HIV-1 Antisense RNA Is Detected In Infected Cells In Vivo

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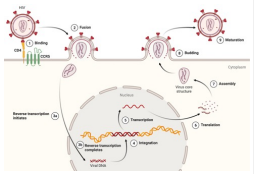
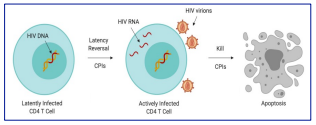
Background

Globally, 1.3 million people were newly infected with Human Immunodeficiency Virus Type 1 (HIV-1) in 2022. The HIV pandemic has resulted in 40.4 million AIDS - related deaths to date.

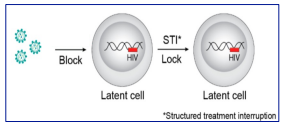
Efforts to develop a cure for HIV are underway. Two possible approaches towards a cure are: 1) The **Kick and Kill** approach attempts to reactivate latent proviruses to stimulate the to immune system and kill infected cells, and 2) The **Block and Lock** model aims to permanently silence proviruses via transcriptional machinery to prevent virus production from infected cells.



Kick and Kill

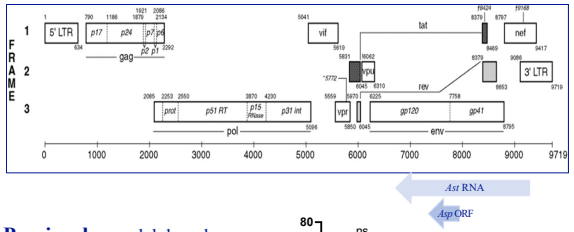


Block and Lock



Natural Antisense Transcript (Ast) Encoded in HIV-1

Studies have shown the expression of *Ast* in a number of HIV-1 expressing cell lines. Ast RNA has been demonstrated to induce viral latency in vitro.



Previously, our lab has shown that a median of 26 copies of Anti-Sense Transcripts (*Ast*) per 100 infected cells were detected to expressed *Ast* at a given point in time, similar to the fraction of sense env HIV-1 transcripts. This finding confirmed the presence of *Ast* in people living with HIV-1 (PLWH) and suggests its potential role as a regulatory RNA.

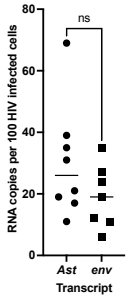


Figure 1: Levels of *Ast* and env transcripts. Comparison of levels of *Ast* and env transcript expression from 4 donors with longitudinal sampling. Mann-Whitney (ns, not significant)

Non-controllers: Limited natural control of HIV-1 replication without Antiretroviral Therapy (ART) (90%).
Viremic controllers: Naturally controls viral replication with 50-2000 HIV-1 RNA copies/mL of plasma without ART (<5%).
Elite controllers: Naturally controls viral replication with < 50 HIV-1 RNA copies/mL of plasma without ART (<5%).

We hypothesize that there are higher levels of *Ast* in natural controllers of HIV-1 replication compared to non-controllers, suggesting that *Ast* expression is a mechanism of virologic control.

Rationale: If *Ast* promotes a "Block and Lock" strategy, HIV viral transcription will be suppressed.

Aims of Study:

- To investigate the association between the levels of plasma viremia and levels of *Ast*.
- To measure levels of *Ast* in chronically untreated people living with HIV-1 with varying levels of natural virologic control.

Methodology

1. Collect PBMC from PLWH
2. RNA isolation & DNase treatment
3. cDNA synthesis & RNase H treatment
4. Precipitate with EtOH/NaOAc, resuspend in Tris-HCL
5. Endpoint dilute cDNA
6. Prepare digital PCR mix with primers and probe targeting HIV-1 Ast
7. Perform *Ast* digital quantitative PCR

Figure 2: The steps of the *Ast* detection and quantification in patient samples. PCR is performed at an endpoint for *Ast* molecules. The denominator is the total # of infected cells assayed. The numerator is the # of infected cells that contain detectable *Ast*.

Preliminary Results

Analysis of Ast from ACH-2 cell control

Mann-Whitney two-tailed test indicated $p < 0.0001$

Median RT+ 0.92 *Ast* molecules/100 infected cells

Median RT- 0.04 *Ast* molecules/100 infected cells

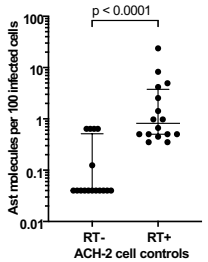


Figure 3: Comparison of *Ast* levels in RT+ experimental group and RT- control group of ACH2. RT- are experimental wells that excluded the reverse transcriptase (RT) enzyme from the cDNA synthesis. The purpose of the RT- wells is to ensure that DNase treatment was complete. In our ACH-2 cell model control for HIV latency, there were rare positives in the RT- wells, indicating that very few molecules of HIV DNA remained. The number of RT+ wells that were positive was significantly higher than the RT- demonstrating that *Ast* is present in some infected ACH-2 cells, likely contributing to HIV-1 latency in this tissue culture model.

Detectable levels of Ast in Donors (Pre- and Post- ART)

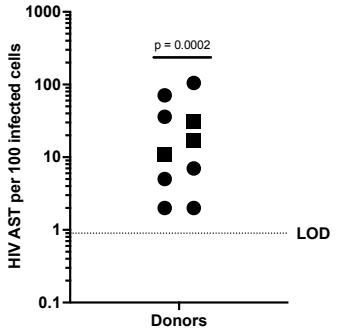


Figure 4 : Detection of HIV AST. Digital PCR measurement of HIV AST copies per 100 infected PBMC. Untreated donor (circles), treated donors (squares), not detected (open-shapes).

Conclusions

- Digital PCR assay can detect and quantify levels of *Ast* in HIV-1 infected cells
- *Ast* may be expressed in PLWH and leads to the question of whether HIV-1 *Ast* expression contributes to viral latency.
- Overexpression of *Ast* might lead to a "block and lock" strategy of a HIV-1 cure.
- These are preliminary results show that HIV-1 *Ast* was detected in one PLWH to date. Testing more infected cells and donors is essential.

Future Steps

- Digital PCR assays must be performed on more donor samples and cells from each group.
- *Ast* detection in unstimulated cells in donors on *Ast* regimen supports the investigation of its role as a regulatory RNA *in vivo*. Therefore, *Ast* expression in donors on ART must be studied.
- Little is known concerning the role of CD4+ cells with HLA-DR play in maintaining persistent HIV-1 during effective antiretroviral therapy (ART).
-To address this issue, we will examine cellular activation/exhaustion markers by sorting patient samples into DR+ and DR- specifically looking into patient in acute infection state, patient on short term ART and patient on long-term ART.

Acknowledgements and References

We thank the Office of AIDS Research, the Center for Cancer Research, and the National Cancer Institute for funding this research. We are very grateful to the donors for participating in our study. We also thank Susan Toms, Valerie Turnquist, and Terri Burdette for administrative support and John Coffin, John Mellors, Fabio Romerio, and Wei Shao for consultation.

Puronen CE, Ford ES, Uldrick TS. Immunotherapy in People With HIV and Cancer. Front Immunol. 2019 Aug 28;10:2060. doi: 10.3389/fimmu.2019.02060. PMID: 31555284; PMCID: PMC6722204.