HIV Transcription Profile in Blood, Gut, Liver, and Genital Tract in Suppressed Women

Sara Moron-Lopez[1,2], Guorui Xie[1,3], Peggy Kim[2], Joseph K. Wong[1,2], Jennifer Price[1], Najwa Elnachef[1], Ruth Greenblatt[1], Phyllis Tien[1,2], Nadia Roan[1,3], 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] Gladstone Institutes, San Francisco, CA, USA

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BACKGROUND

- HIV persists in primary and secondary lymphoid tissues [1].
- Higher frequency of infected CD4+ T cells in gut compared to blood [1,2]
- Gut and blood differ in the levels of unspliced HIV transcripts [1,2]
- HIV latency is regulated by blocks to elongation, completion and multiple splicing in blood [3]
- Gut and blood differ in constitutive blocks to HIV transcription [4]

However, most of these studies have been performed in HIV-infected men and it is known that sex-specific differences affect various aspects of HIV infection and progression [5,6]

AIM: To characterize the HIV reservoir and the transcription profile in blood, liver, gut and genital tract from HIV-infected ART-suppressed women

METHODS

Sample processing and parameters studied

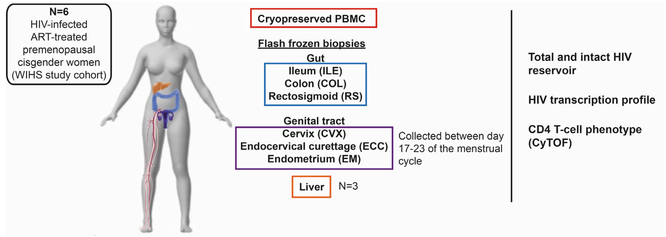

Figure 1. Participants, collected samples and parameters studied.
We analyzed cryopreserved PBMC and flash frozen biopsies from gut (ileum, colon and rectosigmoid), genital tract (cervix, endocervical curettage and endometrium) and liver from 6 HIV-infected ART-suppressed premenopausal women (WIHS study cohort).
We quantified total and intact HIV reservoir and the HIV transcription profile by ddPCR (Yukl lab) and the CD4 T-cell phenotype by CyTOF (Roan lab).

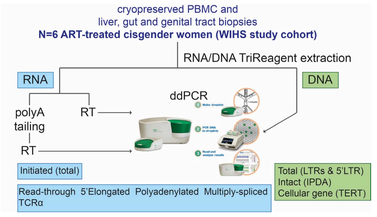

Figure 2. Sample processing for HIV quantification.
Nucleic acids from cryopreserved PBMC, and flash frozen biopsies from liver, gut (ileum, colon and rectosigmoid) and genital tract (cervix, endocervical curettage and endometrium), were extracted using TRI Reagent. Cell-associated HIV transcripts (read-through [U3-U5], initiated (total) [TAR], 5'elongated [R-U5-preGag], polyadenylated [PolyA], and multiply-spliced [Tat-Rev]), total HIV DNA (5'LTR [R-U5-preGag] and LTRs [U3-U5]), intact HIV DNA (IPDA), and the cellular gene TERT were quantified using ddPCR [3,7].

HIV transcription profile

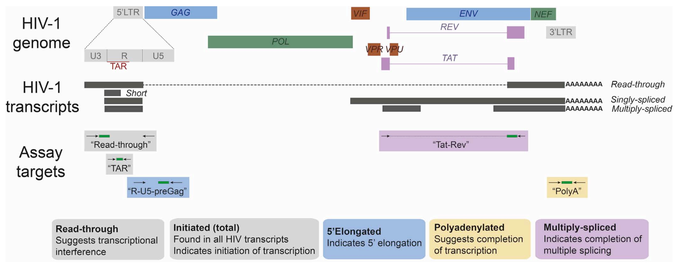

Figure 3. Assays to investigate the HIV transcription profile.
Diagram of the assays used to characterize the HIV transcription profile.

These results suggest *tissue-specific differences in the mechanisms that govern HIV latency*, with **greater suppression of HIV transcription in most tissues than blood**

RESULTS

1. Characteristics of study participants

	HIV-infected ART-treated cisgender women (n = 6)
Time since HIV diagnosis (years, median[IQR])	15.2 [9.3-21.1]
Time on ART (years, median[IQR])	11.9 [8.1-17.2]
PI+2NRTI based ART (n[%])	3 [50]
InI+2NRTI based ART (n[%])	3 [50]
Viral load (HIV-1 RNA cp/ml plasma, median[IQR])	35 [20-81]
Absolute CD4 count (cell/mm^3, median[IQR])	938 [731-1,046]
CD4/CD8 ratio (median[IQR])	1.0 [0.8-1.3]

2. The gut, genital tract and liver are all sites of HIV persistence in women

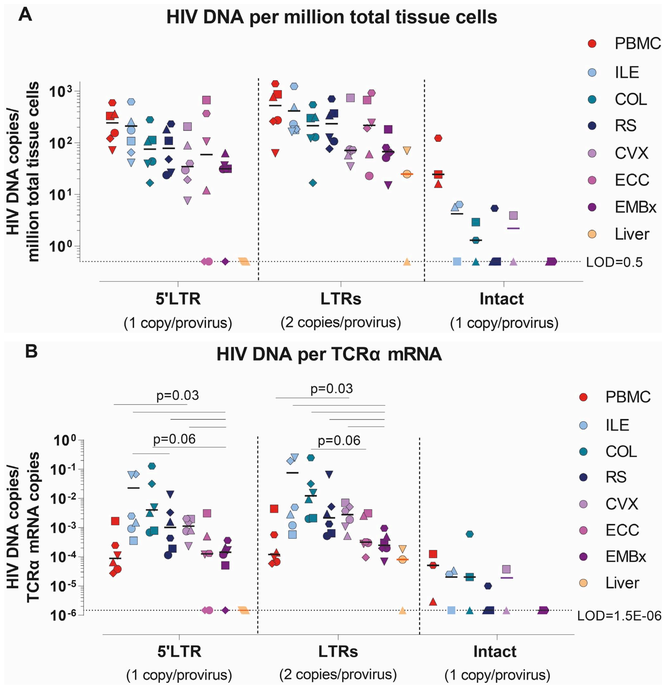

Figure 4. Total and intact HIV reservoir. Level of total (5'LTR and LTRs) and intact (IPDA) HIV DNA **(A)** per million total tissue cells, **(B)** per expression of TCRα mRNA. Colors represent different tissues and symbol shapes different participants. Individual values and median are shown.

3. HIV-infected cells in the blood and endometrium showed higher levels of HIV transcription than gut, cervix and liver

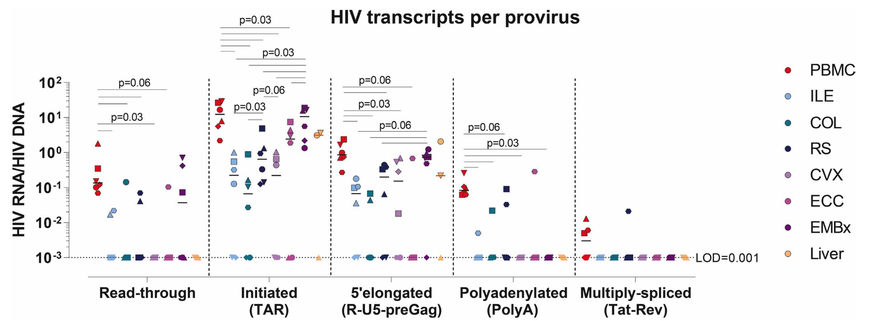

Figure 5. HIV transcription profile.
Level of HIV transcripts per provirus in PBMC and ileum, colon, rectosigmoid, cervix, endocervical curettage, endometrium and liver biopsies. Colors represent different tissues and symbol shapes different participants. Individual values and median are shown. Results were analyzed using the Wilcoxon signed-rank test..

4. Total and memory CD4+ T-cell phenotypes differ between the blood, gut and endometrium

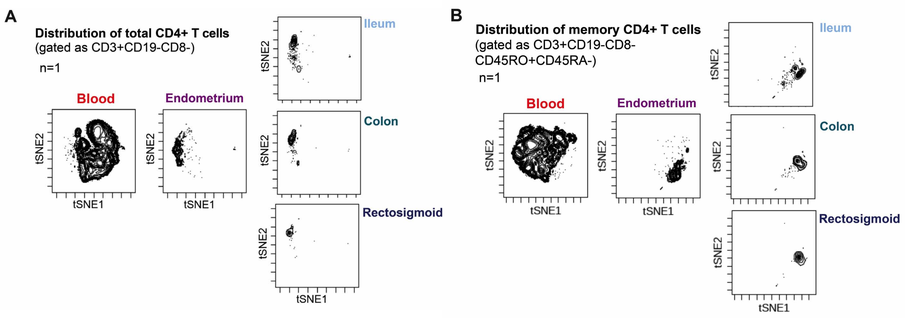

Figure 6. Total and memory CD4+ T-cell phenotypes by CyTOF.
tSNE (t-distributed Stochastic Neighbor Embedding) plots represent the 2-dimensional distribution of total **(A)** and memory **(B)** CD4+ T-cell phenotypes in PBMC and endometrium, ileum, colon and rectosigmoid biopsies.

CONCLUSIONS

- The **gut, genital tract and liver** are **all sites of HIV persistence in women**
- The **female genital tract contains a large pool of HIV-infected cells**, similar to the gut-associated lymphoid tissue
- **HIV-infected cells in the blood and endometrium showed higher levels of HIV transcription** than those in gut, cervix and liver
- **Total and memory CD4+ T-cell phenotypes differ between blood and endometrium**. Therefore, blood contamination in endometrium does not account for the similarities observed in HIV transcription

Limitations
1. Low number of study participants - Preliminary results that have to be confirmed in a larger cohort
2. HIV DNA and RNA levels were measured in total tissue cells instead of in CD4 T cells - Fewer infected cells, which may lead to imprecision in the data

Future directions
1. Immunophenotyping of tissue cells by CyTOF (Roan lab)
2. Measure frequency and phenotype of HIV DNA+ and HIV RNA+ cells in tissues (IHC, DNA/RNA scope; Hunt lab)
3. Consider additional virological studies on sorted CD4+ T cells from blood and tissues

References
1. Wong Curr Opin HIV AIDS (2016); 2. Yukl JID (2010); 3. Yukl STM (2018); 4. Telwatte PLoS Path (2018); 5. Griesbeck Clin Science (2016); 6. Scully Curr HIV/AIDS Rep (2018); 7. Bruner 2019 Nat

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Contact:
Name: Sara Moron-Lopez
Address: 4150 Clement St
San Francisco, CA 94121
Email:
sara.moronlopez@gmail.com;
sara.moron-lopez@ucsf.edu