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Mechanistic differences underlying HIV latency in the gut and blood contribute to differential responses to latency-reversing agents

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Abstract

Objective—While latently HIV-infected cells have been described in the blood, it is unclear whether a similar inducible reservoir exists in the gut, where most HIV-infected cells reside. Tissue-specific environments may contribute to differences in the mechanisms that govern latent HIV infection and amenability to reactivation. We sought to determine whether HIV-infected cells from the blood and gut differ in their responses to T cell activation and mechanistically-distinct latency reversing agents (LRAs).

Design—Cross sectional study using samples from HIV-infected individuals (n=11).

Methods—Matched PBMC and dissociated total cells from rectum+/−ileum were treated *ex vivo* for 24h with anti-CD3/CD28 or LRAs in the presence of antiretrovirals. HIV DNA and "read-through", initiated, 5'elongated, completed, and multiply-spliced HIV transcripts were quantified using droplet digital PCR.

Results—T cell activation increased levels of all HIV transcripts in PBMC and gut cells, and was the only treatment that increased multiply-spliced HIV RNA. Disulfiram increased initiated HIV transcripts in PBMC but not gut cells, while ingenol mebutate increased HIV transcription more in gut cells. Romidepsin increased HIV transcription in PBMC and gut cells, but the increase in transcription initiation was greater in PBMC.

Conclusions—The gut harbors HIV-infected cells in a latent-like state that can be reversed by T cell activation involving CD3/CD28 signaling. Histone deacetylation and protein kinase B may contribute less to HIV transcriptional initiation in the gut, whereas protein kinase C may contribute more. New LRAs or combinations are needed to induce multiply-spliced HIV and should be tested on both blood and gut.

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Conference presentations:

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Keywords

HIV infections; humans; rectum; protein kinase C; histones; romidepsin; disulfiram

Background

Latent HIV infection is widely regarded as the main barrier to curing HIV. While latent infection was originally described in blood CD4+ T cells[1–3], most HIV-infected cells reside in tissues such as the gut[4, 5], where differences in infected cell types[6], immune activation[4, 7, 8], and other factors could influence HIV latency. We recently showed that the blocks to HIV expression differ between gut and blood, with blocks to HIV transcriptional completion and splicing in both sites but 10-fold less HIV transcriptional initiation in the gut[9, 10]. While *ex vivo* T cell activation has been shown to reverse HIV latency in blood cells[1–3], it is unclear to what degree activation can reverse the blocks to virus expression in the gut, where a much higher proportion of CD4+ T cells show constitutive markers of "activation"[4, 7]. Likewise, it is unclear which cellular mechanisms govern the different blocks to HIV transcription, or whether agents aimed at reversing latency will show differing effects in the gut or between gut regions[4, 11].

Multiple different "latency reversing agents" (LRAs) have been shown to increase HIV expression *in vitro* (Table S1)[12]. Some have been tested in human trials, including cytokines, disulfiram (a protein kinase B [PKB] agonist), histone deacetylase inhibitors (HDACi, including vorinostat, panobinostat, and romidepsin), protein kinase C (PKC) agonists (topical ingenol mebutate), toll-like receptor agonists, mTOR inhibitors, immune checkpoint blockers, and others[12]. Most of these trials showed increases in HIV RNA in blood cells and/or plasma, generally without reduction in HIV DNA[12], but few studies have investigated the effect of these LRAs on tissues.

Given the greater block to HIV transcriptional initiation in the gut[10], and one prior trial showing that vorinostat induced less HIV transcription in gut than blood[13], we hypothesized that many LRAs would exert less effect on the gut. To investigate this question and probe the tissue-specific mechanisms that suppress HIV transcription, we tested the ability of activation and LRAs to induce HIV transcription in peripheral blood mononuclear cells (PBMC) and cells from ileum and rectum of ART-suppressed individuals. Since LRAs can exert differential effects on the various blocks to HIV transcription[9], and increases in polyadenylated and multiply-spliced HIV RNA are likely essential for latency reversal[9], we tested the degree to which each treatment can increase HIV transcriptional initiation, elongation, polyadenylation, and splicing. Activation increased all HIV transcripts in PBMC and both gut sites, while disulfiram, romidepsin, and ingenol mebutate exerted differential effects on cells from blood and gut. No single LRA increased multiply-spliced HIV RNA in cells from either blood or gut, although the combination of ingenol mebutate plus romidepsin increased multiply-spliced HIV transcripts in PBMC.

Methods

Study Participants

Fresh gut biopsies (rectosigmoid and/or ileum) and blood were obtained from 11 HIV-infected individuals (Table S2). The study was approved by the Institutional Review Board for the University of California, San Francisco (approval #11–07551). All participants provided written informed consent.

Cell isolation

PBMC were recovered by Ficoll density gradient centrifugation[4] from fresh venous blood. Freshly-isolated gut biopsies were obtained by colonoscopy and dissociated into total gut cells using collagenase[4]. LRAs were tested on dissociated gut cells rather than biopsies in order to achieve a more representative cell mix (from 20–28 biopsies) with more equal numbers of total and HIV-infected cells in each well, allow accurate cell counts and viabilities, and circumvent diffusion-limited penetration of nutrients and treatments into biopsies in the absence of blood circulation.

Latency-reversing agent treatments

PBMC and gut cells were resuspended in RPMI with L-Glutamine, penicillin, streptomycin, antiretrovirals, and either 10% FBS (PID7258 and PID1775) or 20% human AB serum (Sigma-Aldrich; used for subsequent participants in an effort to increase viability). Aliquots of 5–6×10^6 PBMC or gut cells were cultured in 6-well plates (10^6 cells/ml) and treated for 24h with LRAs (in DMSO), DMSO alone, or anti-CD3/CD28+IL-2. LRAs were used at previously-published concentrations[9, 14] and included 50nM chaetocin (DNA methyltransferase inhibitor), 30nM panobinostat (HDACi), 40nM romidepsin (HDACi), 2μM JQ1 (bromodomain BET inhibitor), 1μM disulfiram (PKB agonist), 20nM ingenol 3,20 dibenzoate, and 12nM ingenol mebutate (ingenol-3-angelate or PEP005; PKC agonist). Cell counts and viabilities were assessed post-cell isolation and 24h post–treatment by trypan blue staining.

Time course experiments

PBMC and peripheral CD4+T cells (6×10^6 cells/well) were treated with DMSO, ingenol mebutate (12nM), romidepsin (40nM), ingenol mebutate (12nM)+romidepsin (40nM), or anti-CD3/CD28 for 24h and 48h.

Nucleic acid extraction and reverse transcription

After LRA treatment, cell RNA/DNA were extracted using TRI reagent[9]. Reverse transcription was performed using poly-dT plus random hexamers to avoid bias toward reverse transcription of the 3′ end (which can occur with poly-dT alone), the 5′ end (as can occur with random hexamers), or any one gene (as can occur with gene-specific primers)[9].

Droplet digital PCR (ddPCR)

HIV DNA (R-U5-pre-Gag region) and copies of the housekeeping gene TERT (telomere reverse transcriptase) were measured in duplicate by ddPCR[9]. Duplicate aliquots of cDNA

were used to quantify total initiated (TAR), 5'elongated (R-U5-pre-Gag), mid-transcribed (unspliced; Pol), polyadenylated (PolyA) and multiply-spliced Tat-Rev transcripts using ddPCR[9, 10, 15]. Levels of each RNA region were expressed as copies/ug RNA (~10^6 PBMC) and copies/provirus (HIV RNA/HIV DNA), and then further normalized as fold change relative to DMSO[9].

Statistics

Differences between treatments or tissues were evaluated by the Wilcoxon signed rank test using GraphPad Prism version 8.0. Where indicated, correction for multiple comparisons were performed (Benjamini-Hochberg). Wells with no positive droplets (most common for Tat-Rev) were assigned a value of 0.05 for purposes of calculating the median and *P* values in Fig. 2–5 and S2–S6.

Results

Cell viability was comparable for most *ex vivo* treatments

Viabilities at 24h (medians: PBMC>93%, rectum>86%, and ileum>82%; Fig. S1) were comparable for all treatments except disulfiram. The use of 20% human AB serum increased the median cell viabilities for all tissues (medians: PBMC=96.1%; rectum=87.7%; ileum=86.1%; Fig. S1).

Differential effect of LRAs on HIV transcription in PBMC

PBMC (n=11 participants) and matched total rectal (n=9) and ileal (n=7) cells were treated *ex vivo* for 24 hours with LRAs plus antiretrovirals. Levels of HIV "read-through", initiated, 5'elongated, polyadenylated, and multiply-spliced Tat-Rev transcripts were measured by RT-ddPCR (Fig. 1). In PBMC, each LRA exerted different effects on HIV transcription (Fig. 2). Disulfiram (PKB agonist), panobinostat (HDACi), and romidepsin (HDACi) increased total initiated HIV transcripts relative to DMSO (median fold changes: 1.3-, 3.0- and 3.8-fold, respectively; $p<0.05$ for all; Fig. 2A–B, S2). No significant increase in any HIV transcript was detected with either chaetocin or JQ1 (fold changes ≤1.2; Fig. 2C–D). Only panobinostat and romidepsin elicited appreciable increases in 5'elongated transcripts (2.9- and 5.1-fold, respectively, vs. <1.4-fold for disulfiram). No LRA elicited appreciable increases in polyadenylated or multiply-spliced HIV transcripts (fold changes ≤1.4). T cell activation (anti-CD3/CD28) elicited the greatest increase in HIV transcripts in PBMC (initiated: 3.8-fold; 5'elongated: 2.9-fold, polyadenylated: 3.7-fold; multiply-spliced: 7.9-fold; Fig. 2F), and was the only treatment to increase multiply-spliced HIV, suggesting that the tested LRAs do not maximally induce HIV transcription.

T cell activation induces HIV transcription in gut cells

T cell activation increased all HIV transcripts in both PBMC and rectal cells (n=9), including total initiated (Fig. 3A; PBMC: 4.3-fold; rectum: 2.6-fold), 5'elongated (PBMC: 3.2-fold; rectum: 4.0-fold), polyadenylated (PBMC: 6.6-fold; rectum: 5.0-fold), and multiply-spliced Tat-Rev transcripts (PBMC: 18.4-fold; rectum: 5.2-fold) [$P<0.05$ for all]. In ileal cells (n=7), activation increased initiated (1.7-fold), 5'elongated (3.1-fold), and multiply-spliced Tat-Rev HIV RNA (18.7-fold) [$P<0.05$ for all] and tended to increase

polyadenylated transcripts (3.4-fold, P=0.063; Fig 4A). As in the PBMC, T cell activation was the only treatment that increased multiply-spliced Tat-Rev in ileal or rectal cells (Fig. 3–4). To increase our power to detect differences between the blood and gut, we performed an additional analysis in which we compared the fold changes in HIV transcripts with LRA treatment relative to DMSO in all gut samples (mean of rectum and ileum) to the fold change in PBMC (Fig. S3). T cell activation increased 5'elongated HIV transcripts more in gut cells compared to PBMC (Fig S3A; 3.9-fold vs. 2.9–fold, respectively; *P=0.032*).

Disulfiram and romidepsin exert differential effects in PBMC and gut

We hypothesized that LRAs exert differential effects on cells from blood and gut. Given the constraint of gut cell numbers recovered from each participant, gut cells were tested with anti-CD3/CD28 and three mechanistically-distinct LRAs that have been shown to increase HIV transcription *in vivo*: romidepsin, disulfiram, and ingenol mebutate. Disulfiram elicited a small increase in initiated (1.3-fold) and 5'elongated (1.5-fold) transcripts in PBMC (P<0.05 for both), whereas no increase in HIV transcripts was detected in rectal cells or ileal cells by either normalization (Fig. 3B–4B, S4B–S5B). However, given limited cell numbers, disulfiram was only tested in ileal cells from 3 individuals, versus 7 for rectum and PBMC.

In contrast, romidepsin increased initiated (PBMC: 3.8-fold; rectum: 1.4-fold), 5'elongated (PBMC: 5.1-fold; rectum: 2.7-fold), and polyadenylated (PBMC: 1.2-fold; rectum: 2.5-fold) HIV transcripts in both PBMC and rectal cells (n=9; P<0.05 for all; Fig. 3C). When HIV RNA was normalized per provirus to correct for differences between tissues in infected cell frequencies, romidepsin increased initiated and 5'elongated HIV transcripts relative to DMSO in PBMC and ileal cells, and increased elongated and polyadenylated transcripts in rectal cells (P<0.05 for all; Fig. S4–5). However, romidepsin increased total initiated HIV transcripts more in PBMC than rectal cells (P=0.0078; Fig. 5C). No statistically significant differences were observed between PBMC and ileal cells treated with any LRA (Fig. S6). When both gut sites were included in the analysis, romidepsin increased initiated HIV transcripts to a greater extent in PBMC (median fold increase=3.8) than in gut cells (median fold increase=1.7; P=0.014; Fig S3B). This combined gut site analysis also revealed that disulfiram increased total initiated HIV transcripts more in PBMC than gut cells (median fold increase =1.3 [PBMC] and 1.1 [gut cells]; P=0.039; Fig. S3C). Together, these data suggest that disulfiram has a relatively modest effect on HIV transcription and may not be effective in the gut, while romidepsin exerts a quantitatively greater effect in peripheral blood compared to rectal and ileal cells.

Ingenol mebutate increases HIV transcription more in gut cells than PBMC

The PKC agonist ingenol mebutate elicited little effect on HIV transcription in PBMC, but increased initiated (2.3-fold) and polyadenylated (2.9-fold) HIV RNA in rectal cells relative to DMSO (n=9; P<0.05 for both; Fig. 3D). When HIV RNA levels were normalized per provirus, we observed comparable increases in initiated and polyadenylated transcripts (P<0.05; Fig. S4D) and a trend toward an increase in 5'elongated transcripts (P=0.055) in rectal cells. Similarly, ingenol mebutate increased initiated and 5'elongated transcripts in total ileal cells but not in PBMC from matched participants (n=6; P<0.05; Fig. 4D). When normalized to provirus, ingenol mebutate tended to increase initiated HIV transcripts

(*P*=0.063; Fig. S5D) in the ileal cells but not PBMC. Likewise, when both gut sites were included in the analysis, ingenol mebutate tended to increase 5' elongated transcripts more in gut cells (1.8-fold) than in PBMC (1-fold, *P*=0.067; Fig S3D). These findings suggest that ingenol mebutate increases HIV transcription more in rectal and ileal cells than PBMC.

Effects of LRAs may differ by cell type and treatment duration

In a prior study[9], ingenol mebutate increased polyadenylated and multiply-spliced Tat-Rev HIV transcripts in peripheral CD4+ T cells after overnight resting and 24h treatment (compared to time zero). Accordingly, we hypothesized that the treatment and/or duration of exposure could differentially affect CD4+ T cells compared to PBMC. To investigate this question, PBMC and CD4+ T cells were treated with ingenol mebutate, romidepsin, or both drugs for 24h and 48h. Ingenol mebutate increased 5'elongated, polyadenylated, and multiply-spliced Tat-Rev transcripts more in CD4+ T cells than PBMC, and the increases were generally greater at 24h than 48h (Fig. S7B–D). In contrast, romidepsin increased 5'elongated HIV transcripts more at 48h than 24h, with less consistent differences between PBMC and CD4+T or in the degree to which other transcripts increased at 24h vs. 48h. These data highlight that the induction of specific HIV transcripts by LRAs may vary as a function of exposure duration and cell type.

Combination treatment with ingenol mebutate and romidepsin increases multiply-spliced HIV more than either treatment alone

Given that ingenol mebutate and romidepsin reverse blocks to HIV transcription by distinct mechanisms, we reasoned that the combination could have synergistic effects. At 24h, the combination of ingenol mebutate plus romidepsin increased 5'elongated, polyadenylated, and multiply-spliced HIV transcripts more than either agent alone or even αCD3/αCD28 (Fig. S7). While we could not formally assess synergism[16, 17], these data indicate the potential for synergistic activity. For instance, at 24h in PBMC, romidepsin had minimal effect on multiply-spliced Tat-Rev and ingenol mebutate increased Tat-Rev by 6-fold, while the combination increased Tat-Rev by 14-fold. This effect was even more profound in CD4+ T cells at 24h, where the combination increased Tat-Rev by ~48-fold, compared to 4-fold for romidepsin and 12-fold for ingenol mebutate alone. These data reinforce the notion that LRA combinations may be more effective at reversing blocks to HIV transcription[18–21] and suggest a combination that can potently increase multiply-spliced HIV transcripts.

Discussion

Few studies have investigated the effect of LRAs on lymphoid reservoirs such as the gut, where most HIV-infected cells reside[4, 5]. We found that T cell activation can induce HIV expression in cells from both ileum and rectum, but that LRAs exert differential effects on cells from blood and gut.

Consistent with other studies that employ dissociated tissue cells or tissue cell lines[4, 22–24], we used dissociated total gut cells instead of intact biopsies. Our previous data demonstrate that tissue dissociation has no effect on HIV DNA levels and minimal effect on the HIV transcription profile[10]. Using 20% human AB serum, gut cell viability was maintained at

levels similar to those obtained immediately post-cell isolation, and the HIV transcription profile in the DMSO-treated cells resembled that observed previously from flash-frozen biopsies[10].

The functional definition of latent infection using *ex vivo* T cell activation has only been described in cells from blood[1–3] and lymph nodes[25]. We used anti-CD3/CD28 to determine whether T cell activation can induce HIV expression in infected gut cells. As in PBMC, anti-CD3/CD28 increased all HIV transcripts in cells from both ileum and rectum, suggesting that the gut harbors a population of HIV-infected cells in which HIV expression can be induced by activation. Given the lack of methods to perform viral outgrowth assays from gut cells, we were unable to determine whether activation induced release of infectious virions from gut cells. However, infectious virus has been cultured from gut biopsies without activation[26], and in some individuals, the virus that rebounds in the plasma after interruption of ART has been shown to match sequences from the gut[27]. Taken together, these findings suggest that the gut contains a very large population of HIV-infected cells in a latent-like state, representing a critical barrier to HIV cure.

The extent to which HIV expression in gut cells can be induced by anti-CD3/CD28 is somewhat surprising given the high frequency of gut cells that express surface proteins traditionally associated with activation, such as CD38 and HLA-DR[4, 7, 8]. It is possible that activation induces HIV expression in the rare gut cells without constitutive expression of these markers, that these markers are less indicative of activation in gut T cells, and/or or that “activated” gut T cells behave more like resting cells that can respond to activating stimuli. Future studies should investigate how gene expression differs between T cells from blood and gut that do or do not express these activation markers.

The ability of LRAs to induce HIV transcription differed between the blood and gut, suggesting differences in the molecular mechanisms that suppress HIV transcription in these two sites. Disulfiram caused a modest increase in HIV transcriptional initiation in PBMC but exerted no detectable effect in cells from ileum or rectum. The power to detect an effect may have been limited due to the low number of study participants and/or cell numbers, especially for the ileum. However, we observed no effect in rectal cells despite using the same number of study participants and cell numbers as in PBMC (for which HIV DNA levels are comparable to total gut cells), suggesting that disulfiram has less effect on rectal cells. Data from cell line and primary cell models suggest that disulfiram promotes intracellular depletion of PTEN protein to upregulate the protein kinase B/Akt signaling pathway and subsequently induce HIV transcription[28, 29]. The lesser effect of disulfiram on rectal cells suggest that this protein kinase B/Akt pathway may contribute less to the constitutive suppression of HIV transcription in the rectum. The generally modest effect of disulfiram may also explain why a prior trial with disulfiram induced a transient increase in plasma virus in only a small subset of participants with higher plasma disulfiram concentrations [30]. However, the increase in plasma viremia in these individuals does suggest that disulfiram reversed latency in some cells *in vivo*. It is possible that these are rare cells from blood or tissues that were not sampled in this study, or that there were differences in the study participants or the effects of disulfiram *in vivo*.

The HDACi romidepsin increased initiated, 5'elongated, and polyadenylated HIV transcripts in PBMC and gut cells, but caused less increase in HIV transcriptional initiation in gut cells. Romidepsin may increase HIV transcription by reversing epigenetic modification of the viral LTR or changing the expression of cellular genes that impact HIV transcription, including the protein kinase B/Akt pathway and downstream signaling components such as GSK-3β, mTOR, p70S6K, and 4E-BP1[31]. The observed effects of romidepsin in PBMC accord with results from some clinical trials[32–34], but differ from some *in vitro* studies using CD4+ T cells, which generally showed less induction of polyadenylated HIV RNA[9, 35]. The fact that romidepsin increased HIV transcriptional initiation in both blood and gut cells suggests that histone deacetylation may contribute to a reversible block to HIV transcription in both sites. However, romidepsin caused less increase in HIV transcriptional initiation in gut cells, suggesting that histone deacetylation contributes less to suppression of HIV transcriptional initiation in the gut and is not the main mechanism responsible for the lower levels in gut cells[10]. These data accord with observations from a clinical trial with the HDACi vorinostat, which caused less of an increase in unspliced HIV RNA in cells from the rectum compared to blood[13].

The PKC agonist ingenol mebutate (ingenol-3-angelate, PEP005) increases HIV transcription by inducing the pS643/S676-PKCδ/θ-IκBα/ε-NF-κB signaling pathway. In contrast to results from *in vitro* treatment of CD4+ T cells[9], ingenol mebutate showed little effect on PBMC from most individuals; this discrepancy could reflect differences in the cell types, study participants, and/or methods. Despite the lack of effect in PBMC, ingenol mebutate increased initiated, 5'elongated, and polyadenylated HIV transcripts in one or both gut sites, in accordance with results from skin biopsies of patients treated with topical ingenol mebutate[36]. The greater effect of ingenol mebutate on gut compared to blood cells suggests that the PKC pathway contributes more to suppressing HIV transcription in the gut.

Tissue-specific differences CD4+ T cell subsets could also contribute to differential effects of LRAs. Compared to the blood, where naïve (T_N) and central memory (T_{CM}) cells are more frequent and T_{CM} contain a large fraction of the HIV DNA[37], effector memory (T_{EM}) and transitional memory (T_{TM}) cells predominate in the ileum and rectum of both HIV- and ART-suppressed HIV+ individuals[7] and contain most HIV DNA[38, 39]. T_{CM} and T_{TM} are more efficiently reactivated by ingenol mebutate than agents such as romidepsin, panobinostat, JQ1, and bryostatin-1, while romidepsin increases the proportion of cells transcribing HIV RNA in T_{CM}, T_{EM}, T_{TM}, and T_N[21]. Consequently, differing cell compositions within the gut and blood could contribute to tissue-specific responses to LRAs.

Given the project start date and constraints regarding cell numbers and drug availability, we did not test some of the newer LRAs, such as TLR7/9 agonists, immune checkpoint blockers, galectin 9, inducers of histone crotonylation, SMAC mimetics (birinipant), or birinapant with ingenol mebutate[12, 40]. Furthermore, our transcription profiling approach does not discriminate between defective and intact proviruses, so we cannot conclude that the induction of transcription comes from cells harbouring replication-competent virus. The fraction of the total HIV reservoir that can be reactivated to produce virions by LRAs ranges from 1.5%[41, 42] to 31%[21]. Considering only 2–10% of proviruses are genetically-

intact[43, 44], a large proportion of detected transcripts may originate from defective proviruses.

The ability to induce expression of polyadenylated and multiply-spliced transcripts is likely essential for effective latency reversal and killing of HIV-infected cells. No single LRA consistently increased multiply-spliced Tat-Rev transcripts in PBMC or gut cells. These results are not completely explained by proviral defects in Tat or Rev, since activation increased Tat-Rev in cell aliquots from the same participants. While the induction of plasma viremia in some individuals treated with disulfiram[30] and romidepsin[32] suggests that these agents may reverse latency in at least some cells from some individuals, our results suggest that these LRAs may not reverse blocks to splicing in most infected cells from blood or gut, which may explain their limited effects in reversing latency and reducing HIV DNA or latently-infected cells in vivo.

Although the yield of gut cells was insufficient for testing combinations of LRAs, the combination of romidepsin and ingenol mebutate increased multiply-spliced Tat-Rev in PBMC and CD4+T cells. HDACi have been shown to synergize with PKC agonists in inducing unspliced HIV RNA *in vitro*[19] and could potentially inhibit deleterious pro-inflammatory cytokines that are induced by PKC agonists[45, 46]. A recent study demonstrated that the combination of ingenol mebutate (100nM) and romidepsin (40nM) induced the highest frequency of cells expressing HIV RNA and protein[21]. Although we used an 8-fold lower concentration of ingenol mebutate (12nM), the combination of ingenol mebutate plus romidepsin induced multiply-spliced Tat-Rev in blood cells more than either agent alone. Since HDACi may have more effect on blood cells and PKC agonists may have more effect on gut cells, future studies should investigate whether this combination can induce multiply-spliced HIV transcripts and/or reduce HIV DNA in both blood and gut. However, clinical use of PKC agonists may be limited by bioavailability and/or side effects.

More effective and tolerable LRAs or combinations are likely required to elicit sufficient reactivation of latent proviruses in blood and tissues, and may need to be combined with other therapies to augment killing of HIV-infected cells. Findings from this study and others suggest that it will be critical to evaluate the efficacy of these agents in reversing the blocks to HIV transcriptional completion/splicing and reducing infectious proviruses in both blood and tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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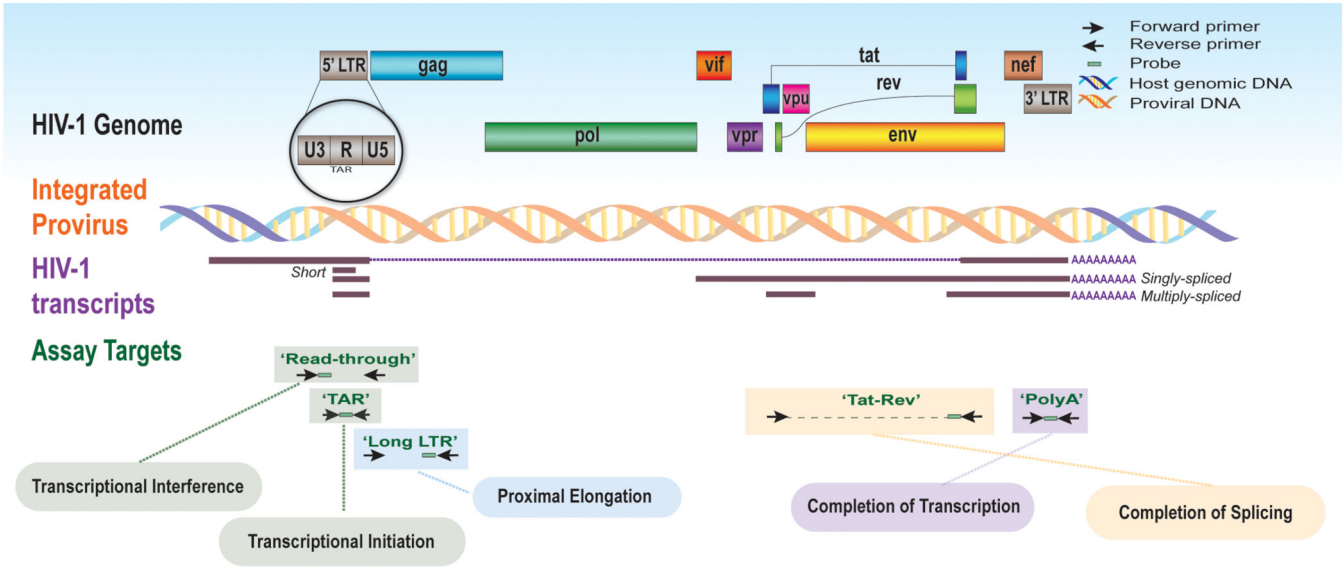

Figure 1. The HIV genome and the targets for transcription profiling assays.

This schematic shows the genetic organization of proviral HIV DNA and the HIV 'transcription profiling' assays targeting specific RNA sequence regions that provide insight into blocks to transcription.

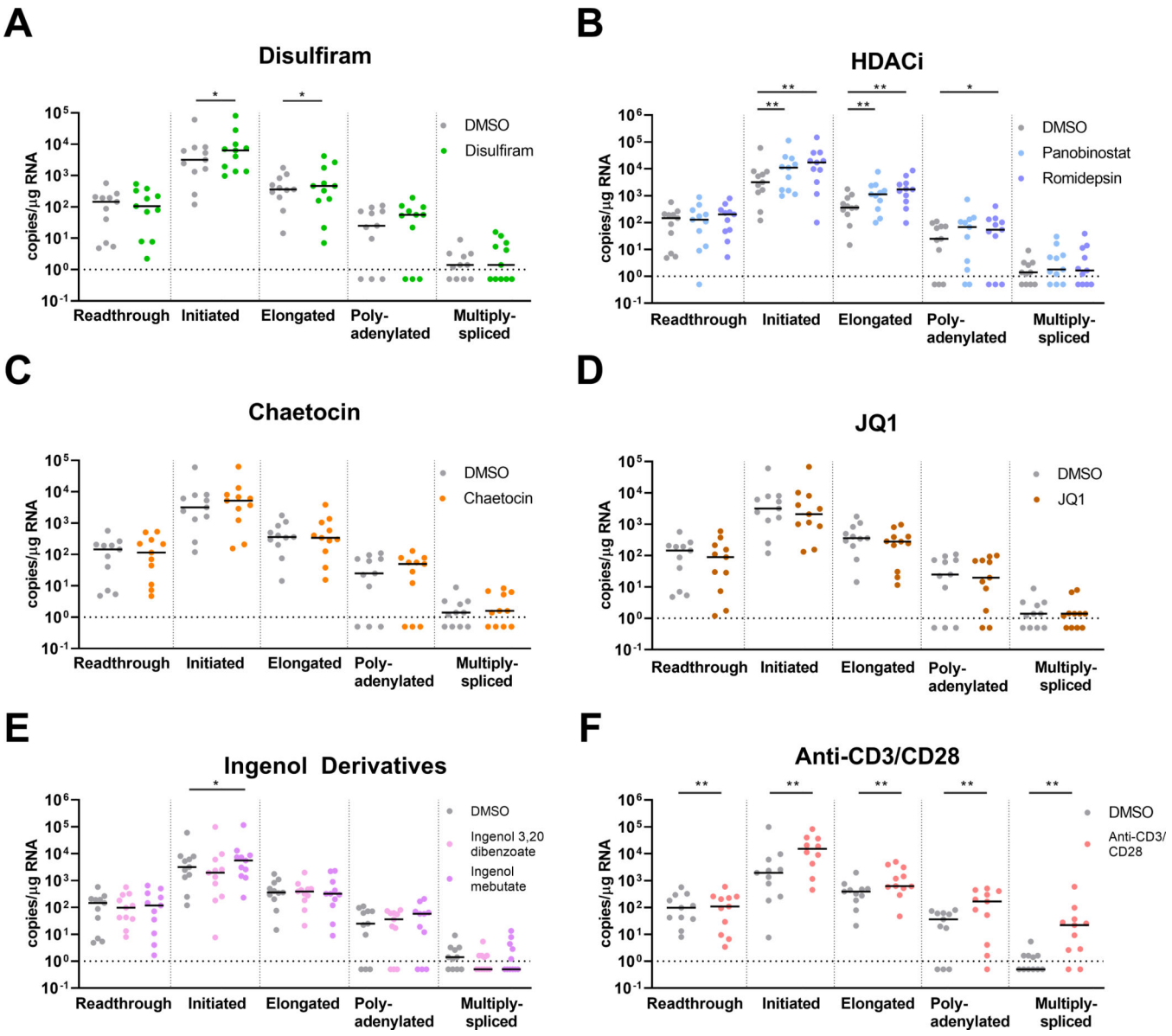

Figure 2. Effect of activation and LRAs on PBMC from ART-suppressed individuals.

PBMC (5×10^6 cells/condition; n=11 participants) were treated for 24h with DMSO alone or **(A)** disulfiram (1µM); **(B)** HDAC inhibitors, panobinostat (30nM) and romidepsin (40nM); **(C)** chaetocin (50nM); **(D)** JQ1 (2µM); **(E)** ingenol derivatives, ingenol 3,20 dibenzoate (20nM) and ingenol mebutate (12nM); or **(F)** αCD3/αCD28 antibodies. HIV RNA levels were measured using RT-ddPCR[9] and normalized to cellular RNA input (copies/µg RNA).

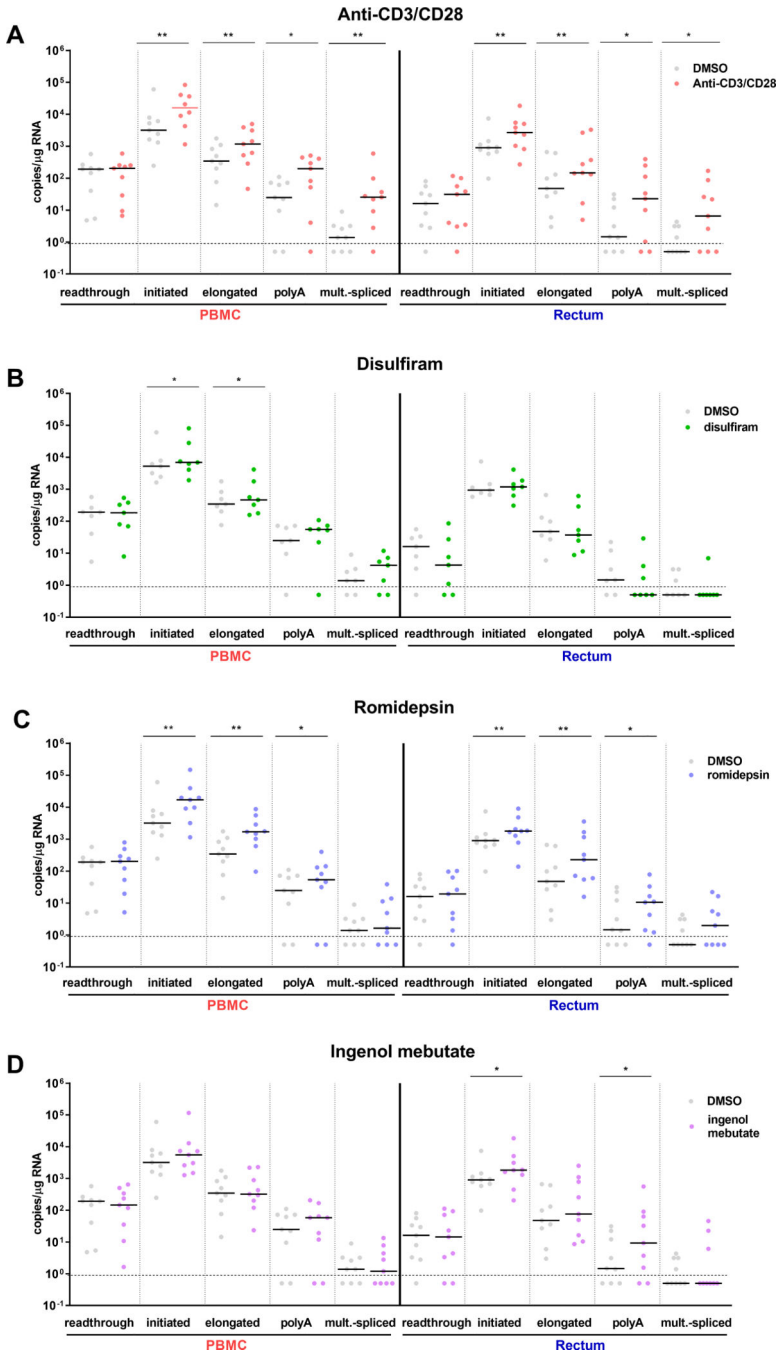

Figure 3. Effect of activation and LRAs on PBMC vs. total rectal cells.

Matched PBMC and rectal cells were treated with DMSO or (A) αCD3/αCD28, n=9; (B) disulfiram, n=7; (C) romidepsin, n=9; and (D) ingenol mebutate, n=9. HIV RNA levels were normalized to copies/µg RNA. * denotes P<0.05 and ** denotes P<0.01.

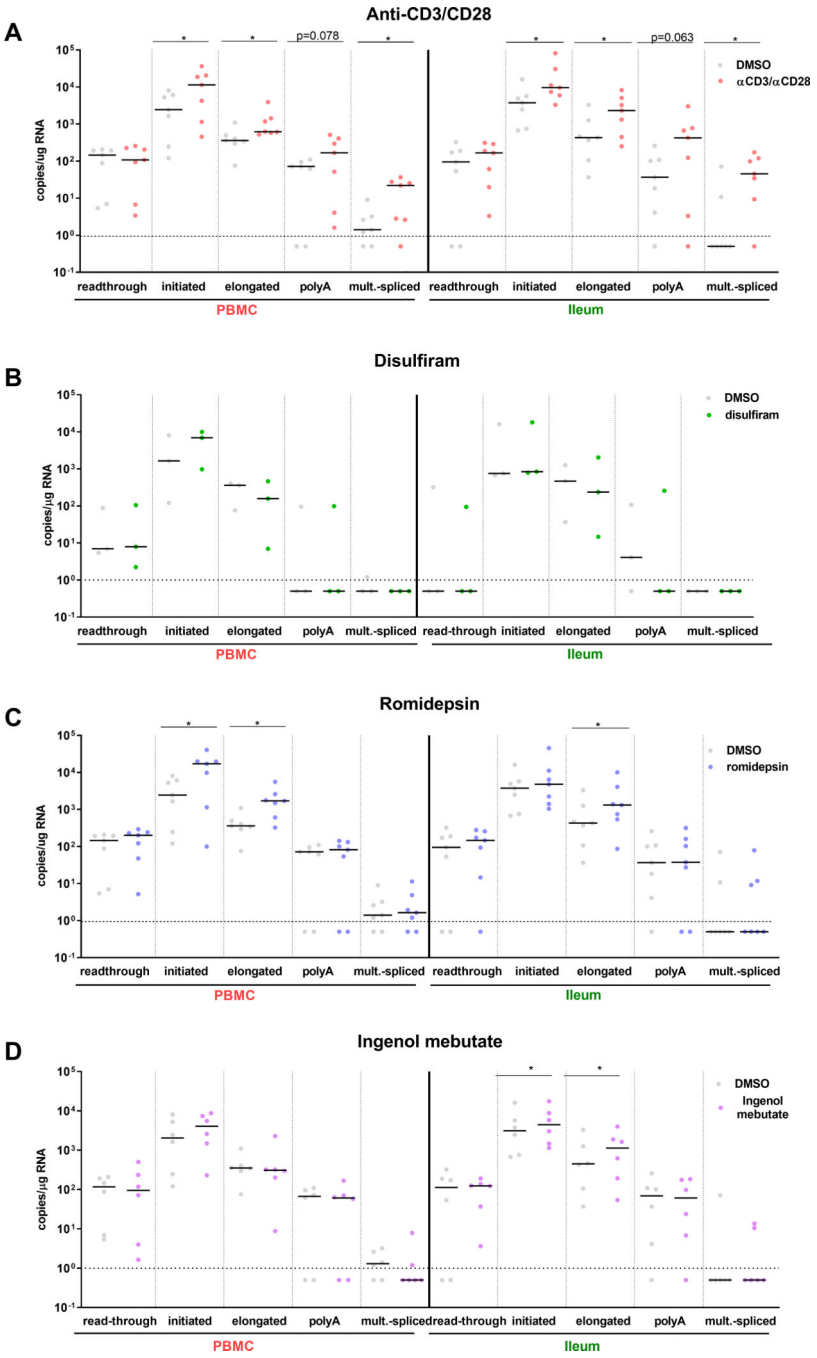

Figure 4. Effect of activation and LRAs on PBMC vs. total ileal cells.

Matched PBMC and ileal cells were treated with DMSO or (A) αCD3/αCD28, n=7; (B) disulfiram, n=3; (C) romidepsin, n=7; and (D) ingenol mebutate, n=6. HIV RNA levels were normalized to copies/µg RNA. * denotes P<0.05.

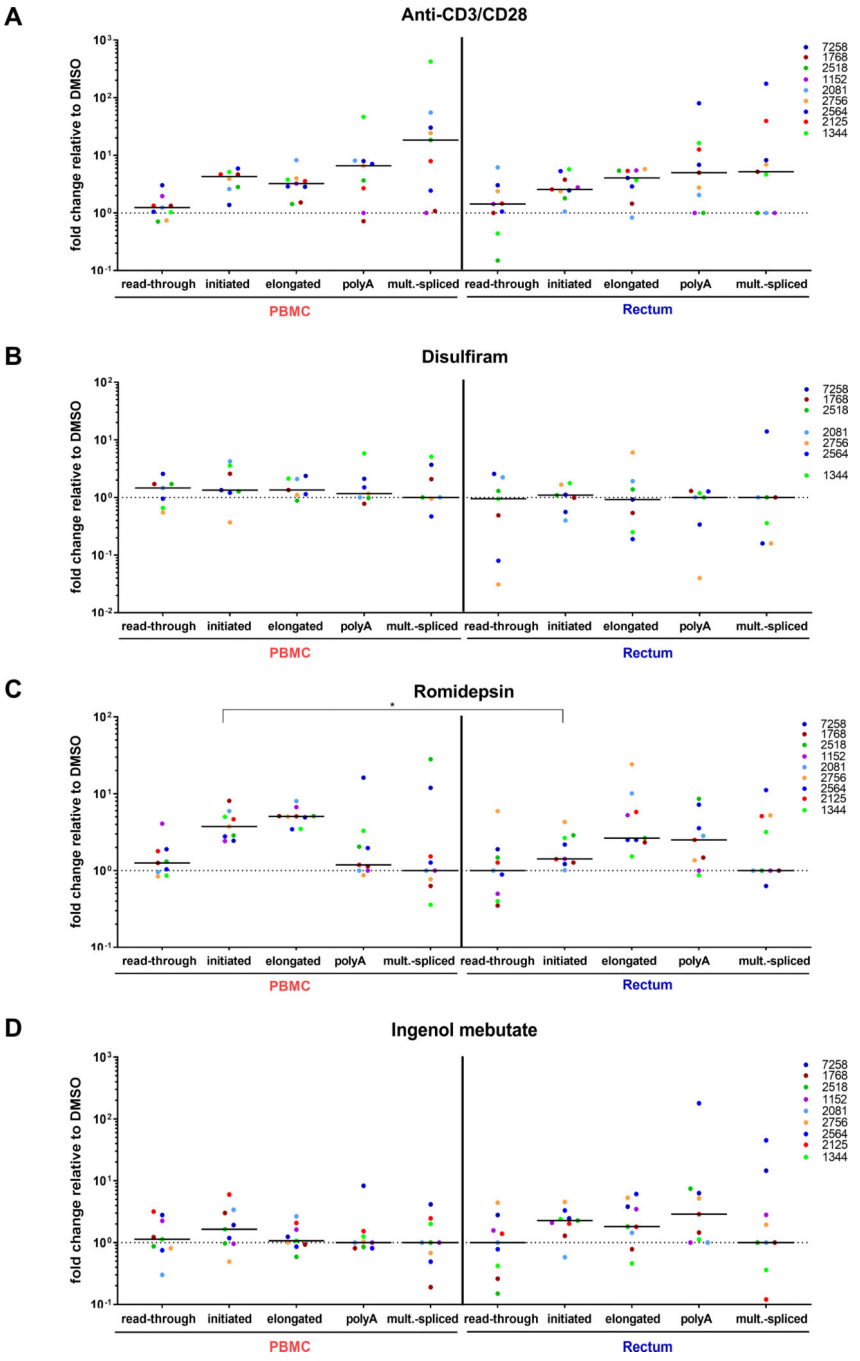

Figure 5. Fold change in HIV transcripts relative to DMSO in PBMC vs. total rectal cells.

The fold changes in HIV transcripts (relative to DMSO) are plotted for matched PBMC and total rectal cells treated with (A) αCD3/αCD28, n=9; (B) disulfiram, n=7; (C) romidepsin, n=9; and (D) ingenol mebutate, n=9. * denotes P<0.05. Colors denote individual participants. Dotted line represents baseline (DMSO; fold change =1).