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

Sachi Pathak1, Tolulope Famuyiwa1, Rachel Šklutuši1, Jennifer L. Groebner1, Rebecca Höh2, Steven G. Deeks2, Jason W. Rausch1, John W. Mellors3, Adam A. Capoferri1, Mary F. Kearney1

1HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD, USA
2University of California San Francisco, San Francisco, CA, USA
3Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

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 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.

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 Antisense 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.

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

Collect PBMC from PLWH

- RNA isolation & DNase treatment
- cDNA synthesis & RNase H treatment
- Precipitate with ETOH/NaOAc, resuspend in Tris-HCl
- Endpoint dilute cDNA
- Prepare digital PCR mix with primers and probe targeting HIV-1 Ast
- Perform Ast digital quantitative PCR

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 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)

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 Art regimen supports the investigation of Ast 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 expression 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.

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