Ex Vivo Bioactivity and HIV-1 Latency Reversal by Ingenol Dibenzoate and Panobinostat in Resting CD4+ T Cells from Aviremic Patients

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The HIV-1 latent reservoir in resting CD4+ T cells represents a major barrier to viral eradication. Small compounds capable of latency reversal have not demonstrated uniform responses across in vitro HIV-1 latency cell models. Characterizing compounds that demonstrate latency-reversing activity in resting CD4+ T cells from aviremic patients ex vivo would help inform pilot clinical trials aimed at HIV-1 eradication. We have optimized a rapid ex vivo assay using resting CD4+ T cells from aviremic HIV-1+ patients to evaluate both bioactivity and latency-reversing potential of candidate latency-reversing agents (LRAs). Using this assay we characterize the properties of two candidate compounds from promising LRA classes, ingenol 3,20-dibenzoate (a protein kinase C agonist) and panobinostat (a histone deacetylase inhibitor), in cells from HIV-1+ ART-treated aviremic participants, including the effects on cellular activation and cytotoxicity. Ingenol induced viral release at levels similar to positive control (CD3/28 receptor stimulation) in cells from a majority of participants and represents an exciting LRA candidate as it combines robust viral reactivation potential with a low toxicity profile. At concentrations that blocked histone deacetylation, panobinostat displayed a wide range of potency among participant samples and consistently induced significant levels of apoptosis. The protein kinase C agonist ingenol 3,20 dibenzoate demonstrates significant promise in a rapid ex vivo assay using resting CD4+ T cells from treated HIV-1 positive patients to measure latent HIV-1 reactivation.
Introduction

Durable blockade of viral replication by combinations of antiretroviral drugs has transformed HIV-1 infection from an untreatable, lethal condition characterized by progressive immune deficiency into a chronic, manageable medical problem for the vast majority of patients with access to therapy(1). Despite the ability of ART to block ongoing HIV-1 replication and allow for restoration of the circulating CD4+ T cell population, HIV-1 eradication does not occur with these drugs due to the presence of long-lived viral reservoirs in resting memory CD4+ T cells(2-4). ART can continuously suppress viral replication for years or even decades, however patients who stop therapy will develop viremia within a matter of weeks and progress to overt immunodeficiency if ART is not resumed(5). This rebound viremia arises from a minority of cells among the resting memory CD4+ T cell population harboring unexpressed HIV-1 proviral DNA that is stably integrated into the cellular genome(6).

The HIV-1 latent reservoir in patients on ART is stable over a period of many years, and does not decay significantly during the lifespan of an infected patient(7). It is generally accepted that eradication of the virus will require elimination of this latent reservoir(8, 9). The absence of specific markers to distinguish latently infected cells from uninfected ones has led to the proposition that substances able to reverse the latent viral state should be used to "purge" the latent reservoir(10). Infected cells could then potentially be cleared via viral cytopathic effects or immune-mediated mechanisms(11).
The inability of currently available in vitro model systems to reliably predict latency reversal ex vivo (12) underscores the importance of evaluating candidate compounds using ex vivo techniques making use of cells obtained from HIV-1 infected patients on ART. The current technique using such cells is known as the viral outgrowth assay or VOA (13). Because the VOA was designed to use serial dilutions of patient cells, this technique requires large numbers of cells that are best obtained via leukapheresis. In addition, the VOA relies on the patient’s endogenous virus to spread to and replicate within indicator cells, a process that can take up to ten days. To circumvent these limitations, we have optimized an assay that can be performed in three days, and requires no more than 180mL of peripheral blood, obtained via venipuncture. Rather than relying on virus spread, this procedure measures a burst in cell-free virus release that is detectable within 48 hours of cell stimulation. This assay is ideal for evaluating the efficacy of candidate LRAs in pre-clinical studies. In this work, we evaluate representative candidates from two promising LRA classes, protein kinase C agonists (PKCa) (14-20) and histone deacetylase inhibitors (HDACi) (21-24).

**Materials and Methods**

**Participant involvement** Aviremic HIV-1 infected patients on ART were recruited for phlebotomy according to an approved institutional review board protocol at the University of Utah. Inclusion criteria for this study required viral suppression (less than 50 HIV-1 RNA copies/mL) for a minimum of six months, ART initiation during chronic HIV-1 infection (greater than six months since seroconversion), and compliance with a stable ART regimen for a minimum of twelve months per participant and...
provider report. A healthy HIV-uninfected donor was recruited via a separate approved IRB protocol. Informed consent and phlebotomy were performed in the Center for Clinical and Translational Science Clinical Services Core at the University of Utah.

Resting CD4+ T cell isolation and ex vivo culture conditions

Peripheral blood mononuclear cells are isolated from whole blood immediately after phlebotomy via density gradient centrifugation, followed by negative selection of resting CD4+ T cells (rCD4) using magnetic bead separation (Miltenyi Biotec and StemCell Technologies). Aliquots of 5x10^6 resting CD4+ T cells are cultured under multiple conditions: a negative control consisting of culture medium and dimethyl sulfoxide (DMSO; compound solvent) alone, CD3/CD28 antibody-coated magnetic beads (Dynabeads® Human T-Activator CD3/CD28, Life Technologies; positive control) and medium containing candidate latency reversing agents at concentrations previously shown to induce viral reactivation (100nM for both ingenol and panobinostat; compounds obtained from Martin Delaney Collaborative of AIDS Researchers for Eradication (CARE), University of North Carolina, Chapel Hill, NC)(17, 18, 22). At 48 hours culture supernatant is collected for real time quantitative polymerase chain reaction (qPCR, described in detail below) and aliquots of 10^5 cells are obtained for flow cytometry in order to evaluate for the presence of biomarkers of drug activity, cellular activation and apoptosis (described in detail below).
Real-time quantitative polymerase chain reaction. Supernatant from each well is collected for quantification of cell-free virions using a two-step quantitative polymerase chain reaction (qPCR) that makes use of a primer and probe set for conserved regions of the 3' LTR of HIV-1 mRNA using recently published methods (25). Culture supernatant undergoes DNase treatment (Quanta Biosciences) followed by cDNA synthesis using qScript cDNA Supermix containing oligo-dT primers and random hexamers according to manufacturer's protocol (Quanta Biosciences). RNA aliquots that do not contain reverse transcriptase (no RT controls) were run in parallel for every sample. Real-time quantitative PCR was subsequently performed in triplicate on cDNA and RNA (no RT control) samples using TaqMan Universal Master Mix II (Applied Biosystems) on a Roche LC480 Real-Time PCR instrument. Primers and probe used were as follows: forward primer (5' to 3') CAGATGCTGCATATAAGCAGCTG, reverse primer (5' to 3') TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGAAGCAC and probe (5' to 3') FAM-CCTGTACTGGGTCTCTCTGG-BHQ1. Cycling conditions were as follows: 50°C for two minutes followed by 95°C for ten minutes for polymerase activation, followed by 45 cycles of 95°C for 15 seconds and 60°C for one minute. Serial ten-fold dilutions of a plasmid containing the HIV-1 3'LTR (VQA plasmid; obtained from Greg Laird and Robert Siliciano) from 10^6 to 10^0 copy per well were amplified in triplicate along with unknowns in order to provide a standard curve and quantify cell-associated viral mRNA. This assay has been shown to be highly specific for the detection of HIV-1 mRNA with a lower limit of detection of 50 HIV-1 mRNA copies/mL (25).
Biomarker flow cytometry  After 48 hours in culture, aliquots of 10^5 cells are fixed using BD Cytofix™ Fixation Buffer (50% by volume; BD Biosciences) for 10 minutes at 37°C. After incubation, DMSO (Fisher Scientific) was added to the sample to a final concentration of 10% and frozen at -80°C. At the time of analysis, samples were thawed on ice and re-suspended in 2 mL of phosphate-buffered saline (PBS). 500μL aliquots were used for each staining and staining control. For acetyl-Histone H3 analysis, samples were pelleted and re-suspended in 100μL of BD Phosflow™ Perm Buffer III (BD Biosciences) while vortexing, and incubated on ice for 30 minutes. Cells were then washed with PBS and incubated in 100μL of PBS + 3% FBS containing 1μL of acetyl-Histone H3-PE (Millipore) and 0.75μL of cleaved caspase 3-AF488 (Cell Signaling Technology) for 1 hour at room temperature protected from light. After incubation, cells were washed with PBS + 3% fetal bovine serum (FBS) and 100μL of 2% paraformaldehyde (PFA) was added prior to flow cytometry acquisition. For CD69 analysis, samples were pelleted and re-suspended in 100μL of BD Cytofix/Cytoperm™ (BD Biosciences) and incubated at 4°C for 30 minutes. Cells were then washed with BD Perm/Wash™ (BD Biosciences) and incubated in 100μL of Perm/Wash™ containing 1μL of CD69-APC (Invitrogen) and 0.75μL of cleaved caspase 3-AF488 for 1 hour at room temperature protected from light. After incubation, cells were washed with Perm/Wash buffer and 100μL of 2% PFA was added prior to flow cytometry acquisition.

Biostatistics  Log response (MIF CD69, % active caspase 3, fold-induction acetyl H3) and log mRNA viral release were analyzed with a linear mixed-effects model. A fixed
growth media effect and a random subject effect were included in the predictor variables. For the response variables, mRNA and CD69, response was log-transformed before analysis to account for larger variability in larger values. mRNA values below limit of quantitation (LOQ) were imputed as $\frac{1}{2}$ LOQ, i.e. 25 copies mRNA/mL. Data were analyzed using SAS™ version 9.3 PROC MIXED.

**Results**

*Participants*  We recruited 12 HIV-1 positive, aviremic participants on ART (participant characteristics shown in Table 1). Two participants, H025 and H026, returned for a second phlebotomy during the study period. We therefore report the results of fourteen independent *ex vivo* experiments using resting CD4$^+$ T cells (rCD4) from twelve individuals. The median age among our cohort was 49.5 years (range 37-60). Median CD4$^+$ T cell count at the time of enrollment was 738 cells/μL (range 247-1166) and time of viral suppression was 31.5 months (range 7-55). All participants initiated ART during chronic infection. We recruited a healthy, HIV-1-uninfected participant for phlebotomy in order to isolate uninfected resting CD4$^+$ T cells as a negative control for the assay.

*Rapid Ex Vivo Evaluation of Anti-Latency activity (REVEAL)*. Reactivation of latent proviruses induces a burst of productive transcription as evidenced by the presence of cell-associated mRNA followed by release of HIV-1 virions into the culture medium. We reasoned that latently infected cells from the peripheral blood of aviremic patients would release a detectable quantity of virions into the medium upon stimulation. To detect the presence of such virions, we relied upon a recently published quantitative
PCR protocol(25). This method uses primers that are specific for authentic viral mRNAs, including genomic viral RNA, and does not amplify products of aberrant read-through originating from adjacent cellular promoters(25).

We isolated between 15 and 60 million rCD4s from 180mL of peripheral blood by routine phlebotomy. We stimulated aliquots of five million rCD4 T cells per control or experimental condition. Based on previously described estimates of the frequency of replication-competent proviruses present in rCD4 T cells in vivo(3, 4, 14, 26), we estimated that five million rCD4 T cell aliquots would ensure the detection of virions released into the supernatant. The variable quantity of rCD4 T cells obtained from 180mL of whole blood from participants limited our ability to test all conditions in all donors; ingenol was tested in all donors and panobinostat was tested simultaneously in six.

The positive control, CD3/CD28 stimulation with antibody-coated magnetic beads, induced viral release in samples from all HIV-1-infected participants (Figure 1) with a geometric mean of 3,868 copies of HIV-1 mRNA/mL (range 1,030-14,518 copies/mL). Viral release in the absence of stimulation (culture medium plus DMSO) was only detected in two aliquots and at very low levels (geometric mean 52 copies/mL, range 14-96 copies/mL; Figure 1). The ratio of means between our positive and negative control was 74.1 (range 11.4 – 481.3 with a probability of difference P<0.0001) representing the average fold increase in viral RNA copies detected between these conditions. Low level viral release in negative control samples may reflect sub-optimal
ART compliance or viral ‘blips’ at the time of phlebotomy(27). Supernatant from the
HIV-1-uninfected donor did not demonstrate any detectable viral mRNA from any
condition. Viral release from rCD4 T cells exposed to CD3/CD28 antibodies was
accompanied by induction of CD69 and a modest increase in histone acetylation
measured by flow cytometry (Figures 2 and 3, respectively).

We observed a greater than 100-fold variability between participants with regard to
viral release in the CD3/28 antibody positive control condition (Figure 1). High
variability in the magnitude of response to stimulation has also been observed in all
published pilot clinical trials with LRAs to date(21, 28-30) and is likely to reflect
underlying differences between individuals with respect to viral reactivation threshold.
This in turn is likely governed by a number of unique characteristics including overall
reservoir size and transcriptional regulatory state of these cells. Aliquots of five million
purified rCD4 T cells cultured for 48 hours consistently provided the dynamic range to
test both latency reversal and bioactivity of candidate LRAs ex vivo. We have termed
these combined protocols the rapid ex vivo evaluation of anti-latency assay, or REVEAL.

We designed the REVEAL assay to amplify genomic RNA from virions in the culture
supernatant. It has long been recognized that virions will pellet out of supernatant
when subjected to ultracentrifugation while naked mRNA will not(31). To verify that
detected RNA was pelletable, supernatants the CD3/CD28 condition of one donor was
subjected to ultracentrifugation followed by qPCR. Initial REVEAL analysis in
unconcentrated supernatant measured a total of 1500 mRNA copies.
Ultracentrifugation of this supernatant led to a 125-fold volume concentration with detection of 875 total mRNA copies in the pellet, representing 58% recovery of RNA signal. Therefore, the RNA detected in the supernatant is pelletable. We also performed p24 ELISA per manufacturer's instructions (HIV-1 p24 Antigen ELISA 2.0, Zeptometrix Corp), in an attempt to detect Gag protein secreted to the culture supernatant at 48 hours. Only samples treated with the positive control (CD3/28 antibodies) showed positive p24 values, although these were below the quantitative threshold of the assay (3.9pg/mL; data not shown). None of the negative control treatments resulted in positive ELISA values. These data further support the viral RNA, and not p24 ELISA, represents a sensitive and quantitative readout for the REVEAL assay. Attempts to co-culture virus with indicator cells resulted in easily detectable viral replication after 14 days in culture by p24 ELISA for the CD3/CD28 condition but not for the medium / DMSO condition. However, sufficient numbers of lymphocytes to allow for a quantitative, limiting-dilution-based assay are not typically available from 180 mL blood draws, and would require leukapheresis. These experiments provide further evidence that stimulation of aviremic patient cells results in release of replication-competent virus.

Reactivation and bioactivity of ingenol 3,20 dibenzoate We have recently observed that ingenol 3,20-dibenzoate, a protein kinase C agonist, induced viral reactivation reproducibly and with similar potency as CD3/28 stimulation in a primary central memory T cell model of latency(32). Therefore, it was compelling to further test ingenol 3,20 dibenzoate in aviremic patient cells. Ingenol at 100nM induced viral release from
resting CD4+ T cells at levels close to those following T cell receptor stimulation in eleven of fourteen independent experiments (Figure 1). The geometric mean for CD3/28 stimulation among all participants was 3,868 HIV-1 mRNA copies/mL (range 1,030-14,518 copies/mL) compared to 811 copies/mL for ingenol (range 216-3,044 copies/mL). Using the linear mixed-effects model for comparison, we determined that the relative fold increases in viral RNA induced by CD3/28 versus ingenol treatments were not statistically significant (P=0.10).

PKC agonists are known to up-regulate cell surface expression of CD69(33), an early marker of T cell activation. Cells exposed to ingenol up-regulated CD69 to levels approximating T cell receptor stimulation by flow cytometry (Figure 2), with a geometric mean intensity of florescence (MIF) for ingenol of 336 (range 176–643) compared to 577 for CD3/28 antibody positive control (range 302–1104). These means were not statistically significant (P=0.24) by the linear mixed-effects model analysis. CD69 MIF for ingenol was significantly higher than the medium alone condition (MIF 1.4, range 0.73–2.7) with a ratio of 241 (range 96–604; P<0.0001).

Reactivation and bioactivity of panobinostat 100nM panobinostat, a histone deacetylase inhibitor, induced viral reactivation from cells in a minority of participants, with viral release detected from cells of two of six individuals tested (Figure 1). The geometric mean of viral release from aliquots exposed to panobinostat was 266 copies/mL (range = 38–1845 copies/mL) with a statistically significant ratio of means
Panobinostat demonstrated inhibition of histone de-acetylation at 100nM in all participant samples tested (Figure 3) independent of latency reversal. Mean fold change in histone 3 acetylation was 3.57 (range 2.78-4.58) compared to medium alone. Unlike ingenol, panobinostat did not induce expression of CD69 (Figure 2; MIF 4.3, range 1.6-11.5) to levels that differed from medium alone (ratio of means 3.1, range 0.94–10.03; P=0.06).

Induction of programmed cell death

Activation of intracellular caspase 3 is an early and reliable biomarker of apoptotic cell death(34) and is the mechanism of apoptosis induced by ingenol compounds(19, 35). Cells exposed to 100nM ingenol demonstrated a modest but statistically significant decrease in the percentage of cells undergoing early apoptosis compared to medium alone (6.03% versus 12.62%, P= 0.0001; Figure 4). Conversely 100nM panobinostat induced apoptosis in a significant percentage of cells compared to medium alone (30.75% versus 12.62%; P<0.0001; Figure 4) under these culture conditions. Cell viability, measured by forward and side scatter gating, is decreased among cells exposed to panobinostat but not in other conditions compared to medium alone (Figure 4c). The lack of apoptosis induction by ingenol 3,20 dibenzoate may reflect the relatively low concentration of ingenol (100nM) and the fact that our cells are not transformed in contrast to previous studies(19, 34).
We describe a rapid ex vivo system making use of resting CD4+ T cells from aviremic HIV-1 positive participants on stable ART to estimate reactivation potential, bioactivity and toxicity of candidate LRAs that we have named the REVEAL assay. After 180mL of whole blood is obtained by phlebotomy, resting CD4+ T cell isolation and 48 hours of cell culture are followed by standardized qPCR(25) and flow cytometry techniques. This assay produces reproducible results characterizing the behavior of candidate LRAs in patient cells ex vivo within three days. An assay using the same RNA PCR output measurement and a similar culture protocol has recently been published(20). Laird et al. observed that PKC agonists (including bryostatin-1 and prostratin, but not ingenol) reliably induce proviral transcription and viral release from patient cells ex vivo alone and in combination with HDAC inhibitors, including panobinostat. Panobinostat alone did not induce significant viral reactivation(20).

Using the REVEAL assay we have observed that ingenol 3,20 dibenzoate activates patient resting CD4+ T cells as measured by up-regulation of CD69 and reactivates latent virus to levels that approximate those of T cell receptor stimulation. Minimal apoptosis was observed after 48 hours in culture. Our results are in keeping with recently published reports of the latency reversing potential and safety of ingenol derivatives in cell lines(18, 36) and an in vivo non-human primate SIV model (unpublished data, presented October 2014 by Janice Clements at NIH Strategies for an HIV Cure, Bethesda, MD, USA). PKC agonists are recognized as a promising class of...
latency reversing compounds (14, 15, 18, 20, 36), and ingenol derivatives in particular deserve further pre-clinical characterization.

Panobinostat was recently tested in an unblinded clinical trial as a latency reactivation agent in 15 aviremic patients on ART (30). Results from this study demonstrate mean increases in cell-associated viral RNA and plasma viremia in this cohort, with significant variations in individual responses. We have observed an analogous phenomenon exposing patient rCD4 cells to 100 nM panobinostat with a minority of participants exhibiting viral release. Laird et al. describe similar results with panobinostat alone (20) as did Cillo et al using the HDAC inhibitor vorinostat (37). Regardless of viral transcription, panobinostat demonstrated marked inhibition of histone deacetylation in cells tested from all patients at this concentration (Figure 3b). This activity is associated with induction of activated caspase 3, an early marker of apoptosis, in our system (Figure 4b).

There are a number of limitations to the ex vivo patient cell assay described here. In an attempt to maintain the baseline quiescent state of the resting CD4+ T cells isolated from participants and to minimize background viral reactivation, no cytokines (including interleukin 2) are added to cell cultures. While the lack of exogenous cytokines improves the signal-to-noise ratio with regard to viral reactivation in the REVEAL assay, it likely comes at the cost of a higher induction of apoptosis (12.62% caspase 3 positive in medium alone at 48 hours, Figure 4b). The significantly lower percentage of cells undergoing activation of caspase 3 in ingenol and αCD3/28
conditions (Figure 4b) may reflect the initiation of endogenous interleukin 2 production as these cells enter an activated state.

The quantity of resting CD4+ T cells obtained from a single phlebotomy allows for relative quantification of LRA responses relative to TCR stimulation, however not enough cells are obtained to simultaneously measure the size of the inducible reservoir for each participant (13). The dynamic range of the REVEAL assay is likely to be partially dependent on the reservoir size for each participant. However, the assay is designed with internal positive and negative controls that allow for direct comparison between these controls and experimental LRA treatments.

The REVEAL assay isolates resting CD4+ T cells in order to evaluate latency reversal, and does not currently account for the potential effects of candidate LRAs on other immune cells, including CD8+ T cells, NK cells and monocytes. These cells may play an important role in latent reservoir eradication in vivo, and the effect of LRAs on these cell types deserves further exploration. Additional areas of active study include dose-response relationships and the potential for synergy between candidate LRAs as recently described by Laird et al (20). The REVEAL assay described here allows for rapid, reproducible evaluation of candidate LRAs and provides an accessible platform to inform pilot clinical trials aimed at HIV-1 latent reservoir eradication.
Figure legends

Figure 1: Quantification of HIV-1 viral release into culture supernatant after 48 hours under four different conditions reveals ingenol 3,20 dibenzoate to have similar potency to CD3/28 stimulation. Aliquots of five million resting CD4^+ T cells from aviremic patients on ART were exposed to CD3/28 antibody coated beads (positive control), medium and DMSO (negative control), 100nM of ingenol 3,20 dibenzoate or 100nM of panobinostat for 48 hours. Quantitative PCR was performed on culture supernatant to detect HIV-1 mRNA release. CD3/28 antibody stimulation resulted in widely ranging, though consistently detectable, HIV-1 mRNA release in all experiments. One HIV-1-uninfected donor was included to ensure the specificity of the assay (A008) and HIV-1 mRNA was undetectable in all conditions. Ingenol demonstrated similar potency to the positive control with regard to viral release (ingenol mean = 811 copies/mL compared to 3868 copies/mL for CD3/28; ratio of means 4.77 with P=0.10), while panobinostat exposure led to viral release in two of six experiments (mean = 266 copies/mL).

Figure 2: Resting CD4^+ T cells from aviremic patients exposed to 100nM of ingenol 3,20 dibenzoate demonstrated an increase in mean intensity of florescence of CD69 (MIF CD69) to levels similar to CD3/28 antibody exposure (ingenol mean 336 compared to 577 for CD3/28; ratio of means = 1.7 with P=0.239). 100nM of panobinostat induced no change in CD69 florescence compared to medium alone (MIF 4.27 versus 1.39; P=0.063). Panel A: representative flow cytometry histograms for the four conditions tested from a single experiment. The solid grey histogram represents the mean florescence intensity (MFI) of isotype control and the black outline histogram
represents the MFI for each condition, and Panel B: the results of six independent experiments.

**Figure 3:** 48 hour exposure to 100nM panobinostat leads to mean 3.57 fold increase in intracellular acetylated histone 3 (Acetyl H3) compared to negative control. **Panel A:** representative flow cytometry histograms from a single experiment measuring the mean florescence intensity (MFI) of intracellular acetylated histone 3 under four different conditions. The solid grey histogram represents the MFI of isotype control and the black outline histogram represents the MFI for each condition **Panel B:** 100nM of panobinostat, a histone deacetylase inhibitor (HDACi), increased acetylation at histone 3 an average of 3.57 fold in six independent experiments compared to medium and DMSO alone (negative control; P<0.0001). 100nM of ingenol 3,20 dibenzoate did not result in any significant change in acetyl H3 from baseline.

**Figure 4:** 48 hour exposure to 100nM panobinostat resulted in significantly higher percentage of resting CD4+ T cells expressing activated caspase 3, an early marker of apoptosis, compared to negative control (30.75% compared to 12.62%; P<0.0001). **Panel A:** a representative panel of flow cytometry histograms from a single experiment demonstrating a three-fold increase in activated caspase 3 expression in cells exposed to panobinostat compared to medium alone condition. Horizontal bars indicate percentage of cells expressing activated caspase 3. **Panel B:** panobinostat led to increased expression of activated caspase 3 in six independent experiments compared to negative control. Cells exposed to 100nM of ingenol 3,20 dibenzoate and CD3/28
antibody stimulation demonstrated a modest but statistically significant decrease in
activated caspase 3 expression compared to medium alone (6.03% for ingenol and
7.18% for CD3/28; P=0.0001 and P=0.001 respectively). Panel C: cell viability
measured by forward and side scatter gating for each condition demonstrates
decreased cell viability in the panobinostat condition but not for ingenol or anti-CD3/28
exposed cells.

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Experiments designed and performed by AMS, AB, LM, VP.
Participant recruitment and data collection by AMS and DS. Biostatistical analysis by AHB. Manuscript and figures prepared by AMS, AB, VP and reviewed by all authors.

Competing interests
The authors declare no conflicts of interest, financial or otherwise, in regard to this research.
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Table 1: Participant Characteristics

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Abbreviations: 3TC, lamivudine; ABC, abacavir; ATVr, atazanavir boosted with ritonavir; DRVr, darunavir boosted with ritonavir; DTG, dolutegravir; EFV, efavirenz; EVGc, elvitegravir boosted with cobicistat; FTC, emtricitabine; H, Hispanic; RPV, ritonavir; TDF, tenofovir; W, non-Hispanic Caucasian

* Absolute CD4+ T cell count measured in cells/μL.

b Consecutive months of documented viral load (plasma HIV-1 RNA) suppression below limit of clinical detection on ART.