Fimepinostat, a novel dual inhibitor of HDAC and PI3K, effectively reverses HIV-1 latency *ex vivo* without T cell activation

Jesper D Gunst[1,2]*, Kathrine Kjær[1,2], Rikke Olesen[1,2], Thomas A Rasmussen[1,2], Lars Østergaard[1,2], Paul W Denton[1,2], Ole S Søgaard[1,2] and Martin Tolstrup[1,2]

[1] Department of Infectious Diseases, Aarhus University Hospital, Denmark
[2] Institute of Clinical Medicine, Aarhus University, Denmark

Abstract

Objectives: To test the potential of fimepinostat (CUDC-907), a dual inhibitor of histone deacetylases (HDAC) and phosphatidylinositol-3-kinases (PI3K), to reverse human immunodeficiency virus type 1 (HIV-1) latency in infected cell lines and in CD4+ T cells from HIV-1-infected donors on long-term combination antiretroviral therapy (cART).

Methods: Latently HIV-1-infected J-lat Tat-GFP and ACH-2 cell lines were stimulated with clinically relevant concentrations of fimepinostat using the HDAC inhibitors (HDACi) panobinostat and romidepsin for comparison. Next, CD4+ T cells from donors living with HIV-1 on long-term cART were stimulated *ex vivo* and cell-associated unspliced HIV-1 RNA was measured to quantify changes in HIV-1 transcription. Finally, the impact of fimepinostat on T cell activation (CD69 expression) and proliferation (Ki67 expression) was determined using peripheral blood mononuclear cells from uninfected donors.

Results: We found fimepinostat to be a potent latency-reversing agent. This was true in two latently infected cell lines as well as *ex vivo* in CD4+ T cells isolated from donors living with HIV-1. Relative to therapeutic dosing levels, fimepinostat showed latency-reversing potential comparable to romidepsin, which is the most potent HDACi tested in HIV-1 cure-related trials. Interestingly, in contrast to romidepsin, fimepinostat stimulation resulted in decreased T cell activation and had no negative impact on T cell proliferation.

Conclusions: At therapeutic concentration, the dual HDAC and PI3K inhibitor fimepinostat was a potent HIV-1 latency-reversing agent and it did not induce T cell activation and proliferation. The potential of fimepinostat as a latency-reversing agent warrants further investigation.

Keywords: HIV, latency-reversal agent, T cell activation, HDACi, PI3Ki, fimepinostat

Introduction

Latent, integrated HIV type 1 (HIV-1) proviruses persist predominantly in memory CD4+ T cells among individuals living with HIV-1 despite effective combination antiretroviral therapy (cART) [1–3] This latent reservoir is thought to be the main barrier preventing long-term remission off cART.

In the 'shock and kill' approach to cure HIV-1 infection [4], the 'shock' is intended to augment viral transcription leading to apoptosis or immune-mediated clearance of the infected cells. The clinically best-investigated latency-reversing agents (LRAs) in the context of cure-related HIV-1 trials are histone deacetylase inhibitors (HDACi) [5–13], which were developed for the treatment of haematological malignancies. However, HDACi treatment may increase T cell activation and/or homeostatic proliferation, which could lead to unintended clonal expansion of latently infected CD4+ T cells – a key mechanism of HIV-1 persistence [14–16]. Thus, an ideal candidate LRA should have a limited effect on T cell activation and proliferation, as well as acceptable pharmacological and toxicological properties [17–19]. Importantly, non-T cell activating LRA showed ineffective latency reversal in resting CD4+ T cells *ex vivo* [20].

In the present study, we evaluated the latency-reversing potential of fimepinostat, a dual inhibitor of histone deacetylase (HDAC) (classes I and IIB) and PI3K (classes Iα, β and δ) [21], and its impact on T cell activation and proliferation.

*Corresponding author: Jesper D Gunst
Department of Infectious Diseases, Aarhus University Hospital – Skejby, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark
Email: jesper@gunst.dk

Methods

Stimulating reagents

Fimepinostat (CUDC-907, Curis, USA), panobinostat (Selleck Chemicals, USA) and romidepsin (Selleck Chemicals, USA) were all dissolved in dimethylsulphoxide (DMSO) (Merck, USA). DMSO alone at 0.01% was used as a negative control. Phorbol 12-myristate 13-acetate (Sigma-Aldrich, USA) at 25 nM was used as a positive control.

Latently infected cell lines

The following reagents were obtained through the National Institutes of Health (NIH) AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH: J-lat Tat-GFP Clone (clone A1) from Dr Eric Verdin [22,23] and ACH-2 from Dr Thomas Folks [24,25]. Cells were seeded at 2×10^5/well in 100 µL Roswell Park Memorial Institute (RPMI) 1640 media with 10% heat-inactivated fetal bovine serum (hiFBS), penicillin (100 IU/mL) and streptomycin (100 µg/mL) in a 96-well plate (Nunc, Denmark) and incubated with fimepinostat, panobinostat or romidepsin at indicated concentrations. The J-lat Tat-GFP cells, after 48 hours of incubation at 37°C with 5% CO_2, were harvested. Green fluorescent protein (GFP) expression in the J-lat Tat-GFP cells was used as a marker of HIV-1 transcription [22] and analysed in live, single cells. In ACH-2 cells, after 48 hours of incubation at 37°C with 5% CO_2, the supernatants were inactivated for 1 hour with 1% Empigen (Albright & Wilson) and then harvested. Levels of HIV-1 p24 gag were determined by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Aalto Bio Reagents, UK). Stimulations of cells were performed in duplicates. Upon harvest, cells were stained with viability dye (near-infrared [IR] amino reactive dye, Invitrogen, Denmark), and

data were acquired using a BD FACSVerse flow cytometer (BD Biosciences, USA). Viability was determined on percentage of dye negative cells. The 50% cytotoxic concentration was defined as the concentrations where cell viabilities were reduced by 50%. Data were analysed using FlowJo software (v. 10.4, TreeStar Inc, USA).

Primary cells

Blood was collected in 10 mL EDTA (BD-Vacutainer 6144952, UK) from individuals both living with HIV-1-e ($n=4$) and without HIV-1 ($n=10$). Donors living with HIV-1 had been on cART with an HIV-1 RNA of <50 copies/mL for a minimum of 18 months and their most recent CD4+ T cell count of >500 cells/μL. Peripheral blood mononuclear cells (PBMCs) were isolated using SepMate-50 (STEMCELL Technologies, Canada) with Ficoll-Paque (GE Healthcare, Denmark) density separation according to the manufacturers' instructions. PBMCs were cryopreserved in 10% DMSO and stored at –80 °C until use.

CD4+ T cells were enriched from thawed PBMCs from donors living with HIV-1 using a CD4+ T cell isolation kit and magnetic-activated-cell-sorting (MACS) columns using negative selection (catalogue no. 130-096-533, Miltenyi Biotec, Germany) according to the manufacturer's instructions. Cells were seeded at 1×10^6/mL in RPMI 1640 media with 10% hiFBS, penicillin (100 IU/mL) and streptomycin (100 μg/mL) in a 24-well plate (catalogue no. 83.3922.500, SARSTEDT, Germany). Next, cells were incubated with 25 nM fimepinostat and 5 nM romidepsin. After 16 hours of incubation at 37 °C with 5% CO_2, cells were harvested, lysed in RLT+ buffer and stored at –80 °C until RNA was extracted using AllPrep DNA/RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Cell-associated unspliced HIV-1 (CA usHIV-1) RNA was measured using the QX100 Droplet Digital PCR System (BioRad Inc, USA) as described previously [26].

PBMCs from donors without HIV-1 were seeded at 2×10^6/mL in RPMI 1640 media with 10% hiFBS, penicillin (100 IU/mL) and streptomycin (100 μg/mL) in a 12-well plate (Nunc). PBMCs were incubated with fimepinostat and romidepsin at indicated concentrations. After 48 hours of incubation at 37 °C with 5% CO_2, cells were harvested. Using flow cytometry, we determined T cell activation by surface CD69 expression and proliferation using the intracellular marker Ki67. Cells were stained with viability dye (near-IR amino reactive dye, Invitrogen), followed by Fc-blocking (Human TruStain FcX, Biolegend, USA) and surface staining (30 minutes, room temperature) with antibodies against CD3 (BV605 and OKT3, Biolegend), CD4 (PE-Cy7 and SK3, Biolegend), CD8 (PerCP-Cy5.5 and SK1, Biolegend), CCR7 (FITC and G043H7, Biolegend), CD45RA (BV421 and HI100, Becton Dickinson) and CD69 (APC and FN50, Biolegend). After surface staining, cells were fixed, permeabilised and intracellularly stained (45 minutes, 4 °C) for Ki67 expression (PE and B56, BD Biosciences). T cells were identified by size and granularity (lymphocyte gate), followed by the expressions of CD3 and CD4 or CD8. Central memory T cells (TCMs) were defined by $CD45RA^-CCR7^+$, and effector memory T cells (TEMs) were defined by $CD45RA^-CCR7^-$. Gates for CD69 and Ki67 positivity were determined using isotope control antibodies. Only live cells and singlets were included. All samples were acquired on a FACSVerse flow cytometer (BD Biosciences), and data were analysed using FlowJo software (v. 10.4, TreeStar Inc).

Ethical statement

The study was approved by the Danish National Committee on Health Research Ethics (reference number: M-2015-260-15) and the Danish Data Protection Agency (reference number: 1-16-02-424-15). Each participant provided written informed consent.

Statistical analysis

Data are presented as mean ± SD or as mean (95% confidence interval [95%-CI]). The Mann–Whitney U test was used to compare groups. All statistical analyses were performed using GraphPad Prism version 7.00 for Windows (www.graphpad.com).

Results

First, we studied the effect of fimepinostat on HIV-1 reactivation in latently infected J-lat Tat-GFP cells. Fimepinostat at 25 nM and romidepsin at 2.5 nM displayed a similar latency-reversing effect ($P=0.42$), and both trended towards inducing higher levels of GFP expression in J-Lat cells than panobinostat at 12.5 nM (Figure 1a; $P=0.06$ and $P=0.15$, respectively). We used drug concentrations where cell viability was above 50% (Figure 1b, shaded area). At these concentrations, the three drugs showed equal increases in median fluorescence intensity in J-lat Tat GFP cells (Figure 1c). We then investigated whether fimepinostat could induce HIV-1 p24 production in latently infected ACH-2 cells. Dose–response curves of HIV-1 p24 release into the supernatant are shown in Figure 2a. Again, we compared HIV-1 p24 production at the drug concentrations where >50% of the cells were alive (Figure 2b). Fimepinostat at 50 nM trended towards inducing higher HIV-1 p24 production than romidepsin at 5 nM ($P=0.07$), and both induced higher HIV-1 p24 production than panobinostat at 12.5 nM (Figure 2a; $P=0.02$ and $P=0.07$, respectively).

Having demonstrated that fimepinostat induces HIV-1 transcription and p24 production in latently infected cell lines, we next examined its latency-reversing effect in CD4+ T cells isolated from donors living with HIV-1 and on long-term cART. We found that fimepinostat at 25 nM induced HIV-1 transcription *ex vivo* in all examined samples as measured by CA usHIV-1 RNA with a mean of 62 (95% CI: 44–79) copies/10^6 CD4+ T cells (Figure 3a, $P<0.05$) compared with 14 (95% CI: 07–21) copies/10^6 CD4+ T cells in negative controls. This corresponded to a mean fold change (at the individual donor level) of 6.3 (95% CI: 3.4–9.1), which was comparable with romidepsin at 5 nM (Figure 3b; mean fold change 5.0, 95% CI: 2.4–7.6, $P=0.71$).

Finally, we investigated the effects of fimepinostat on T cell activation and proliferation in central memory (TCM) and effector memory (TEM) subsets, which are known to predominantly harbour intact HIV-1 proviruses [27]. In contrast with romidepsin at 5 nM, fimepinostat at 25 nM did not increase CD4+ T cell activation as assessed by CD69 expression (Figure 4a, $P<0.05$ for both TCM and TEM). In fact, fimepinostat stimulation trended towards decreasing levels of CD69 expression compared with negative controls ($P=0.06$ for both TCM and TEM). Interestingly, fimepinostat stimulation led to decreased expression of the proliferation marker Ki67 compared with negative controls ($P<0.05$ for both TCM and TEM), whereas we found no difference in Ki67 expression between fimepinostat- and romidepsin-stimulated cells (Figure 4b, $P=0.11$ for TCM and $P=0.69$ for TEM).

Discussion

In this study, we found that the dual inhibitor of HDAC and PI3K, fimepinostat, potently reverses HIV-1 latency without inducing T cell activation or proliferation. This was substantiated by increases in HIV-1 reactivation and p24 production in latently infected cell lines and by elevated HIV-1 transcription in CD4+ T cells from donors living with HIV-1 with viral suppression. Notably, fimepinostat showed a latency-reversing potential comparable with romidepsin, which is the most potent HDACi tested in HIV-1 cure-related trials [28], but in contrast to romidepsin

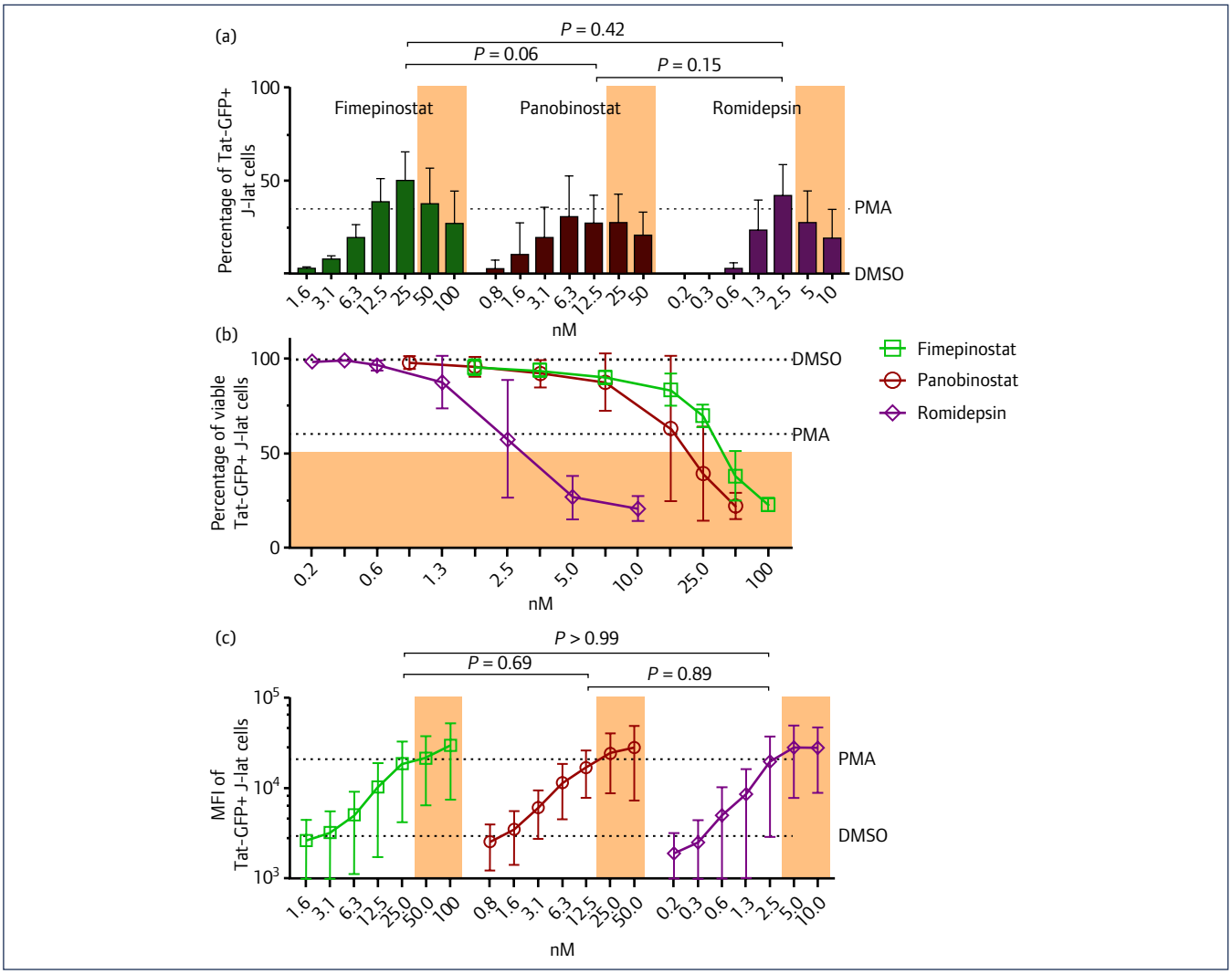

Figure 1. Fimepinostat induced HIV-1 reactivation in latently infected J-lat Tat-GFP cells. (a) HIV-1 reactivation in J-lat Tat-GFP cells at the indicated concentrations in nanomoles of fimepinostat, panobinostat and romidepsin. (b) Effects on the viability of indicated concentrations in nanomoles. Viability was higher in cells treated with fimepinostat than in cells treated with panobinostat and romidepsin. Shaded areas indicate CC50, the cytotoxic concentrations where >50% of the cells were dead. (c) MFI with indicated concentrations in nanomoles. Data are presented as mean ± SD of five (a,c) and two (b) independent experiments. Dotted lines indicate positive and negative controls with PMA at 25 nM and DMSO at 0.01%. DMSO: dimethylsulphoxide; HIV, HIV type 1; MFI: median fluorescence intensity; PMA:, phorbol 12-myristate 13-acetate

and panobinostat, fimepinostat reversed latency without inducing T cell activation. *In vivo*, after 24 hours of the first of multiple doses of romidepsin or panobinostat, a significant increase was observed in CD4+ T cells expressing CD69; reassuringly, this CD4+ T cell activation returned to baseline levels 6 weeks after the last dosing [10,29]. Additionally, stimulation with these HDACi *ex vivo* showed increased CD69 expression on CD4+ T cells up to 48 hours after exposure [30–33]. The less potent HDACi vorinostat has shown no changes in T cell activation on CD4+ T cells assessed by HLA-DR CD38 expression after multiple doses or assessed by CD69 expression up to 24 hours after a single dose *in vivo* [30,34].

In haematological malignancies, fimepinostat is dosed at 60 mg orally in a 5 on/2 off days dosing scheme. This dosing results in a maximum plasma concentration (C_{max}) of 11 ng/mL corresponding to a plasma concentration of 22 nM [35,36]. Panobinostat at 20 mg orally three times/week results in a C_{max} of 11 ng/mL or 31 nM [37]. Romidepsin at 14 mg/m^2 intravenously on days 1, 8 and 15 of a 28-day cycle yields a C_{max} of 377 ng/mL corresponding to a free drug concentration of 56 nM [32]. Thus, the concentrations of fimepinostat at 25 nM and panobinostat at 12.5 nM used in this study correspond well to therapeutic plasma levels, whereas romidepsin at 5 nM corresponds to 35% of clinical drug exposure used in HIV-1 cure-related trials [10,12,32]. All three drugs have mild to moderate side effects, including fatigue and gastrointestinal symptoms, but overall, the safety profiles are acceptable.

Interestingly, we found lower percentages of CD4+ T cells expressing activation marker CD69 upon fimepinostat stimulation compared with romidepsin-stimulated cells. Studies have not shown any correlation between latency reversal potential and T cell activation [30], but T cell activation *in vivo* may lead to proliferation of latently HIV-1-infected CD4+ T cells – a key mechanism of viral persistence during suppressive cART. Thus, latency reversal without T cell activation may reduce the risk of expanding the reservoir in individuals receiving an LRA in HIV-1 cure-related trials.

In the 'prime, shock and kill' HIV-1 curative strategy [38], latently infected cells are sensitised towards apoptosis followed by HIV-1 latency reversal [39]. In this context, inhibition of PI3K has been shown to sensitise cancer cells to HDACi-induced apoptosis [35,40], which would be extremely beneficial in the HIV-1 cure context.

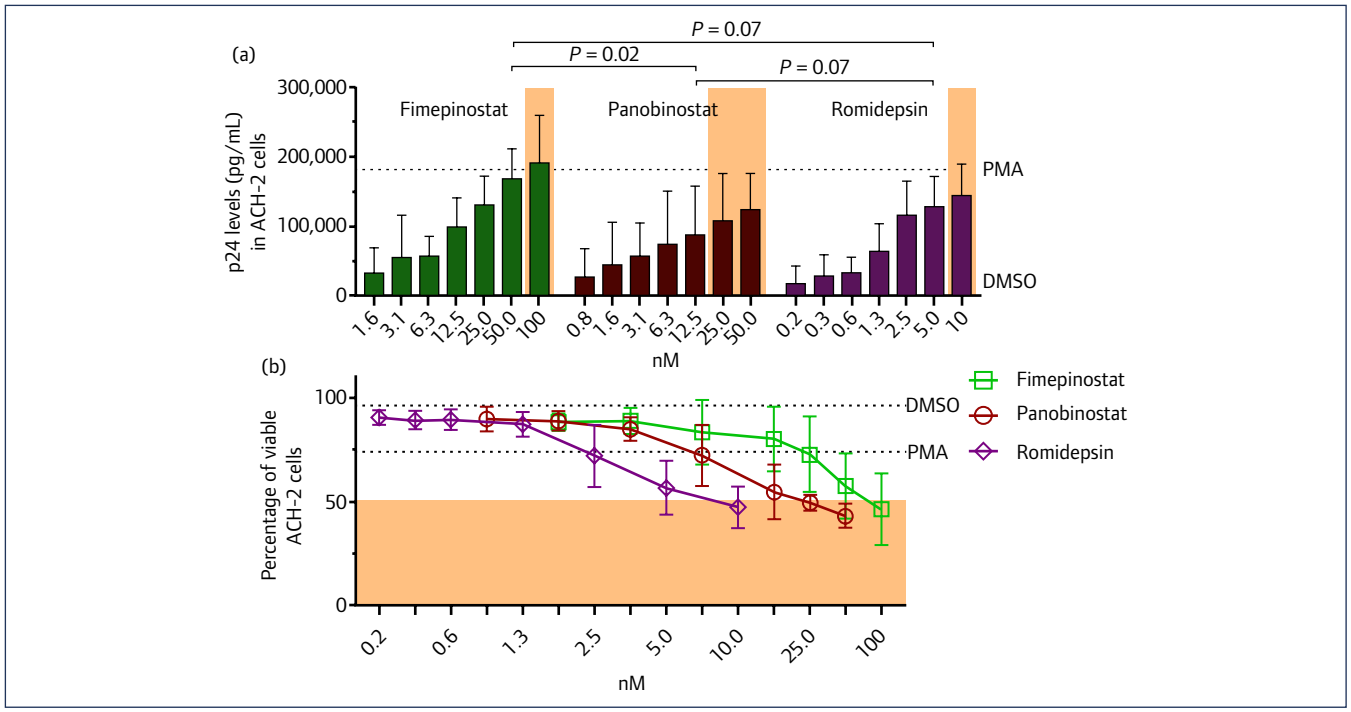

Figure 2. Fimepinostat induced p24 production in latently infected ACH-2 cells. (a) HIV type 1 p24 production in ACH-2 cells in picograms per millilitre with indicated concentrations of fimepinostat, panobinostat and romidepsin in nanomoles. (b) Cells alive after exposure of indicated concentrations in nanomoles. Shaded areas indicate concentrations where >50% of the cells were dead after exposure. Data are presented as mean±SD of four (a) and three (b) independent experiments. Dotted lines indicate positive and negative controls with PMA at 25 nM and DMSO at 0.01%. DMSO: dimethylsulphoxide; PMA: phorbol 12-myristate 13-acetate

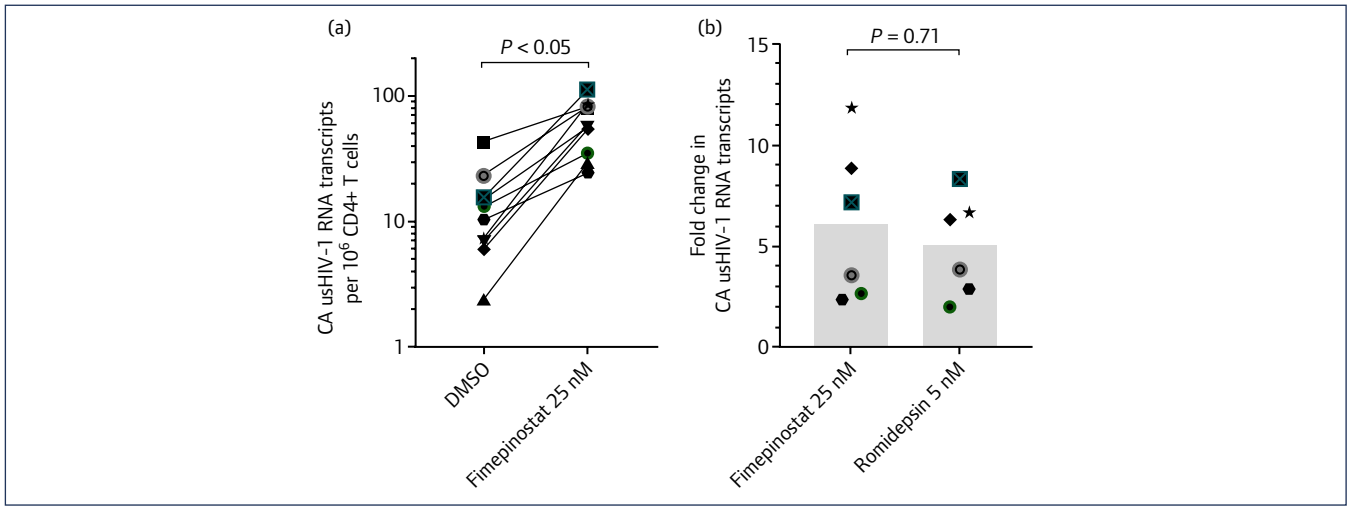

Figure 3. Fimepinostat induced HIV-1 transcription in CD4+ T cells from donors on long-term cART. (a) Absolute quantification of CA usHIV-1 RNA in CD4+ T cells from donors on long-term cART. (b) Fold change of CA usHIV-1 RNA in CD4+ T cells from donors on long-term cART relative to negative control (DMSO). Columns represent the mean. Individual donors, $n=10$ with fimepinostat and $n=6$ with romidepsin; CA usHIV-1: cell-associated unspliced HIV-1; DMSO: dimethylsulphoxide

In conclusion, we found that at therapeutic concentrations, fimepinostat potently reversed HIV-1 latency both *in vitro* and *ex vivo* without causing T cell activation and proliferation. The potential of fimepinostat as an LRA warrants further investigation in future HIV-1 cure-related trials.

Acknowledgements

We thank Lene Svinth Jøhnke for her technical assistance in setting up and conducting the experiments.

Declaration of interest

All authors state no conflicts of interest.

Funding

The participants living with HIV-1 were recruited from the Nordic HIV Latency and Cure Research Collaboration cohort, which has been supported by an educational grant to JDG via the Gilead Nordic Fellowship Programme 2015 and the Scandinavian Society for Antimicrobial Chemotherapy Foundation.

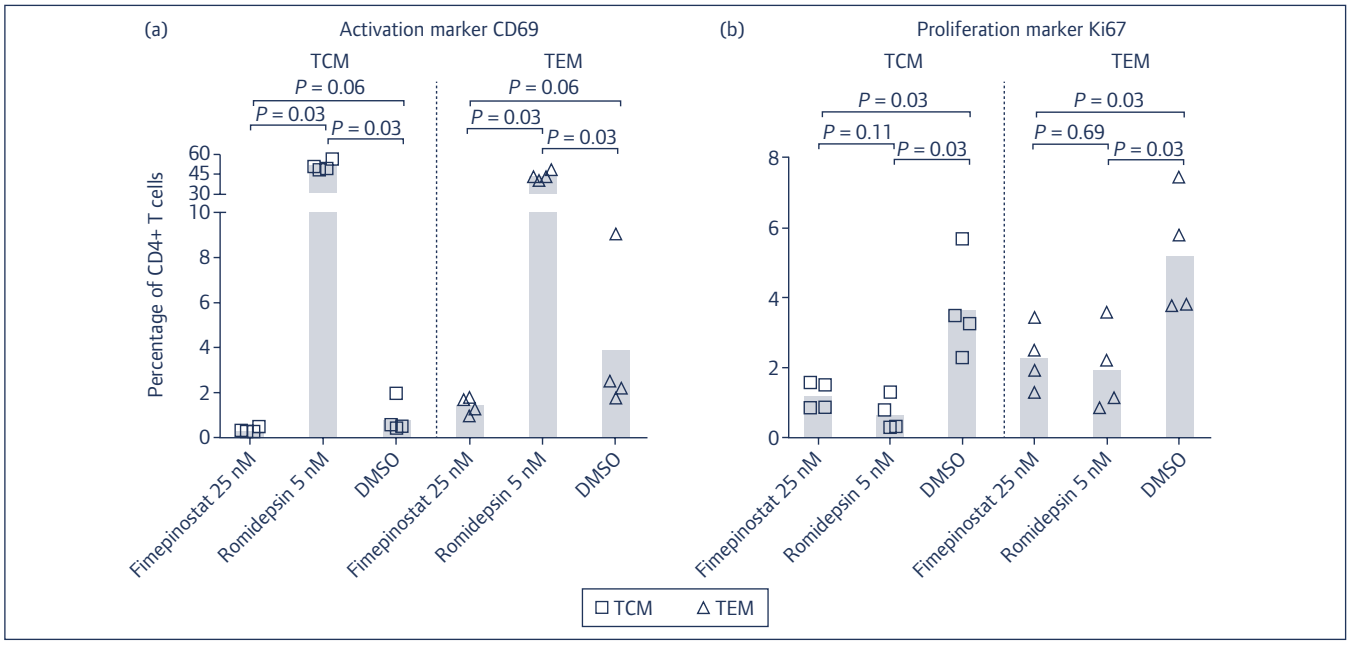

Figure 4. Fimepinostat showed no T cell activation in PBMCs from HIV-1-negative donors. Expression of activation marker CD69 (a) and proliferation marker Ki67 (b) on PBMCs from HIV-1-negative donors after 48-hour incubation with indicated concentrations of fimepinostat, romidepsin and negative control (DMSO at 0.01%) on the percentages of central memory (TCM) and effector memory (TEM) CD4+ T cells, respectively. Columns represent the mean. DMSO: dimethylsulphoxide; PBMC: peripheral blood mononuclear cell; TCM: central memory T cell; TEM: effector memory T cell

References

1. Chun TW, Carruth L, Finzi D *et al.* Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 1997; **387**: 183–188.
2. Finzi D, Hermankova M, Pierson T *et al.* Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 1997; **278**: 1295–1300.
3. Barton K, Winckelmann A, Palmer S. HIV-1 reservoirs during suppressive therapy. *Trends Microbiol* 2016; **24**: 345–355.
4. Deeks SG. HIV: shock and kill. *Nature* 2012; **487**: 439–440.
5. Archin NM, Liberty AL, Kashuba AD *et al.* Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 2012; **487**: 482–485.
6. Spivak AM, Andrade A, Eisele E *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* 2014; **58**: 883–890.
7. Elliott JH, Wightman F, Solomon A *et al.* Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. *PLoS Pathog* 2014; **10**: e1004473.
8. Rasmussen TA, Tolstrup M, Brinkmann CR *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* 2014; **1**: e13–e21.
9. Archin NM, Bateson R, Tripathy MK *et al.* HIV-1 expression within resting CD4 T-cells following multiple doses of vorinostat. *J Infect Dis* 2014; **11**: 1–8.
10. Søgaard OS, Graversen ME, Leth S *et al.* The depsipeptide romidepsin reverses HIV-1 latency in vivo. *PLoS Pathog* 2015; **11**: e1005142.
11. Elliott JH, McMahon JH, Chang CC *et al.* Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV* 2015; **2**: e520–e529.
12. Leth S, Schleimann MH, Nissen SK *et al.* Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): a single-arm, phase 1B/2A trial. *Lancet HIV* 2016; **3**: e463–e472.
13. Archin NM, Kirchherr JL, Sung JA *et al.* Interval dosing with the HDAC inhibitor vorinostat effectively reverses HIV latency. *J Clin Invest* 2017; **127**: 3126–3135.
14. Maldarelli F, Wu X, Su L *et al.* Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. *Science* 2014; **345**: 179–183.
15. Wagner TA, McLaughlin S, Garg K *et al.* HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* 2014; **345**: 570–573.
16. Reeves DB, Duke ER, Wagner TA *et al.* A majority of HIV persistence during antiretroviral therapy is due to infected cell proliferation. *Nat Commun* 2018; **9**: 4811.
17. Xing S, Siliciano RF. Targeting HIV latency: pharmacologic strategies toward eradication. *Drug Discov Today* 2013; **18**: 541–551.
18. Hosmane NN, Kwon KJ, Bruner KM *et al.* Proliferation of latently infected CD4 + T cells carrying replication-competent HIV-1: potential role in latent reservoir dynamics. *J Exp Med* 2017; **214**: 959–972.
19. Chomont N, El-Far M, Ancuta P *et al.* HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 2009; **15**: 893–900.
20. Bullen CK, Laird GM, Durand CM *et al.* New *ex vivo* approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. *Nat Med* 2014; **20** (**January**): 1–6.
21. Qian C, Lai C-J, Bao R *et al.* Cancer network disruption by a single molecule inhibitor targeting both histone deacetylase activity and phosphatidylinositol 3-kinase signaling. *Clin Cancer Res* 2012; **18**: 4104–4113.
22. Jordan A, Bisgrove D, Verdin E. HIV reproducibly establishes a latent infection after acute infection of T cells in vitro. *EMBO J* 2003; **22**: 1868–1877.
23. Jordan A, Defechereux P, Verdin E. The site of HIV-1 integration in the human genome determines basal transcriptional activity and response to Tat transactivation. *EMBO J* 2001; **20**: 1726–1738.
24. Clouse KA, Powell D, Washington I *et al.* Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone. *J Immunol* 1989; **142**: 431–438.
25. Folks TM, Clouse KA, Justement J *et al.* Tumor necrosis factor alpha induces expression of human immunodeficiency virus in a chronically infected T-cell clone. *Proc Natl Acad Sci U S A* 1989; **86**: 2365–2368.
26. Leth S, Nymann R, Jørgensen S *et al.* HIV-1 transcriptional activity during frequent longitudinal sampling in aviremic patients on antiretroviral therapy. *AIDS* 2016; **30**: 713–721.
27. Hiener B, Horsburgh BA, Eden J-S *et al.* Identification of genetically intact HIV-1 proviruses in specific CD4 + T cells from effectively treated participants. *Cell Rep* 2017; **21**: 813–822.
28. Gunst JD, Tolstrup M, Rasmussen TA *et al.* The potential role for romidepsin as a component in early HIV-1 curative efforts. *Expert Rev Anti Infect Ther* 2016; **14**: 447–450.
29. Brinkmann CR, Højen JF, Rasmussen TA *et al.* Treatment of HIV-infected individuals with the histone deacetylase inhibitor panobinostat results in increased numbers of regulatory T cells and limits *ex vivo* lipopolysaccharide-induced inflammatory responses. *mSphere* 2018; **3**: e616–e617.
30. Clutton G, Xu Y, Baldoni PL *et al.* The differential short- and long-term effects of HIV-1 latency-reversing agents on T cell function. *Sci Rep* 2016; **6**: 30749.
31. Rasmussen TA, Søgaard OS, Brinkmann C *et al.* Comparison of HDAC inhibitors in clinical development. *Hum Vaccin Immunother* 2013; **9**: 993–1001.
32. Wei DG, Chiang V, Fyne E *et al.* Histone deacetylase inhibitor romidepsin induces HIV expression in CD4 T cells from patients on suppressive antiretroviral therapy at concentrations achieved by clinical dosing. *PLoS Pathog* 2014; **10**: e1004071.
33. Laird GM, Bullen CK, Rosenbloom DIS *et al. Ex vivo* analysis identifies effective HIV-1 latency-reversing drug combinations. *J Clin Invest* 2015; **125**: 1901–1912.
34. Archin NM, Bateson R, Tripathy MK *et al.* HIV-1 expression within resting CD4+ T cells after multiple doses of vorinostat. *J Infect Dis* 2014; **210**: 728–735.
35. Younes A, Berdeja JG, Patel MR *et al.* Safety, tolerability, and preliminary activity of CUDC-907, a first-in-class, oral, dual inhibitor of HDAC and PI3K, in patients with relapsed or refractory lymphoma or multiple myeloma: an open-label, dose-escalation, phase 1 trial. *Lancet Oncol* 2016; **17**: 622–631.
36. Oki Y, Kelly KR, Flinn I *et al.* CUDC-907 in relapsed/refractory diffuse large B-cell lymphoma, including patients with MYC-alterations: results from an expanded phase I trial. *Haematologica* 2017; **102**: 1923–1930.
37. Fukutomi A, Hatake K, Matsui K *et al.* A phase I study of oral panobinostat (LBH589) in Japanese patients with advanced solid tumors. *Invest New Drugs* 2012; **30**: 1096–1106.
38. Cummins NW, Sainski AM, Dai H *et al.* Prime, shock, and kill: priming CD4 T cells from HIV patients with a BCL-2 antagonist before HIV reactivation reduces HIV reservoir size. *J Virol* 2016; **90**: 4032–4048.
39. Kim Y, Anderson JL, Lewin SR. Getting the 'kill' into 'shock and kill': strategies to eliminate latent HIV. *Cell Host Microbe* 2018; **23**: 14–26.
40. Rahmani M, Aust MM, Benson EC *et al.* PI3K/mTOR inhibition markedly potentiates HDAC inhibitor activity in NHL cells through BIM- and MCL-1-Dependent mechanisms in vitro and in vivo. *Clin Cancer Res* 2014; **20**: 4849–4860.