate HIV transcription[10] using a primary CD4+ T cell model of latency[11]. In latently infected cells lines, increased HIV transcription induced by disulfiram was mediated via depletion of the phosphatase and tensin homolog (PTEN) and activation of protein kinase B (AKT)[10,12]. In a pilot clinical study, 14 days of disulfiram was given at the standard dose of 500mg daily to 16 adults on suppressive ART. Disulfiram was well tolerated, but there was no statistically significant overall change in plasma HIV RNA measured using a sensitive 'single copy assay' (SCA) or in the frequency of infectious virus[13]. However, there was a statistically significant increase in plasma HIV RNA in the period after completion of disulfiram compared to pre-dosing levels and, in a post-hoc analysis, participants with detectable disulfiram concentrations at any time point had a statistically significant increase in plasma HIV RNA[13].

We postulated that a more conclusive evaluation of the effect of disulfiram on latent HIV infection would be facilitated by optimizing drug exposure. We therefore performed a dose escalation study in which three cohorts received increasing doses of disulfiram and were evaluated for safety, tolerability, pharmacokinetics and effect on HIV transcription and plasma HIV RNA.

Methods

Study design

We conducted a prospective proof-of-concept dose escalation study. We hypothesised that an increase in CA-US HIV RNA would be seen within 24 hours, as observed in similar studies of HDACi[6–8] and in our initial pilot study of standard dose disulfiram[13]. A three day course of disulfiram would therefore have an effect on latent HIV infection and minimise toxicity given we planned to administer high doses. We assumed that CA-US HIV RNA is log

normally distributed and that the standard deviation of pre- and post-dose levels is 0.6 log10 (derived from our previous work quantifying CA-US RNA in people with HIV on suppressive ART for at least 3 years[7]). Based on three dose levels, ten patients per group and a null hypothesis of no change in CA-US HIV RNA, we estimated that the study would have approximately 80% power to detect a 0.5–0.6 log (3.1–4.0 fold) difference between pre-treatment and post-treatment peak CA-US HIV RNA within each group at the 0.05 significance level.

Participants

We recruited thirty adults aged 18 years or over, receiving at least three antiretroviral agents, with plasma HIV RNA < 50 copies per mL for at least three years, a recent CD4+ T cell count > 350 cells/μL and able to abstain from alcohol during and for 14 days after disulfiram dosing. We excluded individuals with significant acute illness; hepatic, cardiac, respiratory, neurological or renal disease; diabetes; hypothyroidism; malignancy; transplantation; hazardous alcohol use or use of drug formulations that could contain alcohol. Participants provided written informed consent prior to inclusion in the study and The Alfred Human Research Ethics Committee and San Francisco General Hospital Institutional Review Board approved the study. An independent Safety Monitoring Committee reviewed safety and tolerability data after the second cohort had completed dosing. The study is registered with ClinicalTrials.gov, number NCT01944371.

Outcomes

The primary outcome measure was change in CA-US HIV RNA in total CD4 T-cells[7]. Secondary efficacy endpoints were sensitive measures of plasma HIV RNA and HIV DNA. Safety endpoints were plasma HIV RNA measured using a commercial assay with a lower limit of detection of 20 copies per ml (TAQMAN v2, Roche), adverse events, serious adverse events, dose limiting toxicity and CD4+ T-cell count.

Procedures

Three cohorts of participants (n=10 each) were sequentially enrolled and received disulfiram daily for three days at doses of 500mg (licensed dose), 1000mg or 2000mg. Baseline samples were obtained at three pre-dosing timepoints (screening [B1], immediately prior to the first dose [B3] and a timepoint between [B2].

Liquid chromatography-tandem mass spectrometry was used to quantify disulfiram and its metabolites. The analytes were extracted from plasma via protein precipitation and separated on a Kinetex column (phase C18; diameter, 2.1mm×50mm; Phenomenex, Torrance, USA) using mobile phases (A) Water, 0.1% formic acid and (B) Acetonitrile, 0.1% formic acid. CD4 T-cells were isolated from stored peripheral blood mononuclear cells (PBMC) using a CD4+ T cell isolation kit and magnetic-activated cell sorting columns and CA-US RNA and DNA quantified as recently described[1]. Plasma HIV RNA was measured using an ultrasensitive assay with a dynamic range from a lower limit of detection of 0·19 copy/mL of HIV RNA using 7 mL of plasma, to 10^6 copies/mL[14].

Randomisation and masking

This was a non-randomised study in which enrolled participants were allocated sequentially to the first, second and then third dosing cohort. Laboratory assays were performed by staff masked to dosing cohort. All other study staff were unmasked.

Statistical analysis

Analyses of CA-US HIV RNA modeled the estimated number of copies present in each specimen divided by the input of total RNA in the specimen. Number of copies analyzed was the sum of the estimated number of copies in each replicate. Input RNA was the 18s RNA measurement, multiplied by the number of replicates providing data on number of copies. Analyses of plasma HIV RNA modeled the estimated number of copies present in each specimen divided by plasma volume in the specimen. Specimens with no virus detected were treated as having zero copies.

The primary analysis method was negative binomial regression, with the number of copies being the outcome variable and the input total RNA or plasma volume, respectively, included as an exposure variable, which is equivalent to modeling copies/input. Advantages of negative binomial models over other approaches included the ability to model positively skewed data (i.e. data that violate the normality assumptions underlying many other modelling approaches) that has greater variability at higher values, to readily account for variation in the amount of input total RNA or plasma volume, and to include specimens with observed values of zero without the need for ad hoc adjustments that could introduce bias. The outcome measures for this study are subject to Poisson sampling variability, because <0.1% of a participant's bloodstream was sampled for any given assay measurement and the numbers of target molecules tended to be low; negative binomial regression generalizes the Poisson model to also account for other sources of variability, notably person-to-person differences. Specimens with lower input total RNA or volume provide less information about the patient's true copies/cells or copies/volume in their entire bloodstream. The models produce estimates of relative or fold changes. For example, an estimated effect of 2.0-fold for post-disulfiram versus baseline CA-US RNA indicates that typical within-person changes were 2-fold (double, 100% increase) from baseline to post-disulfiram. The models are able to produce fold-change estimates while including participants with no CA-US or plasma HIV RNA in baseline samples, because they account for how sampling variability can produce a specimen with zero copies. All three pre-dosing time points were included as baseline observations in the primary a priori analysis; figures show the average of the 3 baseline observations. Inclusion of the random intercept terms focused the analysis on within-person changes. These models were used to estimate changes from pre-disulfiram to time points during disulfiram dosing and post-dosing.

The pharmacokinetic model used 239 disulfiram concentrations from 30 HIV patients. An initial base model structure was established using the full pharmacokinetic profile data from disulfiram. A compartmental model with first order absorption and elimination was used to describe pharmacokinetics of disulfiram and it was parameterized using clearance, volume and absorption rate constant. A relative bioavailability parameter was estimated for each dose level relative to the 1000 mg dose. Parameters were assumed to be lognormally

distributed. Diagonal and full variance-covariance block of the parameter distributions were investigated. Additive, proportional, and combined error models were evaluated for residual variability. Individual based area under the curve (AUCs) were estimated using posthoc individual parameter estimates. Data were analyzed using a nonlinear mixed effects (NLME) approach with software (NONMEM, version 7 software, ICON, Dublin, Ireland). The First Order Conditional Estimation with Interaction method was used..

Role of the funding source

The funding sources had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

A total of thirty participants were recruited at two centres between September 2013 and April 2014 (Figure 1). The mean age was 52·8 years and 28 were male. Median baseline CD4 count was 630 cells/μL (Table 1). There was a significant increase in CA-US HIV RNA at multiple timepoints during and following disulfiram dosing in all three dosing cohorts compared to the mean of the three pre-disulfiram time points (negative binomial regression model; Figure 2 and Supplementary Tables 1 and 2, Supplementary appendix pages 4 and 5). When grouped into pre-, during and post-disulfiram time points and analysed by negative binomial regression, the fold increases between the pre-dose period and later periods for each cohort were; 500mg: 1·7 (95% confidence interval [CI] 1·3 – 2·2; p<0·0001) and 2·1 (95% CI 1·5 – 2·9; p<0·0001; overall model p<0·0001); 1000mg: 1·9 (95% CI 1·6 – 2·4; p<0·0001) and 2·5 (95% CI 1·9 – 3·3; p<0·0001; overall model p<0·0001); and 2000mg: 1·6 (95% CI 1·2 – 2·1; p=0·0026) and 2·1 (95% CI 1·5 – 3·1; p=0·0001; overall model p=0·0003; Supplementary Figure 1, Supplementary appendix page 1). There was no statistically significant change in HIV DNA measured at three baseline time points versus day 3 or day 30 (Supplementary Table 3, Supplementary appendix page 6).

Compared to the mean of the three pre-disulfiram time points, plasma HIV RNA during and post-disulfiram did not statistically significantly increase in the participants who received 500mg and 1000mg per day (Figure 2). In the participants who received 2000mg per day, a post-dosing increase in plasma HIV RNA was observed with fold increases on Day 7 of 1·7 (95% CI 1·14 – 2·7; p=0·015) and on Day 30 of 2·0 (95% CI 1·3 – 3·1; p=0·0014; Figure 2). A similar increase was also seen at the time point 2 hours after the final dose of disulfiram. Grouping time points into pre-, during and post-disulfiram periods demonstrated similar results. In the 2000mg cohort, fold changes during and post-disulfiram dosing were 1·3 (95% CI 0·96 – 1·8; p=0·086) and 1·9 (95% CI 1·3 – 2·7; p=0·0014; model p=0·0054; Supplementary Figure 1, Supplementary appendix page 1), respectively.

All participants completed the study, and there were no deaths, drug discontinuations or grade 3 or 4 adverse events (Figure 1). Grade 1 clinical adverse events related to study drug were more common in the higher dosing cohorts with 0, 10 and 14 events in the 500mg,

1000mg and 2000mg cohorts, respectively. There were no Grade 2 clinical adverse events related to study drug (Table 2).

A one-compartment disposition model with first-order absorption best described the pharmacokinetics of disulfiram. The estimated clearance was 0·35 L/hr, (CV%=20%), the volume of distribution was 1·6L and the absorption constant was 0·12 hr^{-1}. The median cumulative plasma drug concentration-time area under the curve (AUC) values for the 500, 1000 and 2000 mg groups were 573, 2845, and 8355 mg-hr/L, respectively. Higher-than-dose-proportional increases in disulfiram exposure were estimated to be due to dose-dependent increases in relative bioavailability (Figure 3).

Of the three baseline samples, the third was collected earlier in the day, immediately prior to the first dose of disulfiram ($p<0·0001$; Figure 4 and Supplementary Figure 1, Supplementary appendix page 1). CA-US HIV RNA was statistically significantly higher at this third time point (B3, estimated effect: 2.6-fold higher, 95% CI 2.2 – 3.3; $p<0·0001$). There was no statistically significant difference in plasma HIV RNA or HIV DNA between these three timeponts (Figure 4). In a post-hoc analysis, random intercept negative binomial regression models were used to evaluate potential explanatory factors for the higher CA-US HIV RNA at B3, including time of sampling, time from sampling to processing and simple markers of a potential stress response (pulse rate, systolic and diastolic blood pressure and temperature). None of these factors were statistically significant predictors of CA-US HIV RNA ($p>0·3$ for all). Although wide uncertainty in our estimates left open the possibility that these factors could influence CA-US HIV RNA, none substantially altered the estimated increase at B3 (smallest estimated B3 effect 2.4-fold, still with $p<0.0001$).

Given the unexpected variation in pre-treatment values we conducted a post-hoc analysis using B3 only as the baseline. Increases in CA-US RNA during and post-disulfiram were still observed, but were modest. Fold changes between the pre-dose period and the later periods for each cohort were; 500 mg: 0·98 (95% CI 0·7 – 1·3; p=0·88) and 1·2 (95% CI 0·8 – 1·7; p=0·33; overall model p=0·24); 1000 mg: 1·3 (95% CI 0·94 – 1·7; p=0·12) and 1·6 (95% CI 1·2 – 2·3; p=0·005; overall model p=0·011); and 2000 mg: 0·89 (95% CI 0·6 – 1·3; p=0·54) and 1·2 (95% CI 0·7 – 1·9; p=0·41; overall model p=0·12; Supplementary Figure 2, Supplementary appendix page 2), respectively.

We performed a post-hoc analysis of the subgroup of participants with baseline (B3) CA-US HIV RNA above the median value and high exposure to disulfiram and its metabolites as indicated by cumulative AUC above the median value. In this subgroup there were statistically significant increases in plasma HIV RNA in the post-disulfiram period, with a 1·56-fold increase on Day 7 (p=0·032) and 1·59-fold on Day 30 (p=0·024; Supplementary Figure 3, Supplementary appendix page 3).

Discussion

In this dose-escalation study, short-term administration of disulfiram was safe and well tolerated, even at doses four times the currently approved dose. Disulfiram therapy resulted in prolonged increases in CA-US HIV RNA at all doses and in plasma HIV RNA at high

dose, consistent with activating HIV latency. As was observed in our pilot study[13] and studies of the HDACi vorinostat and panobinostat[7,8], there was an apparent increase in CA-US HIV RNA that increased further after dosing. CA-US HIV RNA levels were highest on Day 30, 28 days after the last dose. We also observed a statistically significant post-dose increase in plasma HIV RNA in the cohort who received 2000mg/day. Given an excellent safety profile, disulfiram may be suited for future studies of combination therapy to activate latent HIV.

Over the decades in which disulfiram has been used for the treatment of alcohol dependence, clinical practice has included dose titration of up to 6g per day[16] in order to overcome the large inter-individual variation in drug exposure[17]. Using a similar approach, but with dose increases between rather than within individuals, we demonstrated the short-term safety and tolerability of disulfiram at up to four times the current licensed dose, consistent with previous reports of the safety and tolerability of disulfiram in the absence of alcohol[18,19]. This dose escalation study also demonstrated a clear dose-exposure relationship, including evidence of saturation of first pass effect demonstrated by the supra-proportional increase in disulfiram exposure at a dose of 2000 mg, compared to increases observed from 500 mg to 1000 mg, consistent with previous pharmacokinetic studies[17].

With the disulfiram exposure achieved in the highest dosing cohort of 2000mg we observed a clear effect of disulfiram on latent HIV infection, with a significant increase in plasma HIV RNA in this dosing cohort. Furthermore, in a post-hoc analysis, participants with high baseline CA-US HIV RNA (at B3) and high exposure to disulfiram or its metabolites had statistically significant and prolonged increases in CA-US HIV RNA and plasma HIV RNA. In vitro, an increase in HIV transcription in latently infected cells lines with disulfiram was mediated by depletion of the phosphatase and tensin homolog (PTEN) and activation of protein kinase B (AKT), but whether this is the main mechanism in vivo is unknown[10].

We saw statistically significant increases in CA-US HIV RNA within two hours of the first dose of disulfiram, potentially indicating a rapid effect on HIV transcription, but also a prolonged effect in which CA-US HIV RNA levels were qualitatively stable following the last dose and then increased between study days 7 and 30 (5 and 28 days post-last dose). Similarly, increases in plasma HIV RNA in the highest dosing cohort occurred after the third dose, but increases were also seen in the period between days 7 and 30. Similar post-dosing effects were seen in studies of vorinostat and panobinostat[7,8], but these changes in CA-US HIV RNA, at least following vorinostat, were observed together with prolonged persistent changes in host gene expression[7]. Although we have not performed similar studies of host gene expression following disulfiram, we do not expect similar host gene changes given the mechanism of action. It remains unclear why a persistent post dose effect was seen both in CA-US and plasma HIV RNA in this study, and these changes should be closely monitored in future studies of latency reversing agents.

An unexpected finding of the study was large variation in pre-dosing CA-US HIV RNA, with levels statistically significantly higher immediately prior to the first dose than at the two previous time points, in the absence of similar changes in HIV DNA or plasma HIV RNA. We are confident that this was not a technical issue as samples from the B3 timepoint were

not run independently; all samples from each participant were always run together on the same plate. We speculate that the higher US RNA at B3 may be due to changes in HIV transcription due to circadian rhythm or anticipatory stress. Circadian rhythm is governed centrally by the BMAL:CLOCK heterodimer. CLOCK has intrinsic histone acetyl transferase (HAT) activity while BMAL-1 potentiates its HAT function[20]. Acetylation of histones bound to the HIV LTR is well known to activate HIV RNA transcription[21,22] and knockdown of CLOCK results in 10-fold decrease in HIV proviruses per cell[23]. Plasma HIV RNA in untreated HIV-infected individuals[24] and CD4+ T-cell subsets in HIV-uninfected individuals also vary over the day with a higher proportion of naïve T-cells in the morning[25]. Given the substantially lower level of HIV DNA in naïve compared to memory CD4+ T-cells in HIV-infected participants on ART[26], a change in the proportion of naïve to memory T-cells would have affected HIV DNA but not CA-US HIV RNA and the HIV RNA:DNA ratio as observed here.

Alternatively, stress associated with the initiation of disulfiram dosing may have led to a hormonal response that altered HIV RNA transcription. For example, HIV replication *in vitro* increases after addition of both hydrocortisone and norepinephrine to cultures of HIV-infected PBMC[27,28]. The absence of a confirmed biological explanation for the higher CA-US HIV RNA at B3 adds some uncertainty about our evidence for the effect of disulfiram on CA-US HIV RNA, but we believe that plausible causes are unlikely to have persisted throughout the study period, particularly to Day 30, and so would not provide an alternative explanation for all the increases that we observed.

Disulfiram has several attractive features for further investigation as a potential agent for HIV latency reversal. The long-term safety of disulfiram is well understood given the long history of chronic use for alcohol dependence. This contrasts with HDACi, where there are concerns regarding persistent effects on epigenetic modification[7] and HIV-specific immunity[29]. Assessment of the potency of disulfiram *in vivo* is difficult given that it requires cross-study comparisons and a maximum tolerated dose has not been defined, but qualitatively the efficacy of disulfiram on changes in plasma RNA, at the doses investigated in this study, was modest and an order of magnitude less that what was recently observed with the HDACi romidepsin, but still greater than the less potent HDACi vorinostat, which did not lead to statistically significant changes in plasma HIV RNA[6,7]. Furthermore, our data suggest that higher doses of disulfiram may have greater efficacy. This relationship is currently being investigated with more detailed pharmacokinetic/pharmacodynamic modeling. Finally, *in vitro* studies of the combination of disulfiram and an HDACi have demonstrated statistically significant additive induction of CA-US HIV RNA compared to disulfiram alone[30], suggesting the potential for combination therapy to activate latent HIV, for example a combination of prolonged administration of disulfiram at a dose of 2000mg per day with panobinostat. A recent clinical study showed no benefit from adding disulfiram to therapeutic vaccination with recombinant modified vaccinia Ankara-based (MVA-B) HIV-1 vaccine, but disulfiram was dosed at 250mg per day in that study[31].

In this short-term dose escalation study of the effect of disulfiram on latent HIV infection we demonstrated that disulfiram was safe and well tolerated and at high dose an increase in plasma HIV RNA was observed. Given an excellent safety profile and in vitro data

suggesting additive effects when combined with HDACi, disulfiram may be suited for future studies of prolonged or combination therapy to activate latent HIV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

SRL's institution has received funding from Gilead, Viiv, Merck and Tetralogic to support investigator-initiated research and from Gilead, Viiv and Merck for chairing and speaking at educational activities. JHE, JMM and JR's institution has received funding from Gilead, Viiv and Merck for investigator-initiated research and for chairing and speaking at educational activities.

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Research in context

Evidence before this study

We searched PubMed and included all human interventional studies in any language that examined the effect of HIV latency reversing agents on latent HIV infection and were listed between inception and 31st August, 2015. Search terms included “HIV”, “HIV latency”, “latent HIV”, “latency reversing”, “HIV reservoir”, “HIV eradication” and names of specific agents including “vorinostat”, “panobinostat”, “rhomidepsin” and “disulfiram”, as well as the terms “HDAC” or “histone deacytelase inhibitor”. Evidence of activation of HIV transcription in CD4+ T-cells was found for several HDACi in non-randomised studies.

Added value of this study

This study is the largest and first dose escalation study of a latency reversing agent. The study demonstrated a clear dose dependent effect of disulfiram on HIV transcription and a significant increase in plasma HIV RNA in individuals on ART. High dose disulfiram was safe, resulted in a supra-proportional increase in plasma levels of disulfiram and its metabolites and led to a prolonged increase in plasma HIV RNA.

Implications of all the available evidence

A safe and potent activator of latent HIV is a necessary first step towards the elimination of latently infected cells in people with HIV. Given an excellent safety profile and in vitro data suggesting additive effects when combined with HDACi, future studies of prolonged high dose disulfiram, alone or in combination with other latency reversing agents, are warranted.

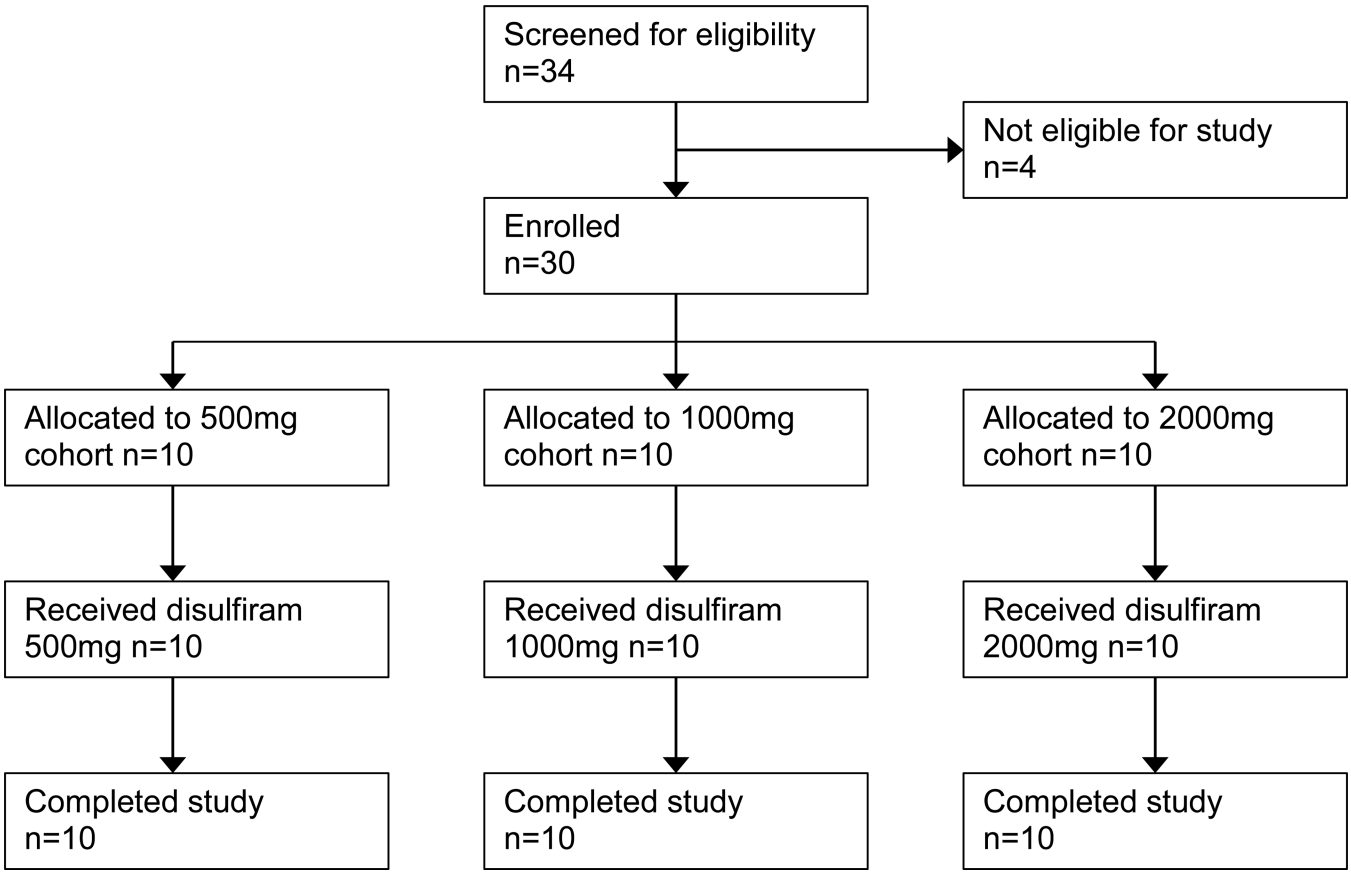

Figure 1. Study profile

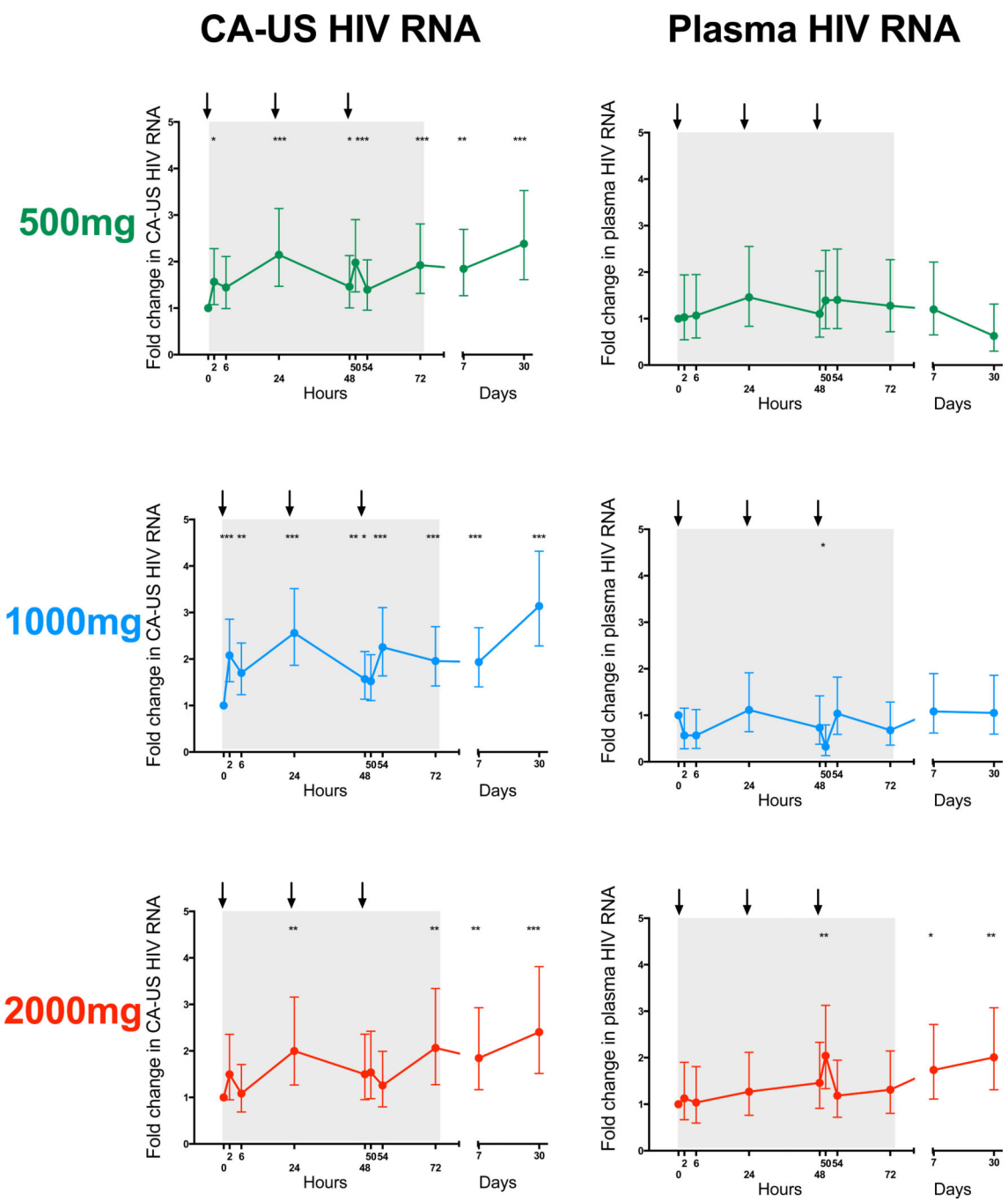

Figure 2. Effect of disulfiram on CA-US HIV RNA in total CD4 T-cells and plasma HIV RNA

Fold change in CA-US and plasma HIV RNA following administration of disulfiram, from negative binomial regression with whiskers showing 95% confidence intervals. Each time point is compared to the mean of three pre-disulfiram time points. Disulfiram was administered at time 0, 24 and 48 hours (vertical black arrows). ***p<0·001; **p<0·01 – 0·001; *p<0·05 – 0·01.

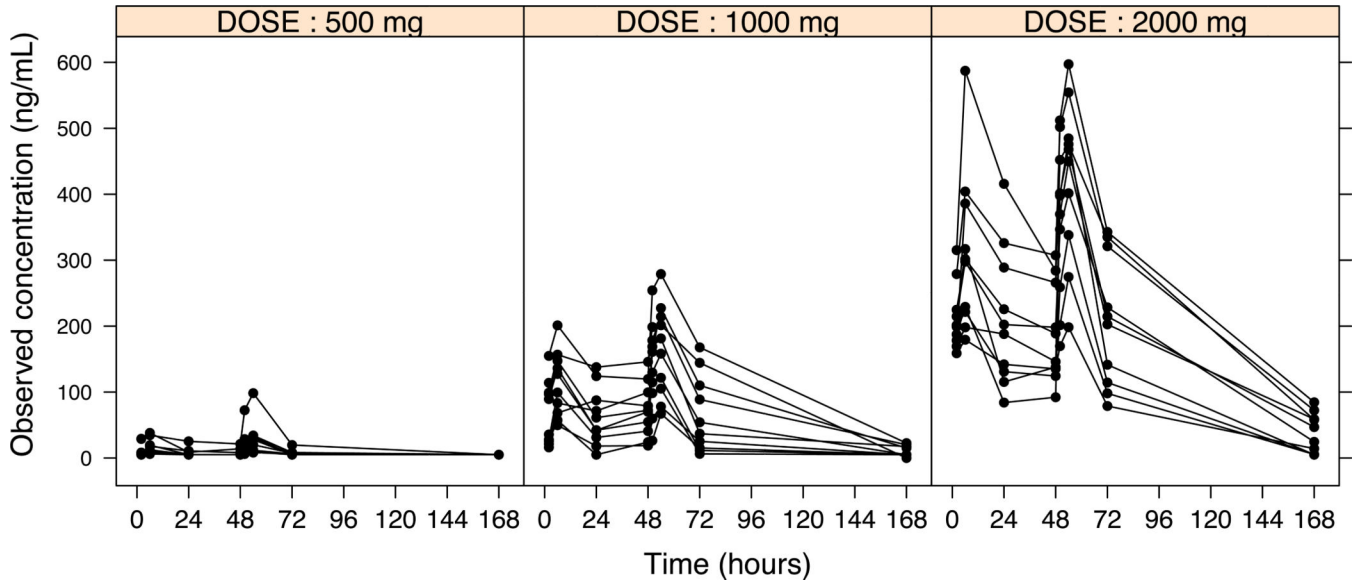

Figure 3. Pharmacokinetic relationship between disulfiram dose and disulfiram drug concentration levels (ng/mL)

Disulfiram concentration-time curves for each dosing cohort demonstrate increases in plasma drug concentrations after disulfiram doses given on days 0, 1 and 2 with declines in drug levels thereafter up to day 7. The median cumulative area under the curve (AUC) values for the 500, 1000 and 2000 mg groups were 573, 2845, and 8355 mg-hr/L. Disulfiram given at 2000 mg demonstrated a 48% higher-than-dose-proportional increase in drug exposure compared to the 500 mg or 1000 mg groups.

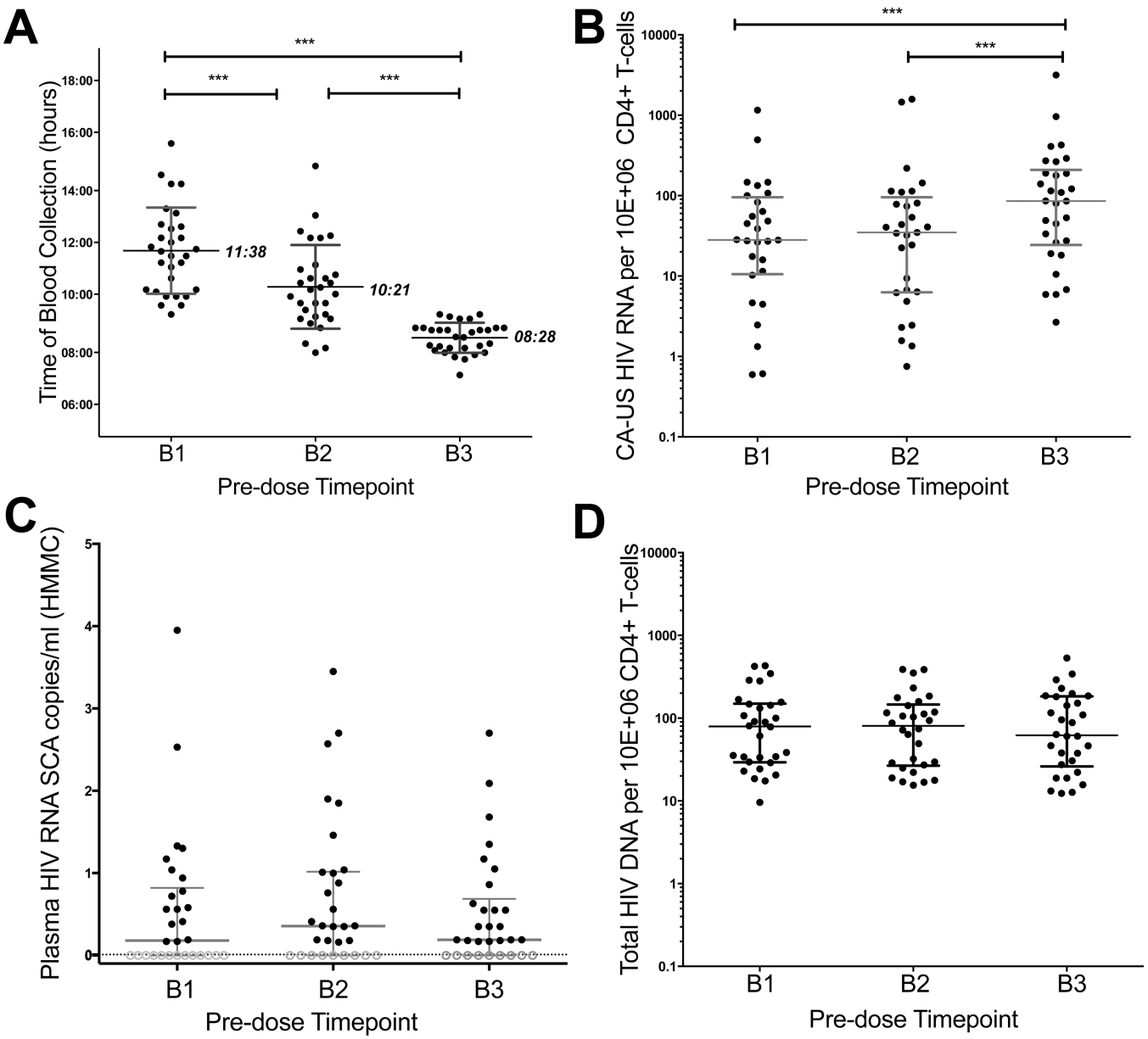

Figure 4. Baseline variability in CA-US HIV RNA

Quantification at each of the three baseline time points: the screening visit (B1), the sample taken immediately prior to the first dose of disulfiram (B3) and a sample taken on a day between B1 and B3 (B2). A) Time of blood sampling; B) CA-US HIV RNA; C) Plasma HIV RNA; and D) HIV DNA. Central lines and whiskers indicate the mean and standard deviation (B); and median and 25th and 75th percentiles (A, C, D). ***p<0·001; **p<0·01 – 0·001; *p<0·05 – 0·01.

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Table 1

Baseline characteristics of study participants

Patient ID	Gender (M = Male, T = Transgender)	Race/Ethnicity[b]	Age (years)	Baseline CD4 (cells/μL)	Regimen[c]	NNRTI, PI, or INI based regimen
1E001	M	AFR	56·2	489	TDF/FTC/EFV	NNRTI
1E002	T	AFR	51·9	714	TDF/FTC/EFV	NNRTI
1E003	M	EUR	41·0	595	TDF/FTC/EFV	NNRTI
1E004	M	AFR	61·3	476	TDF/FTC/EFV	NNRTI
1E005	M	EUR	62·9	479	TDF/FTC/EFV	NNRTI
1R006	M	AFR	53·0	524	TDF/FTC ATV + RTV	PI
1R008	M	EUR	63·4	768	ABC/3TC + ATV + RTV	PI
1R009	M	HIS	53·7	401	TDF/FTC + DRV + RTV	PI
1R010	M	EUR	34·4	781	ABC + TDF + ATV + RTV	PI
1R032	M	EUR	55·0	1180	TDF/FTC + RAL + DRV + RTV	PI / INI
2E011	M	EUR	39·9	663	TDF/FTC/RPV	NNRTI
2E012	M	EUR	54·3	1137	TDF/FTC + NVP	NNRTI
2E013	M	EUR	48·5	651	TDF/FTC/EFV	NNRTI
2E015	M	HIS	49·0	500	TDF/FTC/EFV	NNRTI
2R016	M	EUR	51·6	623	TDF + 3TC + DRV +RTV	PI
2R017	T	AFR	54·4	569	ABC/3TC + LPV/RTV	PI
2R018	M	EUR	60·5	390	TDF/FTC + RAL	INI
2R019	M	EUR	55·2	597	TDF/FTC + RAL + DRV + RTV	PI / INI
2R020	M	EUR	64·9	663	ABC/3TC + RAL	INI
2R033	M	EUR	61·6	543	TDF/FTC +ATV + RTV	PI
3E021	M	AFR	45·9	478	TDF/FTC/RPV	NNRTI
3E022	M	EUR	30·1	772	ABC/3TC + NVP	NNRTI
3E023	M	MIX	66·4	521	3TC + RAL + DRV + RTV + NVP	INI / PI / NNRTI
3E024	M	EUR	67·2	414	TDF/FTC + FPV + RTV	PI
3E025	M	EUR	58·1	442	TDF/FTC/RPV	NNRTI
3R026	M	EUR	58·8	613	ABC/3TC + TDF + DRV + RTV	PI

Patient ID	Gender (M = Male, T = Transgender)	Race/Ethnicity[b]	Age (years)	Baseline CD4 (cells/μL)	Regimen[c]	NNRTI, PI, or INI based regimen
3R027	M	ASI	56·9	857	TDF/FTC/RPV	NNRTI
3R029	M	EUR	51·0	529	TDF/FTC + FPV + RT	PI
3R030	M	EUR	50·6	525	TDF/FTC + NVP	NNRTI
3R034	M	EUR	26·1	1022	TDF/FTC/RPV	NNRTI
Summary Value[a]	**28 (90%) Male**		**54·3 (48·9 – 60·7)**	**582 (487 – 728)**		

[a]Values represent n (% with characteristic) or median (interquartile range)

[b]b AFR = Black/African-American, EUR = White/European-American, ASI = Asian, HIS = Hispanic/Latino, MIX = Mixed Race/Multiracial

[c]Antiretroviral agents separated by "/" were fixed dose combination

NOTE: TDF, Tenofovir; 3TC, Lamivudine; FTC, Emtricitabine; EFV, Efavirenz; NVP, Nevirapine; ABC, Abacavir; 3TC, Lamivudine; DRV, Darunavir; RTV, Ritonavir; LPV, Lopinavir; RAL, Raltegravir; ATV, Atazanavir; RPV, Rilpivirine; FPV, Fosamprenavir

Table 2

Adverse events possibly, probably or definitely related to disulfiram

Dosing Cohort	500mg			1000mg			2000mg			Total
Adverse events	Grade[a]			Grade[a]			Grade[a]			
	1	2	3	1	2	3	1	2	3	
Clinical										
Gastrointestinal										
Abdominal pain	1			3			1	1		6
Diarrhoea	1			3			1	1		6
Constipation							1			1
Gastrointestinal – dry mouth				1			2			3
Bad taste in mouth							2			2
Gastrointestinal-nausea							2			2
Thirst							1			1
Gastrointestinal-nausea					1					1
Oral apthous ulcer				1						1
Neurological										
Neurologic - headache	1			2			3			6
Neurologic - trouble falling asleep				1			1			2
Neurologic - poor coordination							1			1
Neurologic - trouble concentrating							1			1
Dizziness							1			1
Anxiety							1			1
Ocular – trouble focussing							1			1
Ocular – blurred vision on reading							1			1
Olfactory – ‘chemical smell’							1			1
Neurologic - poor memory				1						1
Constitutional / Other										
Irritated throat							1			1

Dosing Cohort	500mg			1000mg			2000mg			Total
Adverse events	**Grade[a]**			**Grade[a]**			**Grade[a]**			
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	
Sore throat							1			1
Systemic-feeling drowsy or sleepy		1		2			1			4
Palpitations							1			1
Systemic-fatigue				1			2	1		4
Light headedness							3			3
Awareness of fast heart beat							1			1
Systemic-ringing in the ears				1						1
Skin-pruritus with no skin lesions				1						1
Lack of morning erection				1						1
Genitourinary-frequent need to urinate				1						1
Sweating	1			1						2
Musculoskeletal-myalgia				1						1
Hypertension	1									1
Laboratory[b]										
Thromobocytopenia							1			1
Hypophosphatemia		3[c]			2[d]			2		7
Increased Creatinine				1						1
Hypercalcemia				1						1
Increased aspartate Aminotransferase	1			1						2
Hypokalaemia	1									1
Hypernatremia	1									1

[a] Grading according to Division of AIDS table for grading the severity of Adult and Pediatric adverse events. August 2009

[b] Laboratory events considered related to study drug if abnormality (onset of abnormal grade or increase in grade) commenced on Day 2, 3 or 4

[c] Two events occurred at the Alfred and were within the reference range for the local laboratory.

[d] Both events occurred at the Alfred and were within the reference range for the local laboratory.