# Characterization of the HIV-1 Transcription Profile after Romidepsin Therapy *in vivo*

**Sara Moron-Lopez**[1,2], Peggy Kim[2], Ole S. Søgaard[3], Martin Tolstrup[3], Joseph K. Wong[1,2], Steven A. Yukl[1,2]

1. University of California San Francisco, San Francisco, CA, USA; 2. San Francisco VA Medical Center, San Francisco, CA, USA; 3. Aarhus University Hospital, Aarhus, Denmark

**Contact:**
**Steven A. Yukl**
steven.yukl@ucsf.edu
4150 Clement Street
San Francisco, CA, 94121
+1(415)221-4810

**#397**

## Introduction

Antiretroviral therapy (ART) cannot eliminate the HIV genomes integrated in latently infected cells, which are a major barrier to cure HIV [1-3].

One strategy to eradicate HIV consists of reactivating viral transcription with latency-reversing agents (LRAs), such as histone deacetylase inhibitors (HDACi).

A recent clinical trial, REDUC part B, analyzed the administration of the therapeutic HIV vaccine Vacc-4x and rhuGM-CSF as local adjuvant, in combination with the HDACi romidepsin. This approach showed an increase in unspliced cell-associated HIV RNA and residual plasma viremia after romidepsin infusions, along with a reduction in total HIV DNA [4]. However, the mechanism by which romidepsin reverses HIV latency *in vivo* remains unclear.

**AIM: To characterize the HIV transcription profile before and after romidepsin therapy in available samples from the REDUC part B study.**

## Methods

### Study design and samples

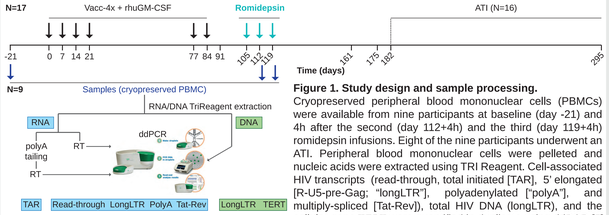
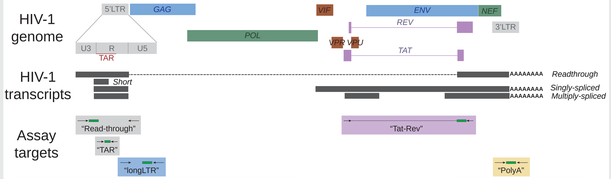



**Figure 1. Study design and sample processing.**
Cryopreserved peripheral blood mononuclear cells (PBMCs) were available from nine participants at baseline (day -21) and 4h after the second (day 112+4h) and the third (day 119+4h) romidepsin infusions. Eight of the nine participants underwent an ATI. Peripheral blood mononuclear cells were pelleted and nucleic acids were extracted using TRI Reagent. Cell-associated HIV transcripts (read-through, total initiated [TAR], 5' elongated [R-U5-pre-Gag; "longLTR"], polyadenylated ["polyA"], and multiply-spliced [Tat-Rev]), total HIV DNA (longLTR), and the cellular gene TERT were quantified in duplicate using ddPCR [5].

### Blocks to HIV transcription and HIV transcription profile




**Blocks to initiation**
- Integration into transcriptionally-silent regions
- Epigenetic modifications
- Lack of host initiation factors
- Lack of viral factors (e.g. Tat)
- Transcriptional interference

**Blocks to elongation**
- Lack of host elongation factors
- Presence of inhibitory host factors
- Nucleosomes
- Lack of viral factors (e.g. Tat)

**Blocks to completion**
- Lack of host distal elongation factors
- Lack of 3' end processing (e.g. polyadenylation)

**Blocks to post-transcriptional modifications**
- Lack of or aberrant splicing
- Lack of nuclear export
- RNA interference

**"Read-through"**
- Suggests transcriptional interference

**"longLTR"**
- Proximal elongation

**"PolyA"**
- Completion of transcription
- Surrogate for HIV protein

**"Tat-Rev"**
- Completion of splicing
- Potential to overcome blocks to initiation, elongation, and export
- Surrogate for productive infection

**"TAR"**
- Found in all HIV transcripts
- Initiation of transcription
- >2-fold excess over longLTR suggests inhibition of elongation

**Figure 2. Blocks to HIV transcription and assays to investigate the HIV transcription profile.**
Diagram of the blocks that regulate HIV transcription and assays used to characterize the HIV transcription profile.

## Results

### Romidepsin increases read-through, total, elongated, and polyadenylated but not multiply-spliced transcripts

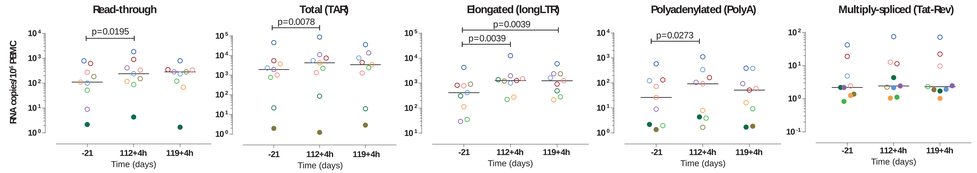



**Figure 3. HIV transcription profile before and after romidepsin therapy.**
Dynamics of each HIV transcript per million PBMC at baseline (day 0) and 4 hours after the second (day 112+4h) and the third (day 119+4h) romidepsin infusions.
Each color represents a different individual from the REDUC part B study. Determinations below the limit of quantification (LOQ) are represented as solid dots.

### Romidepsin increases elongation but not completion or multiple-splicing

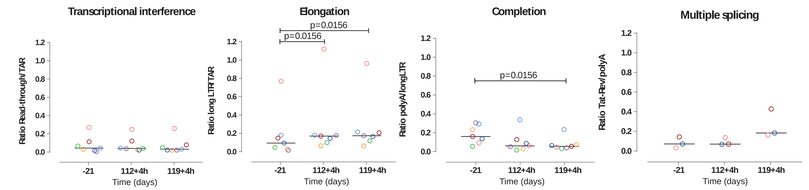



**Figure 4. Blocks to HIV transcription before and after romidepsin therapy.**
Changes in:
transcriptional interference (read-through/total transcripts),
elongation (elongated/total transcripts),
completion (polyadenylated/elongated transcripts), and
multiple-splicing (multiply-spliced/polyadenylated transcripts)
at baseline (day 0) and 4 hours after the second (day 112+4h) and the third (day 119+4h) romidepsin infusions.
Each color represents a different individual from the REDUC part B study.

### HIV DNA and elongated transcripts predict time to viral rebound

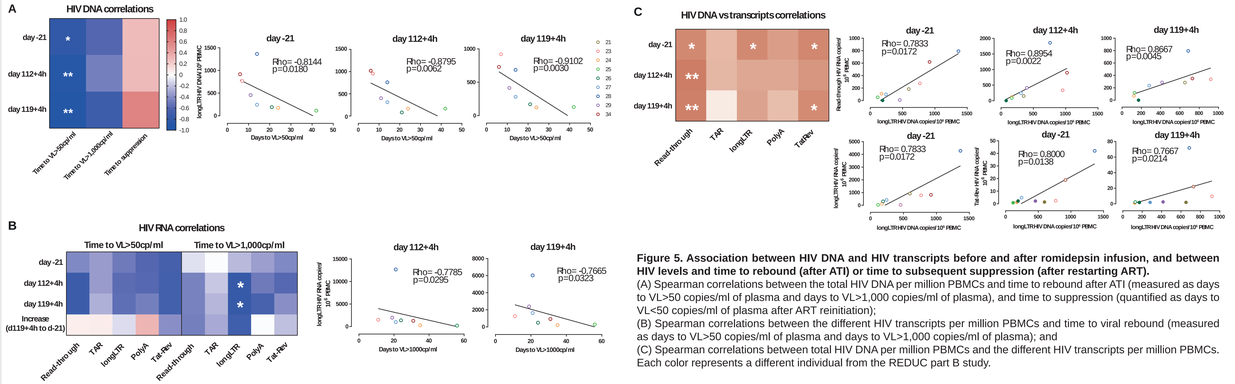



**Figure 5. Association between HIV DNA and HIV transcripts before and after romidepsin infusion, and between HIV levels and time to rebound (after ATI) or time to subsequent suppression (after restarting ART).**
(A) Spearman correlations between the total HIV DNA per million PBMCs and time to rebound after ATI (measured as days to VL>50 copies/ml of plasma and days to VL>1,000 copies/ml of plasma), and time to suppression (quantified as days to VL<50 copies/ml of plasma after ART reinitiation);
(B) Spearman correlations between the different HIV transcripts per million PBMCs and time to viral rebound (measured as days to VL>50 copies/ml of plasma and days to VL>1,000 copies/ml of plasma); and
(C) Spearman correlations between total HIV DNA per million PBMCs and the different HIV transcripts per million PBMCs.
Each color represents a different individual from the REDUC part B study.

### Detection of different TAR sequences after romidepsin infusion

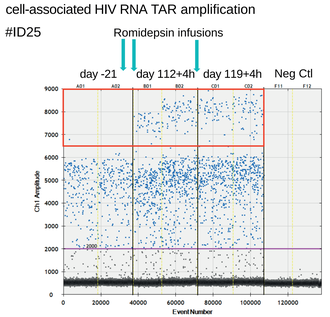



**Figure 6. Droplet digital PCR (ddPCR) plot showing the detection of initiated HIV transcripts (TAR RNA) with different amplitudes (likely different sequences) after romidepsin infusion.**
One dimension ddPCR plot corresponding to the amplification of HIV RNA TAR sequences from participant #ID25 in channel 1 (FAM).
The plot represents the droplet fluorescence amplitude detected at baseline (day 0) and 4 hours after the second (day 112+4h) and the third (day 119+4h) romidepsin infusions.
Red square highlights the cloud of droplets with higher fluorescence amplitude, detected after romidepsin therapy.

## Conclusions

1. After romidepsin infusions, we observed:
   - Reactivation of transcriptionally silent proviruses (Fig. 6),
   - An increase in HIV transcriptional initiation and especially elongation, but not completion or multiple splicing (Fig. 3-4),
   - An inverse correlation between time to rebound after ATI and levels of both total HIV DNA and elongated HIV RNA (Fig. 5).

2. Romidepsin may play a role in strategies to reverse latency, but new approaches are needed to increase HIV transcriptional completion and multiple splicing, which are likely necessary for productive infection and immune recognition/killing of HIV-infected cells.

3. Therapies that increase HIV transcription but do not lead to killing of infected cells may actually shorten time to rebound after ATI.

### Limitations

1. The parent study had sequential study interventions (vaccination and then romidepsin), and we did not have access to samples between vaccination and romidepsin.
2. Samples were available from only 9 of 17 trial participants, of whom only 1 had an increase in viral load after romidepsin.
3. The presence of non-B subtypes may have affected HIV levels and detection frequencies.

### References

1. Chun TW et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. PNAS (1997).
2. Wong JK et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science (1997).
3. Finzi D et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science (1997).
4. Leth S 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).
5. Yukl S et al. HIV latency in isolated patient CD4+T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing. STM (2018).

### Acknowledgments

This work was supported by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (1R01AI132128, R56AI116342, R33AI116218, R56AI091573, U19AI096109), the National Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health (1R01DK108349), the U.S. Department of Veternas Affairs (IK2 CX000520, I01 BX000192), and the American Foundation for AIDS Research (amfAR) Institute for HIV Cure Research (109301).