Combination Latency Reversal With High Dose Disulfiram Plus Vorinostat in HIV-infected Individuals on ART (DIVA)

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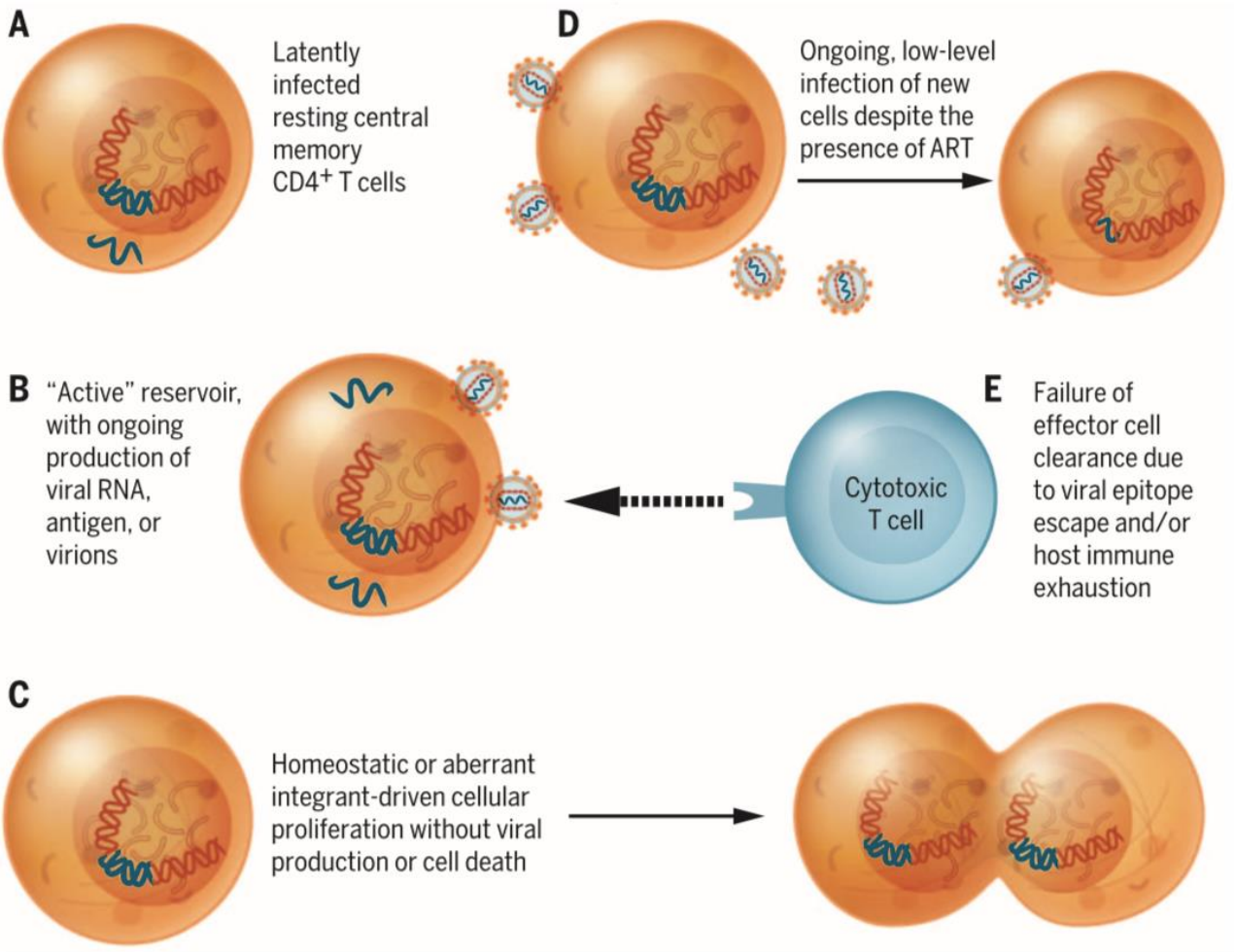
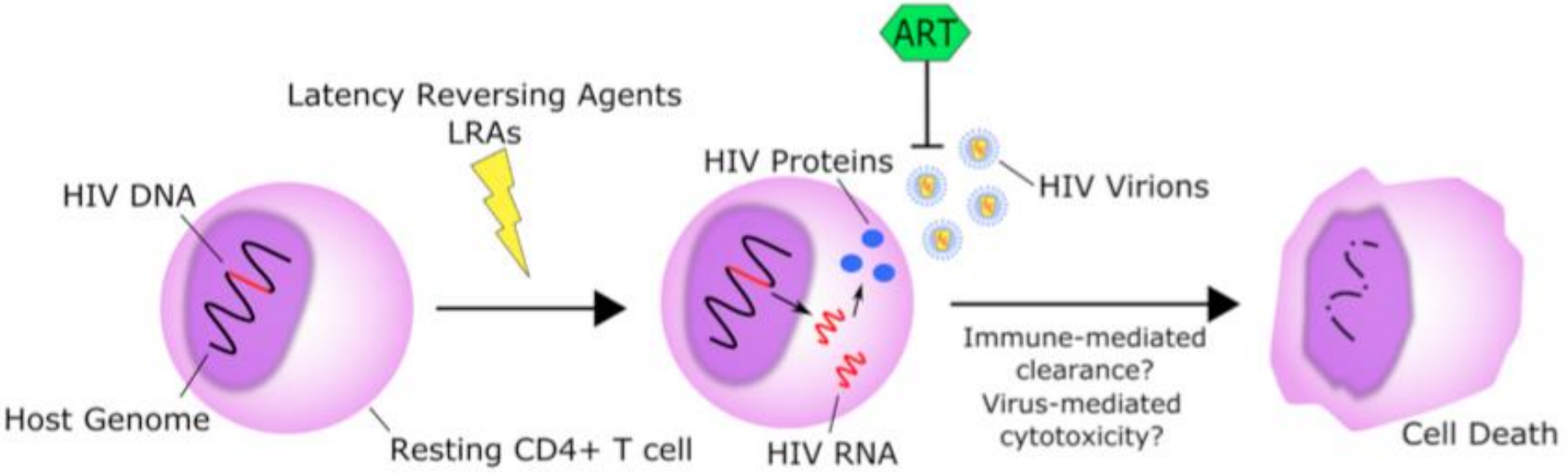
HIV Latency and Barriers to Eradication



Margolis, Science 2016

Latency Reversal



Kim, Cell Host & Microbe, 2018

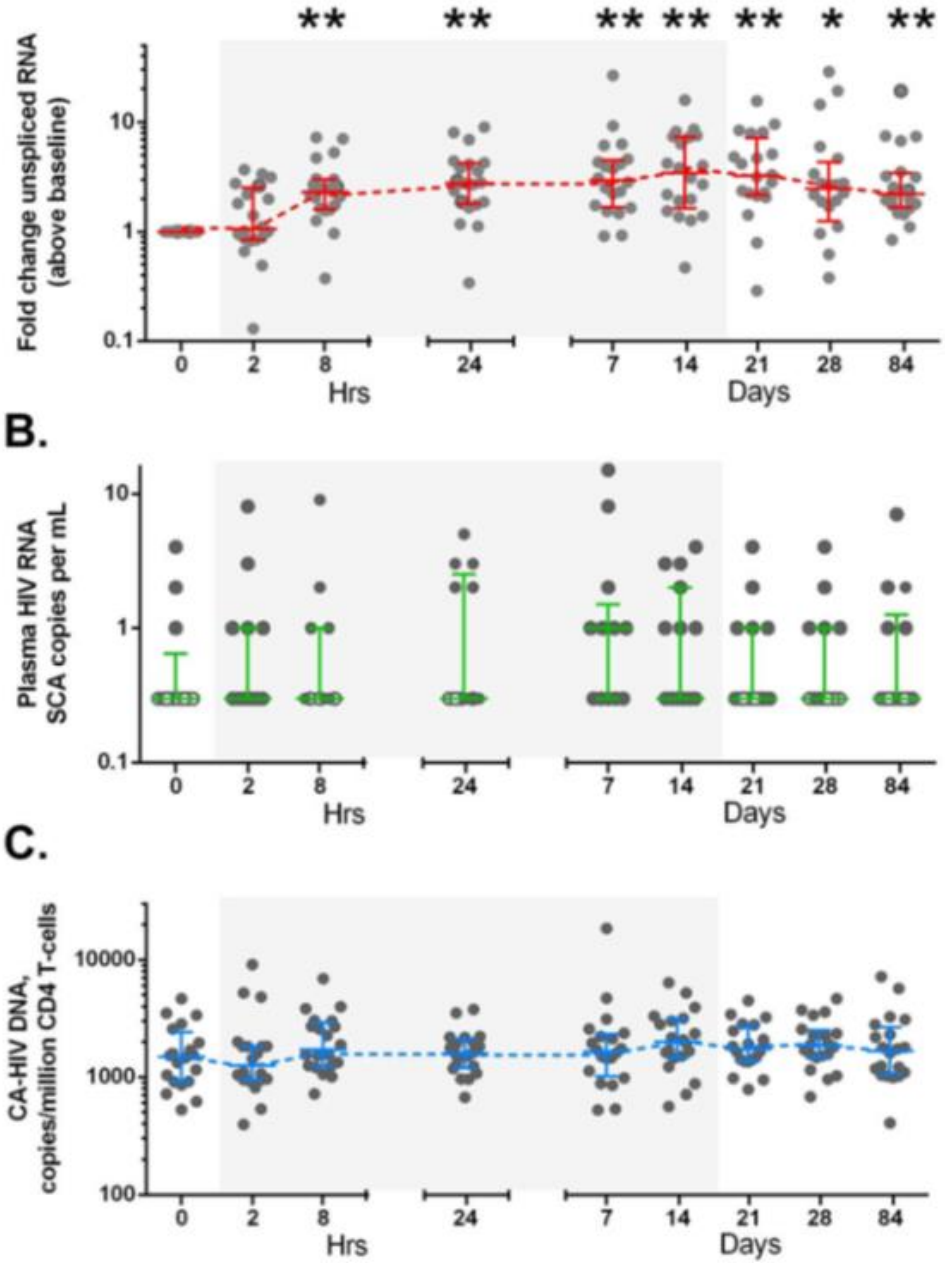
Latency reversal in trials

Latency reversing agent	Site of action	Dosing	US HIV RNA	Plasma RNA	HIV DNA	Negative studies
Vorinostat	HDACi	Single dose[1], Intermittent[2], Continuous[3]	↑	↔	↔	
Panobinostat	HDACi	Intermittent dose[4]	↑	+/-	↔	
Romidepsin	HDACi	Weekly dose[5]	↑↑	↑↑	↔	McMahon CROI 2019
Disulfiram	AKT activation	High dose 2g/day[6]	↑	↑	↔	
Bryostatin	PKC agonist	Low dose 10-20ug/m^2	↔	↔	↔	
Lefitolimod	TLR9 agonist	Twice weekly[8]	↓	↑↑	↔	
Pembrolizumab	PD-1 blockade	Every 3 weeks[9]	↑	↔	↔	
GS986 and GS9620	TLR7 agonist	Dose escalation[10]	ND	↑↑↑	↓	Riddler IAS 2019

1 Archin Nature 2012; 2 Archin JID 2014; 3 Elliott Plos Pathogens 2014; 4 Rasmussen Lancet HIV 2014; 5 Sogaard Plos Pathogens 2015; 6 Elliott Lancet HIV 2015; 7 Gutierrez AIDS 2016; 8 Vibholm CID 2017; 9 Uldrick CROI 2019; 10 Lim Sci Trans Med 2018

Vorinostat (HDACi)

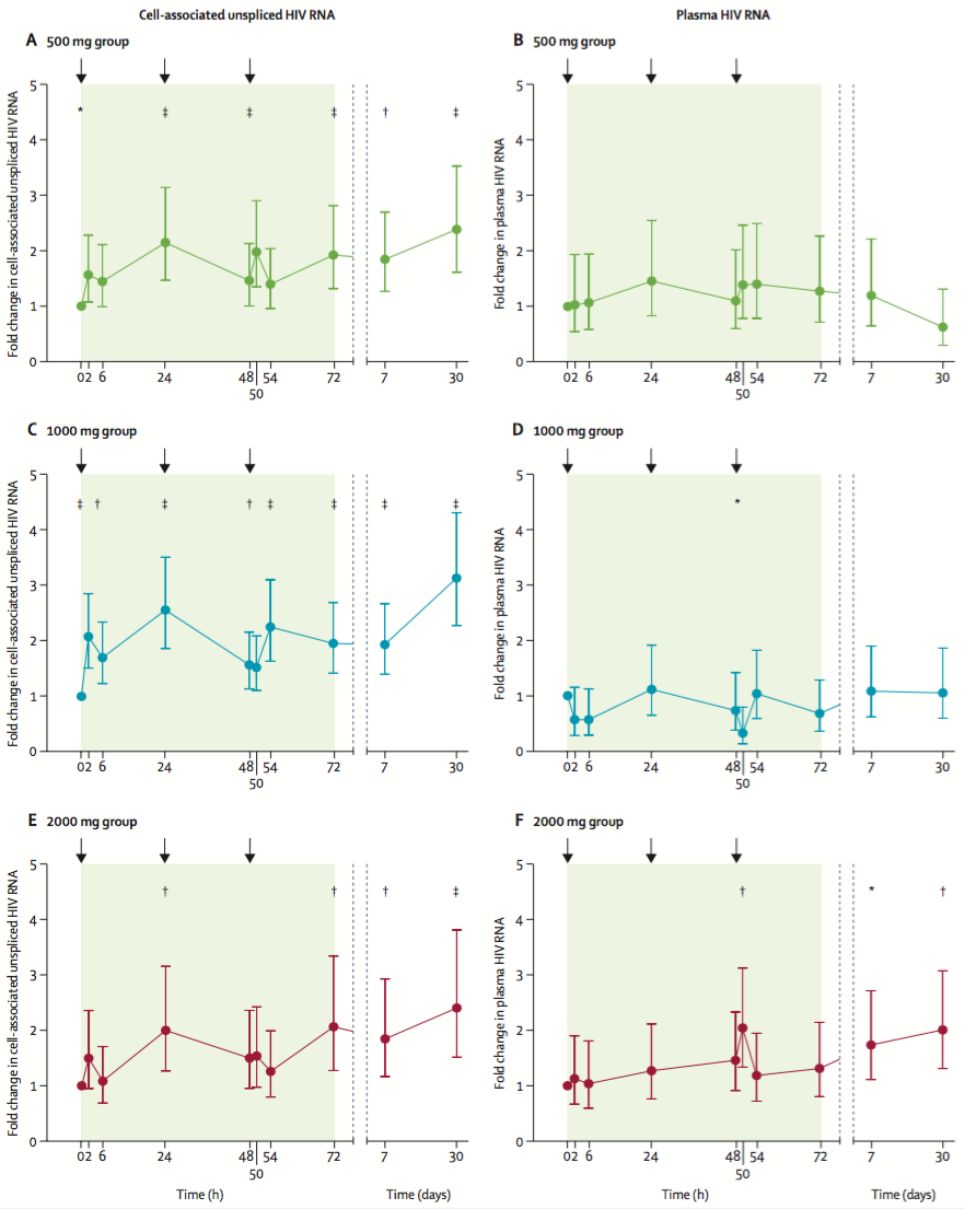
- 20 HIV infected individuals receiving 14 days of 400mg daily Vorinostat
- 7 fold in increase in CA-US HIV RNA ($p<0.001$)
- No changes in HIV RNA or HIV DNA



Elliott Plos Pathogens 2014

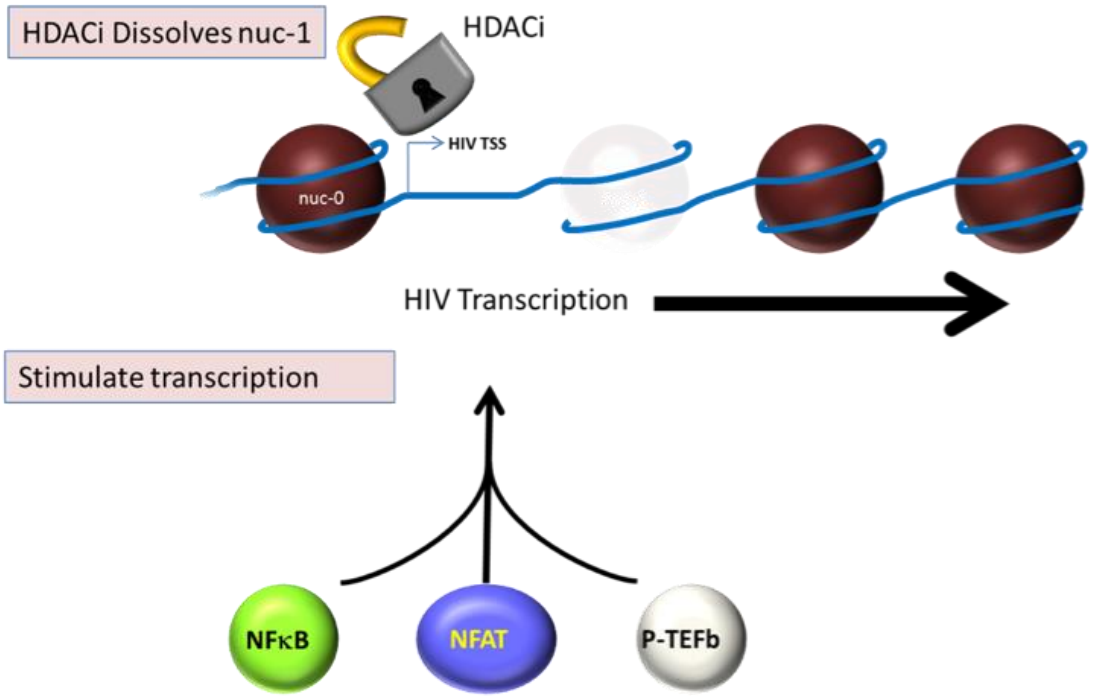
Disulfiram – Dose escalation

- Licensed dose 200-500mg daily
- 3 days DSF
- Well tolerated
- CA-US RNA. Approximately 2 fold ↑ at all doses (p< 0.001)
- HIV RNA. At 2000 mg only
  - 1·7 fold ↑ to day 7 (p=0·015)
  - 2 fold ↑ to day 30 (p=0·0014)
  - Similar ↑ 2h post day 3 dose



Elliott Lancet HIV 2015

Synergism of LRAs with HDACi



Multiple synergistic combinations

HDACi + 5 aza-C (methylation inhibitor)[1]
HDACi + DNA methyltransferase inh[2, 3]
HDACi + bryostatin [4]
HDACi + disulfiram [4]
HDACi + screening drug library (Merck)[5]

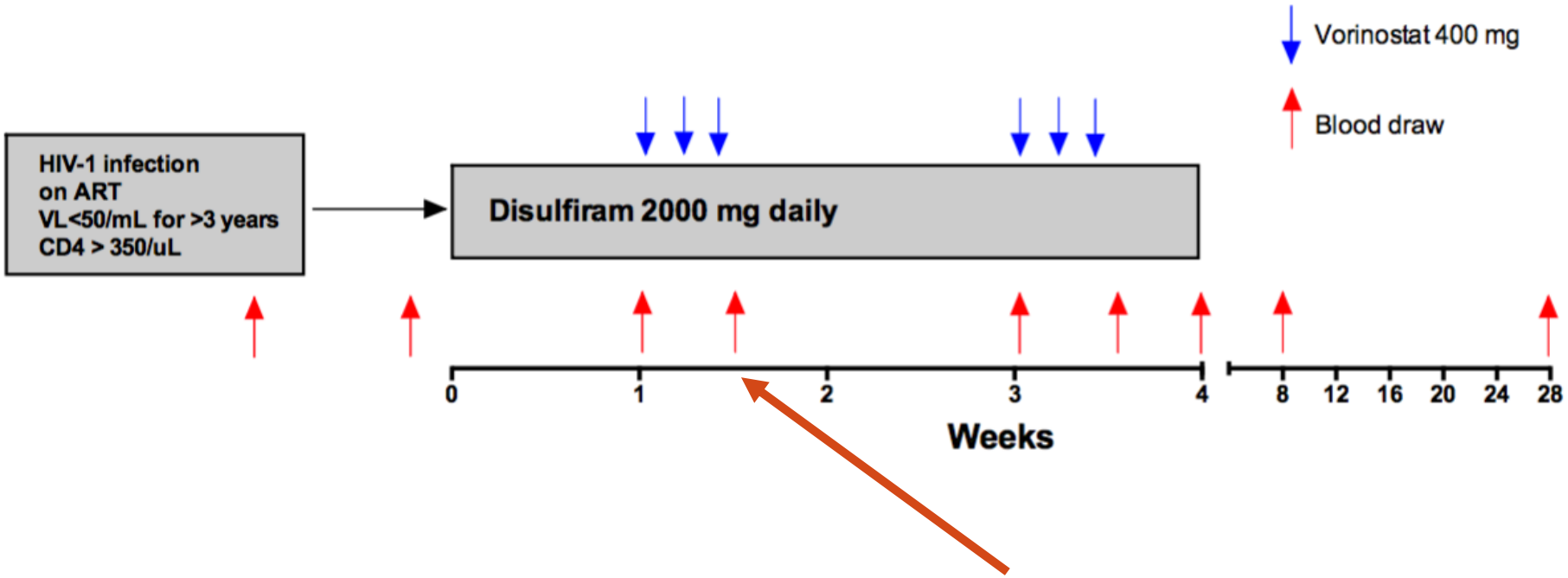
1 Bouchat EMBO Mol Med 2016; 2 Bouchat AIDS 2012; 3 Tripathy MK J Virol 2015; 4 Laird GG J Clin Inv 2015; 5 Barnard R., CROI 2017

Hypotheses

- Targeting different latency pathways will reverse HIV latency more potently than a single agent

- Administration of 28 days of high dose 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.
  - Be safe and well tolerated

Design - Open label single arm clinical trial



- High dose DSF (2g/day) for 28 days + intermittent VOR
- Single arm study (n=15)

Primary endpoint
Plasma HIV RNA on day 11 relative to baseline

Main Inclusion / Exclusion criteria

- Age 18-65 years with documented HIV-1 infection
- Receiving cART with plasma HIV RNA <50 copies/mL for >3 years
- CD4+ T cell count >350 cells/uL at screening
- Willing to abstain from alcohol consumption from one day before to 14 days after completing 28 days of disulfiram
- Women of child bearing potential – contraception

Exclusion criteria

- Participation in LRA study in prior 12 months
- Active HBV or HCV infection

Participant 1 (P1)

67 yr old male

HIV

- Dx 1990s, CD4: 762 cells/µL
- Viral load: <20 copies/ml
- Current ART: ABC/3TC/DTG

Hypertension

Familial hypercholesterolaemia

D8: well, nil concerns, commenced 3 days VOR

D11: reports fatigue since starting DSF, loss of appetite but no other symptoms, missed D10 doses of DSF and VOR

D14 and D16: well, nil concerns

Missed D22 visit

D24: Brought to clinic by a friend. Verbose, sometimes tangential, perseverating but orientated. Also – felt cold, sore throat, back ache

Stopped taking all meds (including ART) ? days to up to a week

Exam - mild ataxia. Admitted to hospital, study drugs ceased

That evening: Some disorganised thoughts, unusual stories, mild confusion and paranoia

Investigations

- Mildly elevated liver enzymes (ALT 61 AST 37 GGT 119 ALP 149), stable renal function, normal inflammatory markers
- Lumbar puncture no red or white blood cells, normal glucose, slightly elevated protein 0.59 g/L (normal range 0.15 – 0.4 g/L)
- Blood, urine and cerebrospinal fluid cultures negative
- Neuroimaging (CT Brain with venogram and angiogram, Carotid Angiogram, MRI Brain): small curvilinear non-occlusive thrombus in left sigmoid sinus, findings consistent with chronic occlusion of left vertebral artery and possibly an old right cerebellar infarct

Neurology review - not a classical presentation but clinical findings could possibly be explained by sagittal sinus thrombosis

ABC/3TC/DTG changed to TAF/FTC + DTG in setting of MRI findings and cardiovascular risk with abacavir

Symptoms resolved in hospital and discharged home D29. Plan for 3 months anticoagulation followed by lifelong aspirin

As a potential alternate cause (sagittal sinus thrombosis) other than study medications and onset of symptoms after starting disulfiram the events deemed probably related to disulfiram, Grade 3 in severity and a serious adverse event

Remainder of follow up asymptomatic

Participant 2 (P2)

61 yr old male

HIV

- Dx > 10 years ago
- CD4: 1085 cells/µL
- Viral load: <20 copies/ml
- Current ART: TAF/FTC + RAL

Osteopaenia

BCC / SCCs

OSA

OA knees

Peripheral neuropathy

D8: Reports mild lethargy, diarrhoea, dysgeusia for 1 week

Close contact had gastroenteritis with significant diarrhea, P2 reported symptoms improving (particularly diarrhea).

Continued with DSF and commence VOR

D9: collapse in shower, presented to ED, thought to be vasovagal in setting of hypovolaemia from diarrhoea, bloods unremarkable, observed overnight, discharged home

D11: Pressured speech, ‘disconnection’ between mind and body, fatigued, emotionally labile, Oriented, provided good history.

Normal exam apart from ataxia

Study drugs stopped and went home

Last doses taken D10 (Therefore 10 days DSF and 2 days VOR)

D11: Admitted in the evening due to significant increase in emotional lability. Orientated, tangential speech. Paranoid themes. Episode of transient haematuria

No localising infective symptoms

Investigations Bloods unremarkable (Bilirubin 36 - same as pre-study 40-50). CT brain: normal

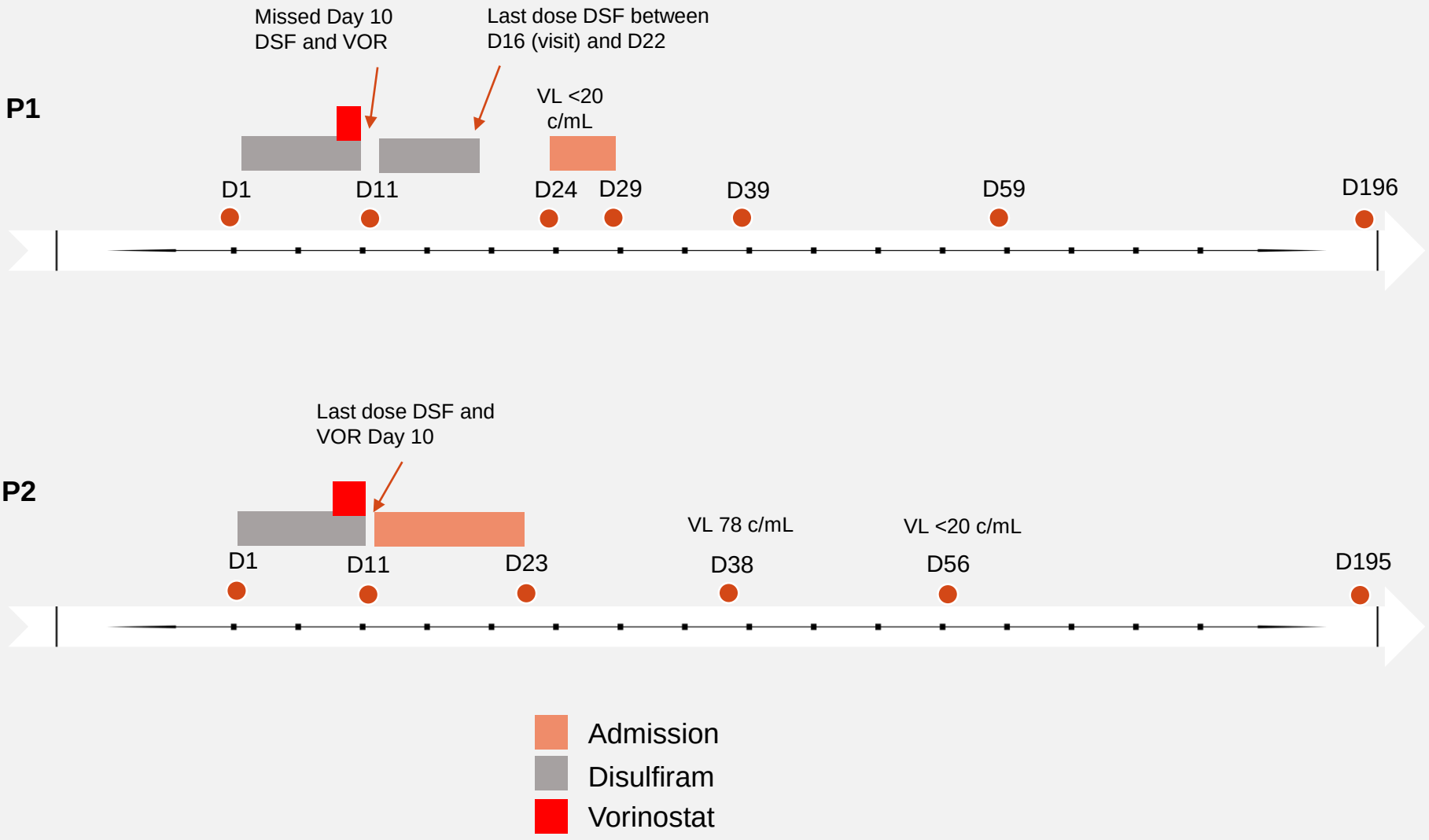
D11 → D12 – Ongoing emotional lability. Sleep deprived. Agitated, Paranoid ideation

D13 → 14 improvement in emotional lability, fatigue, paranoid thoughts, ataxia. Still pressured speech, emotional

Not for MRI / LP and participant keen to avoid this

D23: Mental state at baseline. Diagnosed with small renal calculus. Discharged home

Timelines


P1
Missed Day 10
DSF and VOR
Last dose DSF between
D16 (visit) and D22
VL <20
c/mL
D1
D11
D24
D29
D39
D59
D196
P2
Last dose DSF and
VOR Day 10
VL 78 c/mL
VL <20 c/mL
D1
D11
D23
D38
D56
D195
Admission
Disulfiram
Vorinostat

Summary of Clinical Events

2 Serious Adverse Events of Altered mental status
P1 - possibly related to Disulfiram resulting in treatment discontinuation
P2 - probably related to Disulfiram resulting in treatment discontinuation, and possibly related to Vorinostat or unexpected drug interaction between Disulfiram and Vorinostat

Neurotoxicity – hypomania, disordered thoughts, paranoia

Improved within 10 days of cessation of study drugs

? Related to accumulated DSF – toxicity

? unexpected drug interaction of VOR to DSF (although no rationale for this based on known PK of both drugs)

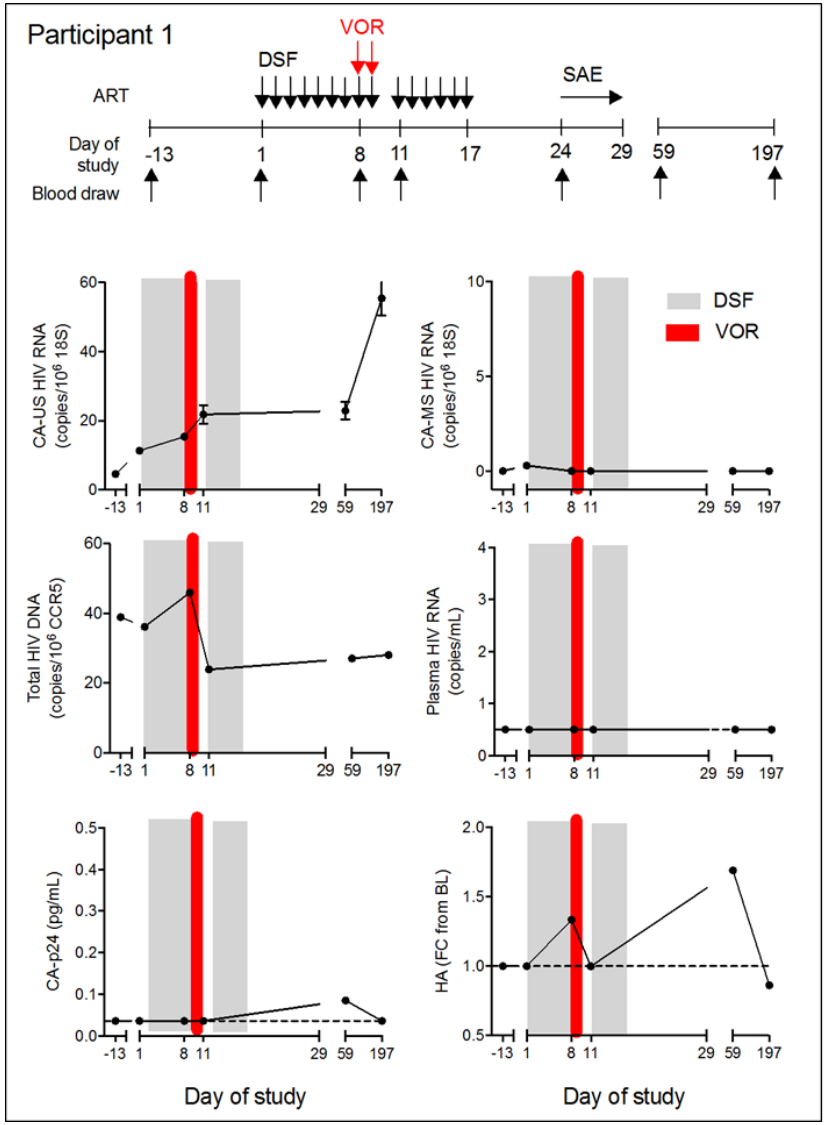
Other Endpoints

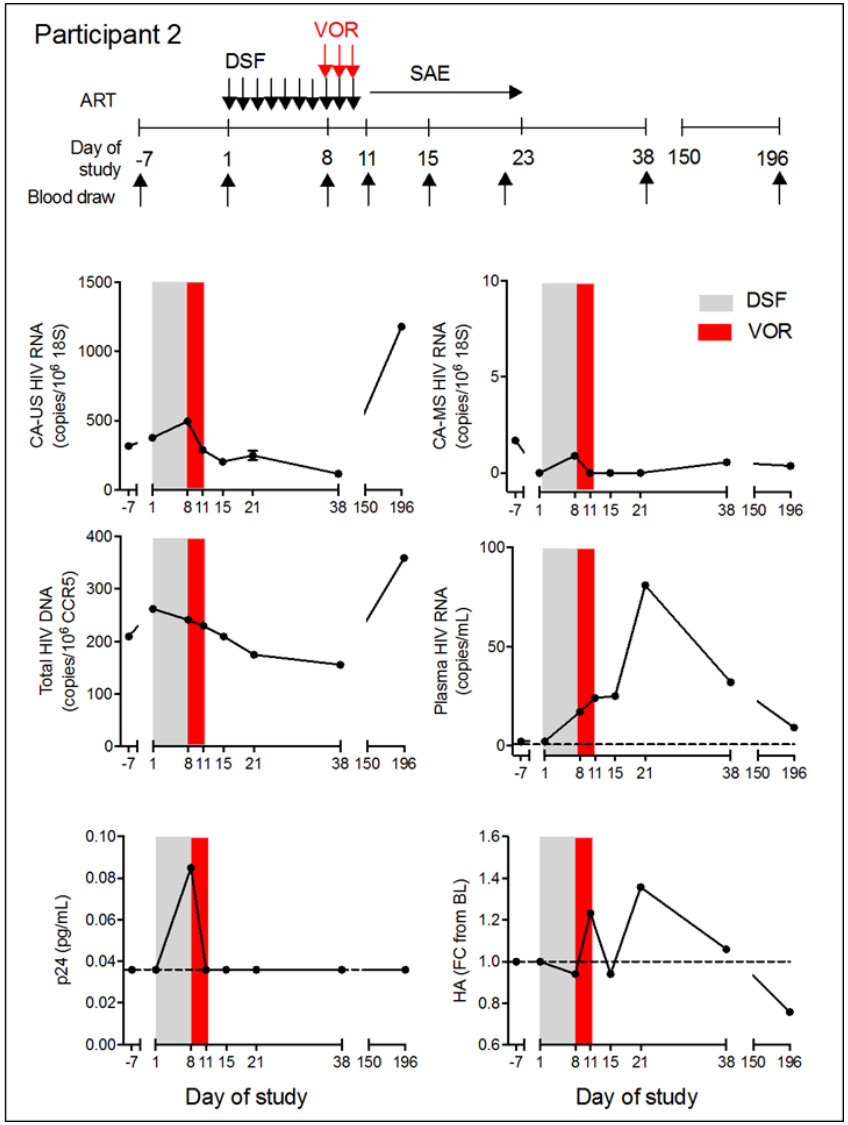
HIV transcription: HIV RNA, cell-associated unspliced and multiply spliced HIV RNA, p24 expression in blood CD4+ T cells

HIV reservoir: Cell-associated total and integrated HIV DNA in peripheral and TILDA in blood CD4+ T cells

PK/PD: Concentrations of ART, vorinostat and disulfiram in plasma

Gene expression: RNA-Seq

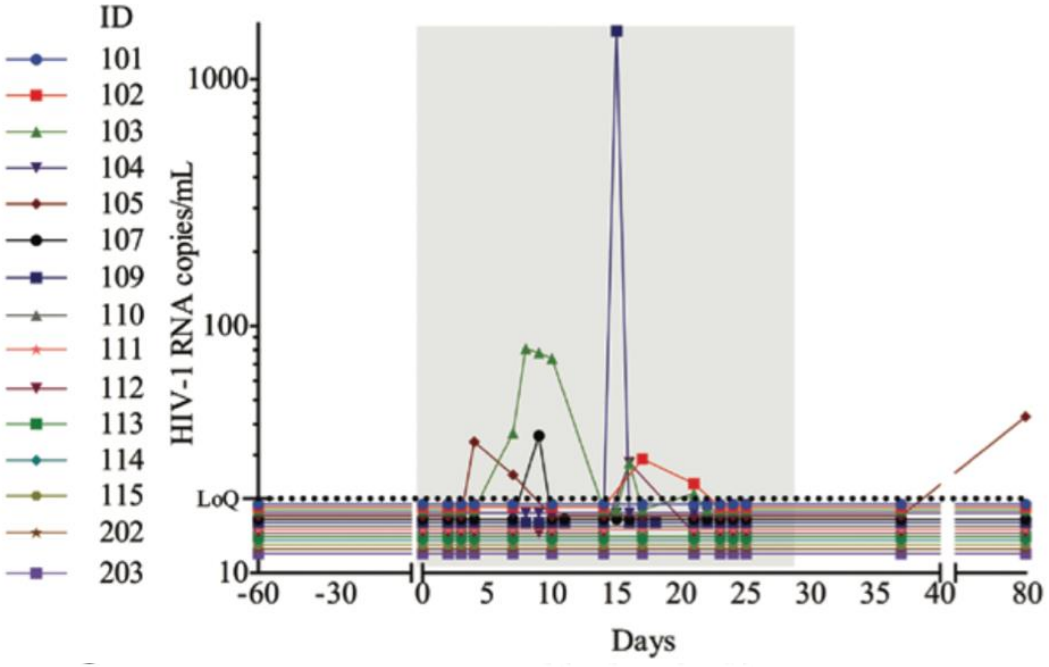




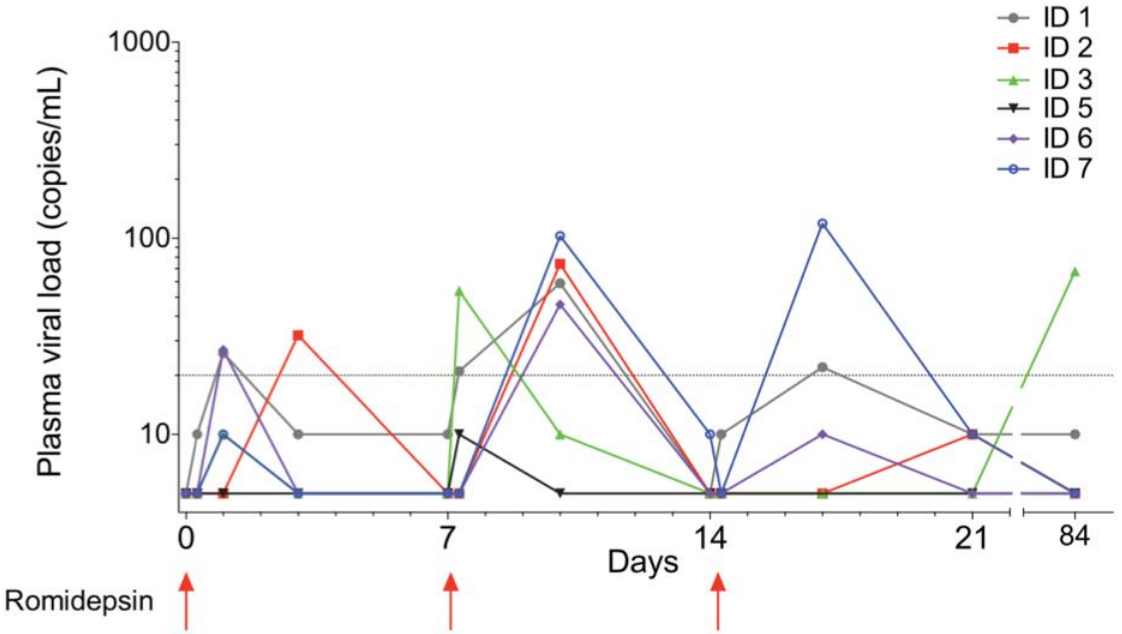
Inpatient from day 11 – 23
Day 38 HIV RNA 78 c/mL (Cobas Taqman)

HIV viremia in LRA trials

TLR9 agonist
Vibholm CID 2017



Romidepsin
Sogaard PLoS Pathog 2015



Vorinostat levels

Subject	Time	Concentration (ng/mL)	Last VOR Dose
P1	Day 1	< 2.00	
	Day 8	< 2.00	
	Day 11	6.52	48 hours prior
	Day 58	< 2.00	
P2	Day 1	< 2.00	
	Day 8	< 2.00	
	Day 11	7.06	24 hours prior
	Day 15	< 2.00	
	Day 21	< 2.00	
	Day 37	< 2.00	

Levels 24 and 48 hours post dose possibly higher than reported elsewhere

- Median plasma VOR conc. 24 hrs post single dose 400mg VOR was < 1 ng/mL[1]
  - Median maximum was 244 ng/mL after median 2 hours[1]
  - Similar levels seen with 3 days a week dosing in weekly cycles[2]
- Similar levels in people with cancer[3]

1 Archin Nature 2012 2 Archin JID 2014 3 Rubin Clin Cancer Res 2006

Disulfiram PK

Participant	Visit	Disulfiram	Carbamathione
P1	Day 1	BLOD	BLOD
	Day 8	BLOQ	BLOD
	Day 11	BLOQ	BLOD
	Day 59	BLOD	BLOD
P2	Day 1	BLOD	BLOD
	Day 8	BLOQ	BLOQ
	Day 11	BLOQ	BLOQ
	Day 15	BLOD	BLOD
	Day 21	BLOD	BLOD

DSF and carbamathione levels. BLOQ (Below limit of quantification; 0.5 - 10 ng/mL for DSF), BLOD (Below limit of detection; < 0.5 ng/mL for DSF)

- DSF easily detectable in tablets taken by participants
- DSF levels detectable but below level of quantification for P1 and P2 at days 8 and 11
- Metabolite carbamathione only detectable in P2 at days 8 and 11
- Median time to processing for PK was 157 minutes, samples not transported on ice
- In dose escalation study. Median time to processing 80 minutes for 118 samples (p=.003 Wilcoxon rank-sum, comparing the 2 studies on time to processing)

Disulfiram PK - dose escalation study

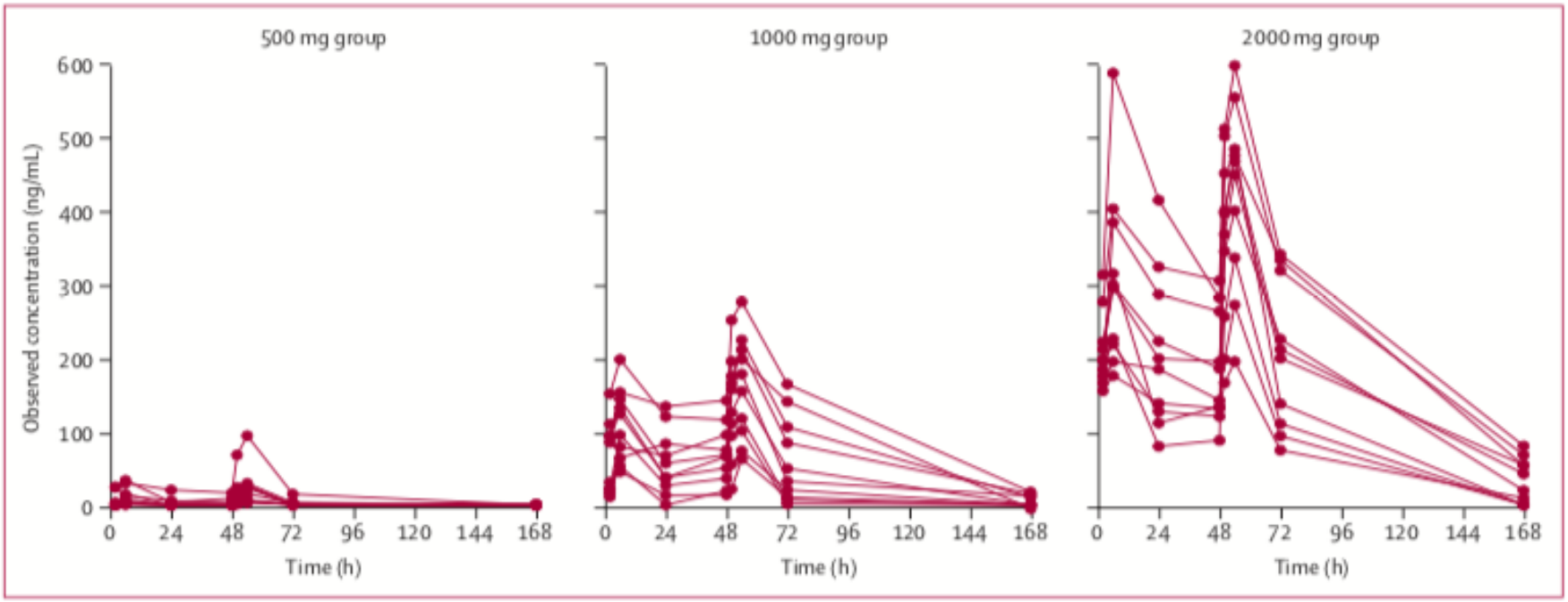


Figure 3: **Pharmacokinetic association between disulfiram dose given and disulfiram plasma drug concentrations**
Disulfiram concentration-time curves for each dosing cohort.

Elliott, Lancet HIV, 2015

Antiretroviral levels

Participant	Visit	ABC (mg/l)	3TC (mg/l)	FTC (mg/l)	DTG (mg/l)	RTG (mg/l)
P1	Day 1	3.3	2.15		4.75	
	Day 8	3.65	1.62		4.00	
	Day 11	1.96	1.75		3.29	
	Day 24	< 0.015	0.0236		0.0385	
	Day 58	< 0.015	< 0.015	0.536	3.00	
	Day 196	< 0.015	< 0.015	1.58	4.75	
P2	Day 1			0.236		0.518
	Day 8			0.295		0.335
	Day 11			0.48		0.427
	Day 15			0.343		0.130
	Day 21			0.292		0.326
	Day 37			0.222		0.937
	Day 196			0.248		1.52

- Levels consistent with adherence to therapy
- P1 not taking ART on D24
- P1 switched regimen for from ABC/3TC/DTG to TAF/FTC + DTG after D24

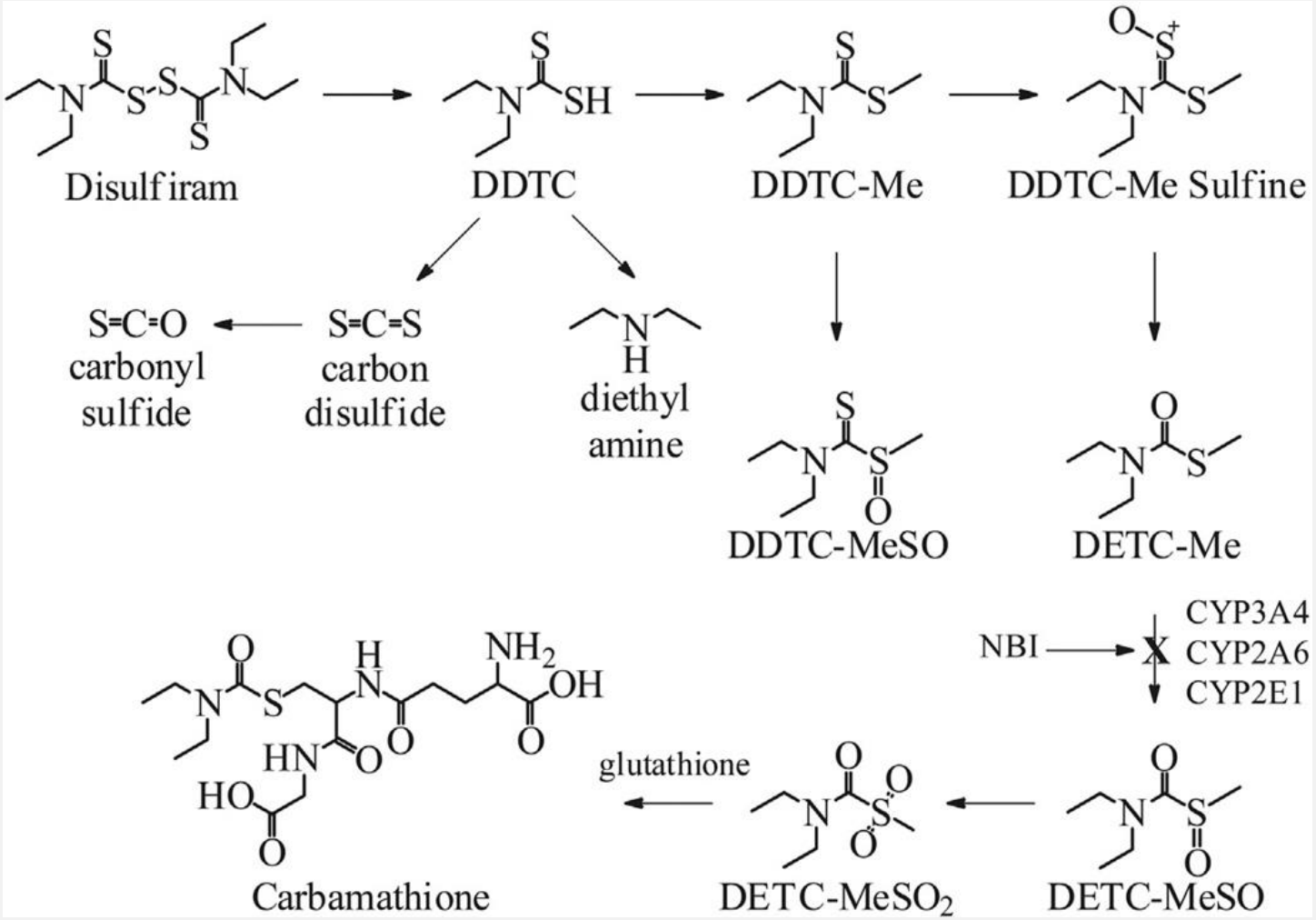
- Typical trough levels: DTG 1.1 mg/L, RTG 0.194 mg/L
- NRTI trough levels are usually below the limit of quantification

Discussion - Disulfiram Dose escalation study

- 500mg, 1g and 2g/day for 3 days in cohorts of 10 participants
- No grade 3 or 4 adverse events
- Neurological events were all grade 1 and more common in the 2g/day (n=11) cohort than the 1g/day (n=4) or 500 mg/day (n=1) cohorts
  - E.g. headache, poor sleep, dizziness, poor coordination, anxiety
- Systemic fatigue (grade 1-2) in four participants, three in the 2g/day cohort and one in the 1g/day cohort.

Elliott, Lancet HIV, 2015

Disulfiram Metabolism



Neuropharmacology. 2013 Dec; 75:95-105

Disulfiram neurotoxicity

- Toxic disulfiram metabolites are diethyldithiocarbamate (DDC) and its metabolite carbon disulfide (CS_2)
- DDC chelates copper → impairs activity of dopamine beta-hydroxylase, (catalyzes metabolism of dopamine to norepinephrine) → depletion of presynaptic norepinephrine and accumulation of dopamine
- Dopamine agonism may be implicated in some of the altered behavior associated with disulfiram toxicity
- Carbon disulfide (CS_2), has neurotoxic effects including rapid onset of headache, confusion, nausea, hallucinations, delirium, seizures, coma, and death

Disulfiram Neurotoxicity

- Early decades of use post 1948 – high dose (above 500mg/d), 2-25% incidence of psychosis[1]
  - Progressive fatigue, forgetfulness, and confusion, can progress to affective changes, ataxia, stupor, and a frank toxic psychosis or encephalopathy

- Describes reports of loading doses of 1-2 gm/day over 3 days then lower maintenance (125mg to 1g/day) with minimal adverse events[1]

- Review of literature 1967[2] – 52 cases, majority (n=41) toxic delirium
  - 3 groups described:
    - Delirium w/o psychosis: recovery <1/52
    - Delirium w/ depression/mania/delusions: recovery 1-3 weeks
    - Psychosis w/o delirium: recovery 3/7-3/52
  - Range of doses, but only 5 cases 0.25mg/d

- Prolonged administration (months) at doses between 800mg and 1.5 g/day in 7 individuals with no neurological toxicity[3]

1 Wright Am J Med 1990 2 Liddon. Amer J Psychiatry. 1967 3 Brewer, Br J Psych 1984

Disulfiram Neurotoxicity as a function of dose and duration of dosing

Rainey, Am J Psych, 1977

TABLE 1
Disulfiram Neurotoxicity as a Function of Level and Duration of Dosage

Study	Toxicity	Dosage (mg/day)	Duration of Dosage	Clinical Findings	Recovery Time After Discontinuation of Medication	Sequelae
Heath and associates (7)	Mild	500–1500	2 weeks	Behavioral: drowsiness, lethargy, loss of libido, headache, depression, psychosis, delirium	1–3 weeks	None reported
Liddon and Satran (1)	Mild	250–500	Months	Behavioral: Same as for 500–1500 mg/day	1–3 weeks	None reported
			Months	Neurological: Variable and fluctuating—meningeal signs, unilateral weakness, hyperreflexia, positive Babinski responses, ptosis, anisocoria Stable—ataxia, motor incoordination, optic neuritis, peripheral neuropathy	1–3 weeks	None reported
Liddon and Satran (1); Kane (6)	Moderate	1500–3000	2 weeks	Behavioral: Same as for mild toxicity	1–2 days	None reported
			2 weeks	Neurological: Variable and fluctuating—same as for mild toxicity	1–2 days	None reported
				Stable—same as for mild toxicity	Weeks	None reported except for peripheral neuropathy, which is possibly permanent
Reichelderfer (8); Gyntelberg and associates (9); Buksowicz (10); Wokittel (11); Schmoigl (12); Brzozowska-Jakowicka and Krasowska (13); Supprian (14); Kochelt and Maksimowska (15)	Severe	>3000	Single dose	Onset: Increasing drowsiness and lethargy, nausea and vomiting with dehydration	Weeks	
				After 24–48 hours: Flaccid paralysis, combative response to painful stimuli, psychotic behavior, facial erythema	Weeks to months	
				After several days to 3 or 4 weeks: Ataxia, incoordination, slurred speech, intellectual impairment	Months to years	With lower doses there may be few sequelae, but with small children or higher doses (>5 g), there may be permanent intellectual impairment, ataxia, and disturbances of fine motor movements
				Associated laboratory findings: Increased urinary acetone (possible acetone in breath), decreased serum cholesterol, possible leukocytosis, EEG consistent with toxic encephalopathy	Weeks	Resolved without apparent sequelae

Conclusions

- The combination was unsafe with significant but reversible neurotoxicity, which we suspect was related to prolonged high dose DSF
- Evidence of latency reversal in both participants
- ART, VOR and DSF detectable in plasma and DSF in participant tablets
- Differences in how PK samples collected and assay used to determine DSF levels may explain inability to detect high DSF levels
- Prolonged high dose DSF, with or without VOR, should not be further investigated as a strategy to reverse HIV latency. Study closed

Acknowledgements

STUDY PARTICIPANTS

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