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

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Summary
Background Activating the expression of latent virus is an approach that might form part of an HIV cure. We assessed the ability of the histone deacetylase inhibitor panobinostat to disrupt HIV-1 latency and the safety of this strategy.

Methods In this phase 1/2 clinical trial, we included aviraemic adults with HIV treated at Aarhus University Hospital, Denmark. Participants received oral panobinostat (20 mg) three times per week every other week for 8 weeks while maintaining combination antiretroviral therapy. The primary outcome was change from baseline of cell-associated unspliced HIV RNA. Secondary endpoints were safety, HIV RNA, total and integrated HIV DNA, infectious units per million CD4 T cells, and time to viral rebound during an optional analytical treatment interruption of antiretroviral therapy. This trial is registered with ClinicalTrial.gov, number NCT01680094.

Findings We enrolled 15 patients. The level of cell-associated unspliced HIV RNA increased significantly at all timepoints when patients were taking panobinostat (p<0·0001). The median maximum increase in cell-associated unspliced HIV RNA during panobinostat treatment was 3·5-fold (range 2·1–14·4). Panobinostat induced plasma viraemia with an odds ratio of 10·5 (95% CI 2·2–50·3; p=0·0002) compared with baseline. We recorded a transient decrease in total HIV DNA, but no cohort-wide reduction in total HIV DNA, integrated HIV DNA, or infectious units per million. Nine patients participated in the analytical treatment interruption, median time to viral rebound was 17 days (range 14–56). Panobinostat was well tolerated. 45 adverse events were reported, but only 16 (all grade 1) were presumed related to panobinostat.

Interpretation Panobinostat effectively disrupts HIV latency in vivo and is a promising candidate for future combination clinical trials aimed at HIV eradication. However, panobinostat did not reduce the number of latently infected cells and this approach may need to be combined with others to significantly affect the latent HIV reservoir.

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question the ability of histone deacetylase inhibitors to disrupt latency to the extent that HIV proteins are expressed on the surface of infected cells, thus enabling immune-mediated elimination to occur.7,16,17

Panobinostat is a highly potent hydroxamic acid pan-histone deacetylase inhibitor in clinical development for the treatment of multiple myeloma.18 Therapeutic concentrations of panobinostat induce viral production in latently infected cells in vitro.19 On the basis of these data, we designed an interventional study to assess whether 8 weeks of cyclic panobinostat treatment added to antiretroviral therapy would increase HIV transcription, increase plasma viraemia, and affect the latent HIV reservoir.

Methods

Study design and participants

We did a single group, phase 1/2 trial at Aarhus University Hospital, Denmark, between Oct 1, 2012 and Jan 16, 2014. We enrolled HIV-infected adults taking antiretroviral therapy with virological suppression (<50 copies per mL, at least two measurements per year) for at least 2 years and CD4 counts above 500 cells per μL. Exclusion criteria included co-infection with hepatitis B or C viruses, clinically significant cardiac disease including QTc prolongation, and current use of a protease inhibitor (because of drug interactions). Ethics committee approval was obtained in accordance with the principles of the Declaration of Helsinki. Each patient provided written informed consent before any study procedures.

Procedures

Patients received oral panobinostat (20 mg) three times per week (on Mondays, Wednesdays, and Fridays) every other week for 8 weeks while maintaining combination antiretroviral treatment. This dose regimen was based on clinical safety and preclinical testing of panobinostat’s effect on HIV production in latently infected cells.18,20 Patients had 13 follow-up visits during panobinostat treatment, at which blood draws were taken, including 2 h and 12 h after receipt of the first dose, and 4 weeks and 24 weeks after completion of panobinostat treatment (appendix p 1). All other samples in the weeks of panobinostat treatment were taken roughly 8 h after a panobinostat dose. At each follow-up visit, we assessed adherence to antiretroviral therapy and panobinostat. We measured plasma concentrations of panobinostat and confirmed compliance with pill counts.

Safety was actively assessed at all study visits by recording of all patient-reported adverse events and serious adverse events. For each adverse event, we assessed its relation to panobinostat treatment and graded its severity according to the Common Terminology Criteria for Adverse Events (version 4.0).21 We assessed the presence of HIV RNA in plasma by nucleic acid testing with a transcription-mediated amplification detection method (Procleix Ulitro Plus, Novartis).22 The results were defined as positive or negative.

Patients had the option of analytical treatment interruption of antiretroviral therapy after completion of panobinostat treatment and analysis of the primary

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ART=antiretroviral therapy. TDF=tenofovir disoproxil fumarate. FTC=emtricitabine. RPV=rilpivirine. RAL=raltegravir. EFV=efavirenz. ABC=abacavir. 3TC=lamivudine. AZT=zidovudine. ATI=analytical treatment interruption. *Exact value not available, the patient had been away, returning to Norway in 2010.

Table 2: Participant characteristics
outcome. Declining to participate in the interruption at study enrolment did not preclude participation in the study. During the analytical treatment interruption, patients were monitored for viral load (twice weekly; Cobas Taqman, quantification limit 19 copies per mL) and CD4 cell counts (weekly). Two consecutive viral load measurements of more than 1000 copies per mL or CD4 count of less than 350 cells per μL prompted resumption of antiretroviral therapy. Because withdrawal of antiretroviral therapy is considered the ultimate test of an intervention targeting the latent HIV reservoir,23 we explored possible correlations between the time to viral rebound during analytical treatment interruption and changes in HIV DNA and viral outgrowth assay estimates of infectious units per million in post-hoc analyses. The appendix shows detailed methods.

Endpoints
The primary outcome measure was change from baseline of HIV transcription as measured by cell-associated unspliced HIV RNA in unfractionated CD4 cells. Secondary endpoints were incidence and severity of adverse events, change from baseline of plasma HIV RNA concentration, change from baseline of total and integrated proviral HIV DNA per 10⁶ CD4 cells, change from baseline in the proportion of cells carrying replication-competent virus as assessed by a viral outgrowth assay, and time to first viral load of more than 1000 copies per mL during the optional analytical treatment interruption. The degree of histone acetylation and two-long terminal repeat circles per 10⁶ CD4 cells were prespecified exploratory endpoints.

Statistical analysis
We designed the study to detect a 0·5 log increase in cell-associated unspliced HIV RNA with 90% power at a 5% significance level. Assuming a standard deviation of 0·5 log,24 we required 11 fully evaluable patients. To account for study withdrawals, we aimed to enrol up to 16 patients. We used repeated ANOVA statistics to assess whether longitudinal changes in numerical outcome measures were overall significantly different from baseline. We tested changes from baseline to specific timepoints with paired t tests or Wilcoxon signed-rank tests depending on the distribution of the data. We analysed binary outcomes with a logistic model with a random effect for each patient to allow for correlations caused by repeated observations. On the log-odds scale, we assumed that the random effects had a normal distribution with mean zero and unknown variance. We included baseline versus on-panobinostat as a fixed, dichotomous explanatory variable to estimate the effect of panobinostat (appendix p 11). We used Spearman’s rank correlation to explore correlations in post-hoc analyses.

This trial is registered with ClinicalTrials.gov, number NCT01680094.

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

**Results**

We enrolled 15 patients and all completed full panobinostat dosing (table 1). The amount of cell-associated unspliced HIV RNA increased during panobinostat treatment (p<0.0001; repeated measurement ANOVA incorporating all data from baseline and on panobinostat), with significant increases at all assayed timepoints compared with baseline (figure 1A, appendix p 3). Amounts of cell-associated unspliced HIV RNA increased rapidly, with a mean increase of 2.4-fold (95% CI 1.8–3.3; p<0.0001) measured 2 h after initiating panobinostat. The maximum increase during panobinostat treatment ranged from 2.1-fold to 14.4-fold for the 15 patients, with a median of 3.5-fold.

Plasma concentrations of panobinostat increased for all 15 patients with the highest concentrations measured 2 h after dosing (figure 1B), consistent with a \( T_{\text{max}} \) of roughly 2 h. Accordingly, mean histone H3 acetylation increased rapidly, by 2.5-fold (95% CI 1.9–3.4; p<0.0001) 2 h after the first dose of panobinostat. Overall, panobinostat treatment resulted in substantial cyclic changes in histone H3 acetylation with increased acetylation detected during panobinostat treatment weeks (figure 1C).

We measured plasma viraemia to assess whether the increases in cell-associated unspliced HIV RNA induced by panobinostat were primarily caused by long terminal repeat-initiated transcription. Measurement of cell-associated HIV RNA transcripts by primers to gag could be confounded by readthrough genomic transcripts. Therefore, our ability to document increases in plasma viraemia at the same time as increases in cell-associated unspliced HIV RNA was crucial for showing that panobinostat effectively disrupts HIV latency in vivo. 30% of baseline samples and 54% of samples from patients taking panobinostat (range 33–73 for all study visits during panobinostat) were positive for plasma HIV RNA (figure 2A, 2B). These data include multiple

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**Figure 2:** Panobinostat treatment and HIV plasma viraemia

Proportion of positive and negative plasma samples at baseline and on panobinostat (A, the two baseline points are grouped together), and the proportion of positive plasma samples throughout the study (B), according to transcription-mediated amplification; the shaded box shows the panobinostat treatment period. (C) HIV RNA positive plasma sample according to transcription-mediated amplification versus amount of cell-associated unspliced HIV RNA. (D) Amount of cell-associated unspliced HIV RNA in transcription-mediated amplification negative versus positive samples.
observations of the same participant. A positive test was more likely on treatment than at baseline (odds ratio 10·5, 95% CI 2·2–50·3; p=0·0002). The probability for detectable plasma viraemia increased from 17·4% at baseline to 68·9% during panobinostat treatment for the average patient. Although four of 15 patients tested positive for plasma HIV RNA at all times (including both baseline measurements), nine of 15 patients tested negative at both baseline measurements and then repeatedly positive during panobinostat treatment (appendix p 8). Detection of plasma HIV RNA was strongly associated with the amount of cell-associated unspliced HIV RNA both during panobinostat dosing (figure 2C) and when all trial samples (ie, at baseline, during treatment, and after treatment) were jointly analysed (appendix p 4). HIV RNA-positive plasma samples had a 4·3-fold (95% CI 2·9–6·4, p<0·0001) higher concentration of cell-associated unspliced HIV RNA than did HIV RNA-negative samples (figure 2D). When plasma was spiked with increasing amounts of HIV DNA, DNA was not detected by a transcription-mediated amplification assay (data not shown).

We did four separate measures of HIV-infected cells in peripheral blood and incorporated them into the posthoc analyses that followed the analytical treatment interruption. We assessed total HIV DNA, integrated HIV DNA, two long-terminal-repeat circles, and infectious units per million CD4 cells. We recorded a decrease in total HIV DNA from baseline to day 14 (p=0·04; figure 3A). However, by day 37, the concentration had returned to that at baseline. Furthermore, integrated HIV DNA (figure 3B) and two long-terminal-repeat circles (data not shown) did not change significantly from baseline. According to viral outgrowth, the proportion of cells carrying replication-competent virus did not change between baseline and after panobinostat treatment. Specifically, the mean infectious units per million was 1·22 (95% CI 0·49–1·95) at baseline and 1·21 (0·66–1·78) 4 weeks after completion of panobinostat (figure 3C, appendix p 9). Thus, despite increased HIV transcription and virion production during panobinostat dosing, our peripheral blood analyses did not detect cohort-wide reductions in the latent HIV reservoir.

Inclusion in the analytical treatment interruption was dependent on the outcome of the primary endpoint analysis, which became available in November, 2013 (36–46 weeks after completion of panobinostat dosing). All 15 patients were eligible and nine decided to participate. The median time after interruption until the plasma viral load reached more than 1000 copies per mL was 17 days (IQR 17–24·5) and seven of nine patients had rebound viraemia (>1000 copies per mL) within 21 days (figure 4A, appendix p 5). Rebound to this concentration took the longest for patients 9 and 4 (figure 4A). We investigated whether these participants had distinguishing characteristics at baseline or during panobinostat treatment that correlated with this delayed viral rebound. We detected no correlation between time to viral rebound during analytical treatment interruption and baseline amounts of total or integrated HIV DNA (figure 4B, 4C). However, we recorded significant correlations between the time to viral rebound and the change in both total and integrated HIV DNA during

![Figure 3: Effect of panobinostat on the latent HIV reservoir](image-url)

The median (IQR) amounts of total (A) and integrated (B) HIV DNA per 10⁶ CD4 cells. The shaded box shows the panobinostat treatment period. (C) Infectious units per million at baseline and 4 weeks after completion of panobinostat treatment (day 84) as assessed by a quantitative viral outgrowth assay.
HIV DNA per 10⁶ CD4 cells. Viral rebound to more than 50 copies per mL and ratios of baseline to post-panobinostat total (D) and integrated (C) HIV DNA per 10⁶ CD4 cells. Time to viral rebound to more than 50 copies per mL and the baseline log-transformed total (B) and integrated (C) HIV DNA per 10⁶ CD4 cells. Time to viral rebound in plasma after interruption of antiretroviral treatment.

Figure 4: Viral rebound during analytical antiretroviral treatment interruption

(A) HIV viral load in plasma after interruption of antiretroviral treatment. Time to viral rebound to more than 50 copies per mL and the baseline log-transformed total (B) and integrated (C) HIV DNA per 10⁶ CD4 cells. Time to viral rebound to more than 50 copies per mL and ratios of baseline to post-panobinostat total (D) and integrated (E) HIV DNA per 10⁶ CD4 cells.

Panobinostat was safe and well tolerated. 45 adverse events were reported, but only 16 were presumed related to panobinostat; these occurred in ten patients and were all grade 1 (table 2). Fatigue was the most common drug-related adverse event. One serious adverse event was recorded during the trial but unrelated to panobinostat (hospital admission for headache before starting study treatment). We recorded minor but reversible changes in leucocyte, neutrophil, monocyte, and thrombocyte counts (appendix p 2). By contrast, CD4 cell counts and total lymphocyte counts were unaffected by panobinostat treatment (appendix p 2). We also repeatedly quantified concentrations of cytomegalovirus DNA (in urine) and Epstein-Barr virus DNA (in blood) during panobinostat treatment, but recorded no evidence of unintended DNA virus reactivation (data not shown).

Discussion

8 weeks of cyclic treatment with panobinostat was safe, well tolerated, and effectively increased HIV transcription in patients taking antiretroviral therapy. Furthermore, panobinostat-induced HIV transcription was temporally associated with increased detection of plasma HIV RNA. Viral rebound followed treatment interruption, although the time to rebound varied between individuals. Together, these results provide evidence that panobinostat can effectively activate HIV from latency in vivo and that it should be further investigated as a curative intervention for HIV (panel).

Three clinical investigations are related to our study.11–15 In each of these trials, the histone deacetylase inhibitor vorinostat was assessed, which requires higher concentrations than does panobinostat to achieve in vitro efficacy.19 In the first trial, a single dose of vorinostat increased cell-associated HIV RNA (by 1·5–10·0-fold; n=8) from baseline.13 Three distinctions between the vorinostat studies and our study exist. First, patients in the single-dose vorinostat trial were preselected for inclusion on the basis of ex vivo assessments of virus induction. By contrast, we did not preselect patients for the capacity of their cells to respond to panobinostat ex vivo. Second, cell-associated HIV RNA was measured in resting memory CD4 T cells in this vorinostat trial, whereas we report it in unfractionated CD4 T cells. Third, whereas no increase in plasma viraemia was reported with vorinostat,14,15 we detected a concurrent increase in plasma viraemia and cell-associated unspliced HIV RNA during panobinostat treatment. We assayed this effect with a transcription-mediated amplification-based assay, which has 50% sensitivity at 3·8 copies per mL and 95% sensitivity at 12 copies per mL.13 This assay has previously been used in studies of potential cures for HIV.17 By contrast with the single-copy assay, the transcription-mediated amplification assay is fully automated and certified. Additionally, treatment with another histone deacetylase inhibitor, romidepsin (5 mg/m²), increased

panobinostat treatment (figure 4D, 4E, appendix p 6). Four patients with the largest reduction in total HIV DNA during the trial (patients 4, 5, 9, and 12) had similar patterns, with DNA reduction as early as 14 days after starting panobinostat that was sustained throughout the remainder of the study period (67–84% decrease overall; figure 5). Of these four, patients 4, 9, and 12 participated in the analytical treatment interruption and as a group had a significantly delayed time to viral rebound compared with the six other participants in the treatment interruption (median 28 days, IQR 21–56 vs 17 days, 16–18; p=0·024). HLA class 1 typing did not show protective alleles (HLA-B5701, HLA-B5703, HLA-B2705) in these three individuals (table 1).26

Figure 4: Viral rebound during analytical antiretroviral treatment interruption

(A) HIV viral load in plasma after interruption of antiretroviral treatment. Time to viral rebound to more than 50 copies per mL and the baseline log-transformed total (B) and integrated (C) HIV DNA per 10⁶ CD4 cells. Time to viral rebound to more than 50 copies per mL and ratios of baseline to post-panobinostat total (D) and integrated (E) HIV DNA per 10⁶ CD4 cells.
concentrations of plasma HIV RNA in patients taking antiretroviral therapy. These increases in plasma viraemia were readily measurable with a routine clinical assay. However, until a full assessment is done of the effects of these drugs on latently infected cells and how they modulate host immune responses, speculation about which histone deacetylase inhibitor is best seems premature.

The fundamental rationale for targeting the latent HIV reservoir through disruption of virus latency is that immune-mediated mechanisms or viral cytopathic effects will eliminate latently infected cells induced to express HIV. Nevertheless, we did not record a sustained decrease in the size of the latent HIV reservoir after treatment with panobinostat. This shortcoming could be a result of insufficient immune recognition and killing by cytotoxic CD8 cells, necessitating additional immune enhancement treatment. Also, histone deacetylase inhibitors might induce or intensify dysfunction of T-cell responses. Alternatively, more potent HIV reactivation might be needed to facilitate elimination of latently infected cells. In this regard, investigation of the safety and efficacy of higher doses of panobinostat, which may be dosed as high as 60 mg, is warranted. Despite the lack of a cohort-wide sustained reduction in CD4 cell viral DNA concentrations, we did detect a reduction in HIV DNA in some participants. Because we included an analytical treatment interruption, we were able to show that reductions in HIV DNA, as measured by the ratio of HIV DNA after panobinostat versus baseline, correlated with delayed time to rebound. These investigations were done post-hoc, so they warrant cautious interpretation, but ongoing studies are focused on identifying the distinguishing characteristics associated with reductions in HIV DNA and could be used to predict outcomes of analytical treatment interruptions in future trials.

The main limitation of our study was the lack of a control group. Thus, naturally occurring longitudinal variation could account for the increased plasma viraemia, the increased cell-associated unspliced HIV RNA, and decreased HIV DNA we report. This explanation is unlikely, however, given that all patients had had stably suppressed viraemia for at least 2 years before enrolment. Furthermore, both histone acetylation and cell-associated unspliced HIV RNA concentrations rapidly and concurrently increased on initiation of panobinostat. The repetitive increases during cyclic dosing provide additional evidence that an increase in HIV transcription was caused by panobinostat. A second limitation was that the cell-associated virological measures were done in CD4 T cells, considered the primary reservoir for HIV, but we did not assess activation of HIV from latency specifically in resting memory CD4 cells or other cell types that might also harbour latent proviruses.
Our findings show that cyclic treatment with panobinostat can activate HIV from latency, as shown by significant increases in HIV transcription and increased plasma viraemia. Thus, panobinostat is a promising candidate for inclusion in future combination clinical trials designed to activate and eliminate the latent HIV reservoir.

Contributors

TAR, MT, LO, and OSS conceived of and designed the study and developed the study protocol. LO provided clinical oversight of the study. ML, SRL, SP, and CD contributed to the study concept and design. MT coordinated trial sample processing and laboratory procedures. TAR and OSS enrolled patients in the study and did follow-up. CRB, AS, and SRL analysed the primary endpoint. RO, SP, and CE declare no competing interests.

Interpretation

We report the first study to investigate the ability of the highly potent histone deacetylation inhibitor panobinostat to activate HIV from latency in vivo. 8 weeks of cyclic treatment with panobinostat was well tolerated and led to significant increases of HIV transcription in patients taking suppressive antiretroviral therapy. Furthermore, panobinostat-induced HIV transcription increased virion production and plasma viraemia. Taken together, these results provide evidence that panobinostat effectively disrupts HIV latency in vivo. Further clinical development of panobinostat for HIV curative interventions is warranted to optimise the drug’s reactivation potential and to explore its effect on the latent HIV reservoir when combined with immune enhancement treatments.

Acknowledgments

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Declaration of interests

Aarhus University has filed a patent application covering the use of panobinostat in HIV-infected patients on which TAR, MT, CRB, LO, and OSS are inventors. SRL has received funding from Merck and Gilead for investigator-initiated research grants and has participated in educational activities for Viiv Healthcare; all payments and honoraria have been paid to her institution. AS, AW, MB, ML, RO, SP, and CE declare no competing interests.

Panel: Research in context

Systematic review

We searched PubMed on May 30, 2014 with the keywords “HIV”, “HIV latency”, “HIV reservoir”, “HIV eradication”, “HIV cure”, and “HDAC inhibitors”. We reviewed and assessed titles and abstracts for relevance. Although many compounds have been investigated in vitro for their ability to reactivate HIV expression, vorinostat is the only histone deacetylase inhibitor that has been assessed in clinical trials thus far. In these studies, administration of vorinostat increased HIV transcription, but did not increase measures of extracellular HIV RNA, nor did vorinostat affect the latent HIV reservoir. These observations have led researchers to question the ability of histone deacetylase inhibitors to disrupt HIV latency to the extent where HIV proteins are expressed on the surface of infected cells, thus enabling immune-mediated elimination.

References


