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

James McMahon PhD FRACP
Department of Infectious Diseases Alfred Health and Monash University
HIV Latency and Barriers to Eradication

A. Latently infected resting central memory CD4+ T cells

B. “Active” reservoir, with ongoing production of viral RNA, antigen, or virions

C. Homeostatic or aberrant integrant-driven cellular proliferation without viral production or cell death

D. Ongoing, low-level infection of new cells despite the presence of ART

E. Failure of effector cell clearance due to viral epitope escape and/or host immune exhaustion

Margolis, Science 2016
Latency Reversal

Kim, Cell Host & Microbe, 2018
# Latency reversal in trials

<table>
<thead>
<tr>
<th>Latency reversing agent</th>
<th>Site of action</th>
<th>Dosing</th>
<th>US HIV RNA</th>
<th>Plasma RNA</th>
<th>HIV DNA</th>
<th>Negative studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>HDACi</td>
<td>Single dose¹, Intermittent², Continuous³</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Panobinostat</td>
<td>HDACi</td>
<td>Intermittent dose⁴</td>
<td>↑</td>
<td>+/-</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Romidepsin</td>
<td>HDACi</td>
<td>Weekly dose⁵</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↔</td>
<td>McMahon CROI 2019</td>
</tr>
<tr>
<td>Disulfiram</td>
<td>AKT activation</td>
<td>High dose 2g/day⁶</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Bryostatin</td>
<td>PKC agonist</td>
<td>Low dose 10-20ug/m²</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Lefitolimod</td>
<td>TLR9 agonist</td>
<td>Twice weekly⁸</td>
<td>↓</td>
<td>↑↑</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>PD-1 blockade</td>
<td>Every 3 weeks⁹</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td></td>
</tr>
<tr>
<td>GS986 and GS9620</td>
<td>TLR7 agonist</td>
<td>Dose escalation¹⁰</td>
<td>ND</td>
<td>↑↑↑↑</td>
<td>↓</td>
<td>Riddler IAS 2019</td>
</tr>
</tbody>
</table>

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 inh²,³
HDACi + bryostatin⁴
HDACi + disulfiram⁴
HDACi + screening drug library (Merck)⁵

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/μL 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
- Last dose DSF and VOR Day 10

P2
- VL 78 c/mL
- VL <20 c/mL

Legend:
- 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

<table>
<thead>
<tr>
<th>Subject</th>
<th>Time</th>
<th>Concentration (ng/mL)</th>
<th>Last VOR Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Day 1</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 11</td>
<td>6.52</td>
<td>48 hours prior</td>
</tr>
<tr>
<td></td>
<td>Day 58</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>Day 1</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 11</td>
<td>7.06</td>
<td>24 hours prior</td>
</tr>
<tr>
<td></td>
<td>Day 15</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 37</td>
<td>&lt; 2.00</td>
<td></td>
</tr>
</tbody>
</table>

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

\textsuperscript{1} Archin Nature 2012  \textsuperscript{2} Archin JID 2014  \textsuperscript{3} Rubin Clin Cancer Res 2006
Disulfiram PK

<table>
<thead>
<tr>
<th>Participant</th>
<th>Visit</th>
<th>Disulfiram</th>
<th>Carbamathione</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Day 1</td>
<td>BLOD</td>
<td>BLOD</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>BLOQ</td>
<td>BLOD</td>
</tr>
<tr>
<td></td>
<td>Day 11</td>
<td>BLOQ</td>
<td>BLOD</td>
</tr>
<tr>
<td></td>
<td>Day 59</td>
<td>BLOD</td>
<td>BLOD</td>
</tr>
<tr>
<td>P2</td>
<td>Day 1</td>
<td>BLOD</td>
<td>BLOD</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>BLOQ</td>
<td>BLOQ</td>
</tr>
<tr>
<td></td>
<td>Day 11</td>
<td>BLOQ</td>
<td>BLOQ</td>
</tr>
<tr>
<td></td>
<td>Day 15</td>
<td>BLOD</td>
<td>BLOD</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
<td>BLOD</td>
<td>BLOD</td>
</tr>
</tbody>
</table>

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)
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

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

- 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.
Disulfiram Metabolism

Neuropharmacology. 2013 Dec; 75:95-105
Disulfiram neurotoxicity

- Toxic disulfiram metabolites are diethyldithiocarbamate (DDC) and its metabolite carbon disulfide (CS₂)

- 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 (CS₂), 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\(^3\)

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Disulfiram Neurotoxicity as a function of dose and duration of dosing

<table>
<thead>
<tr>
<th>Study</th>
<th>Toxicity</th>
<th>Dosage (mg/day)</th>
<th>Duration of Dosing</th>
<th>Clinical Findings</th>
<th>Recovery Time After Discontinuation of Medication</th>
<th>Sequelae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heath and associates (7)</td>
<td>Mild</td>
<td>500-1500</td>
<td>2 weeks</td>
<td>Behavioral: drowsiness, lethargy, loss of libido, headache, depression, psychosis, delirium</td>
<td>1-3 weeks</td>
<td>None reported</td>
</tr>
<tr>
<td>Liddon and Satran (1)</td>
<td>Mild</td>
<td>250-500</td>
<td>Months</td>
<td>Behavioral: Same as for 500-1500 mg/day Neurological: Variable and fluctuating—meningeal signs, unilateral weakness, hyperreflexia, positive Babinski responses, paresthesia, anisocoria Stable—ataxia, motor incoordination, optic neuritis, peripheral neuropathy</td>
<td>1-3 weeks</td>
<td>None reported</td>
</tr>
<tr>
<td>Liddon and Satran (1); Kane (6)</td>
<td>Moderate</td>
<td>1500-3000</td>
<td>2 weeks</td>
<td>Behavioral: Same as for mild toxicity Neurological: Variable and fluctuating—same as for mild toxicity Stable—same as for mild toxicity</td>
<td>1-2 days</td>
<td>None reported</td>
</tr>
<tr>
<td>Reichelderfer (8); Gyntelberg and associates (9); Bukowicz (10); Wokittel (11); Schmoigl (12); Brozowska-Jakowics and Krasowska (13); Supprian (14); Kochelt and Maksimowska (15)</td>
<td>Severe</td>
<td>&gt;3000</td>
<td>Single dose</td>
<td>Onset: Increasing drowsiness and lethargy, nausea and vomiting with dehydration After 24-48 hours: Flaccid paralysis, combative response to painful stimuli, psychotic behavior, facial erythema After several days to 3 or 4 weeks: Ataxia, incoordination, slurred speech, intellectual impairment</td>
<td>Weeks</td>
<td>Months to years</td>
</tr>
</tbody>
</table>

Rebecca, Am J Psych, 1977
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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• Jillian Lau
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