HIV Dynamics and Replication Program
NCI-Frederick

In vivo detection of HIV-1 antisense transcripts in donors before and during ART

313

Adam A. Capoferri[1], Toluleke O. Famuyiwa[1], Rachel Sklutuis[1], Sachi Pathak[1], Jennifer L. Groebner[1], Rui Li[2], Jason W. Rausch[1], Steven G. Deeks[3], John W. Mellors[4], John M. Coffin[5], Fabio Romerio[2], Mary F. Kearney[1]

[1]National Cancer Institute, Frederick, Maryland, USA, [2]The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, [3]University of California San Francisco, San Francisco, CA, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA, [5]Tufts University, Boston, MA, USA

BACKGROUND

- Natural antisense transcripts function primarily in regulating sense gene expression.
- Studies of HIV-1 expression of antisense transcripts (AST) *in vitro* suggest it is driven by a Tat-independent negative sense promoter in the 3' LTR.
- Cell line studies indicate AST promotes HIV latency through recruitment of the Polycomb Repressor Complex 2 (PRC2) to the 5' LTR, resulting in transcriptionally suppressive histone modifications (**Figure 1**).

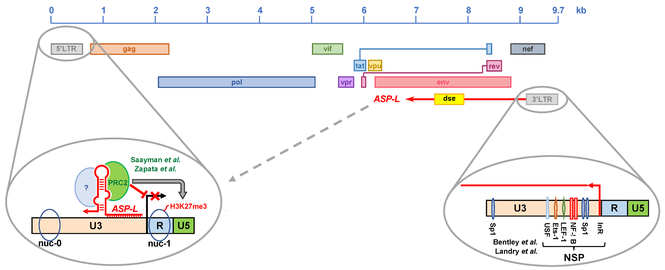


Figure 1. Proposed mechanism transcriptional repression by AST. Li, Sklutuis, Groebner, and Romerio (2021).

METHODS

Peripheral blood mononuclear cells (PBMC) were collected from people with HIV (PWH) who were either on ART (n=3, HIV plasma RNA <50 copies/mL) or untreated donors (n=8, HIV plasma RNA <50-275,000 copies/mL). Donors provided written informed consent.

1. RNA was isolated from HIV-infected PBMC near endpoint, then DNase-treated.
2. RNA was reverse transcribed using a 5'-tagged cDNA synthesis primer complementary to the antisense region of HIV-1 *env* **(Figure 2**).
3. Individual HIV AST cDNAs were counted by digital PCR at limiting dilution or "endpoint".

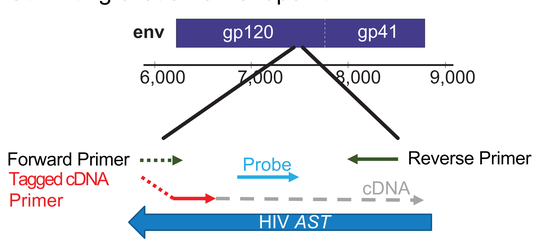


Figure 2. Design for AST detection using a 5'-tagged cDNA synthesis primer.

HIV-1 antisense transcripts can be detected in people living with HIV irrespective of treatment status, lending to a potential role in regulating HIV-1 sense gene expression.

RESULTS

- HIV AST is detectable in donors pre and post-ART with a median of 19 AST copies per 100 infected PBMC (**Figure 3A**), with similar levels between them (**Figure 3B**).
- No difference between sense and antisense transcripts in single infected PBMC (**Figure 3C**).
- HIV AST detected using single genome sequencing matched clonally expanded cells in both blood and lymph nodes (**Figure 4**).

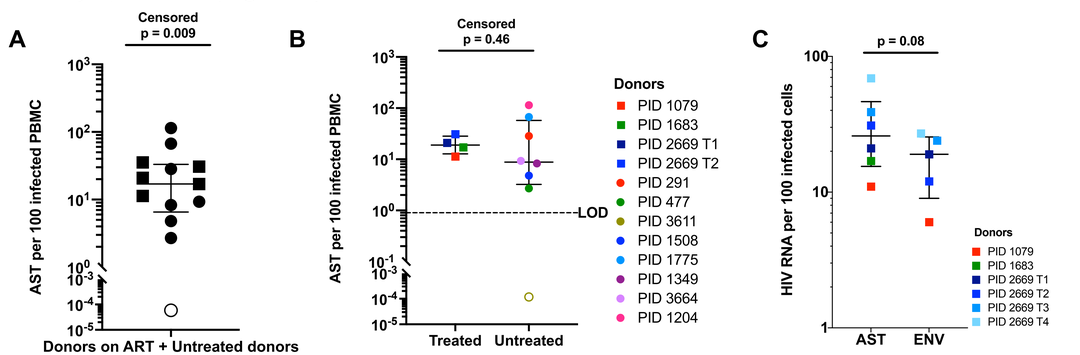
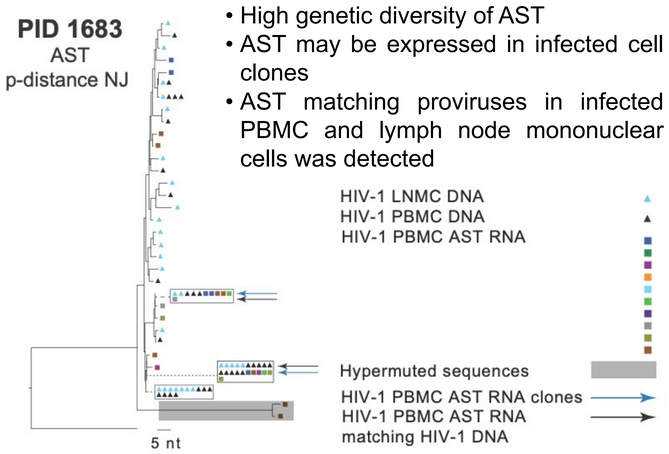


Figure 3. Detection of AST in donors on ART and untreated (not on ART). (**A**) AST molecules per 100 infected PBMC for both groups, one-sample t-test. (**B**) AST molecules per 100 infected PBMC separated by donors on ART vs not on ART, Mann-Whitney U-test. (**C**) Detection of sense *env* vs. AST in donors on ART, paired t-test. Open shapes indicate transcript was undetected, 1/PBMC assayed used as estimate.



- High genetic diversity of AST
- AST may be expressed in infected cell clones
- AST matching proviruses in infected PBMC and lymph node mononuclear cells was detected

Figure 4. Neighbor-joining p-distance tree of PID 1683 of the AST region. AST (colored squares, each representing a separate aliquot of PBMC). Rooted on an AST consensus. Scale set to 5 nt.

CONCLUSIONS

Antisense HIV transcripts…

1) can be detected in single infected PBMC without *ex vivo* stimulation.

2) are expressed in PWH regardless of treatment status.

2) can be clonal and match proviral sequences from PBMC and lymph nodes.

3) are highly diverse indicating that they expressed from many proviruses.

4) are typically detected at a single copy per cell level

The *in vivo* expression of AST irrespective of treatment status warrants further investigation into its potential role as a long non-coding RNA capable of regulating HIV-1 sense gene expression and inducing HIV latency. Understanding the role of HIV AST *in vivo* may inform future strategies for controlling HIV replication without ART.

ADDITIONAL KEY INFORMATION

Acknowledgements:

We thank the Office of AIDS Research, the Center for Center Research, and the National Cancer Institute for funding. We are grateful for the participation of the donors.

Correspondence:

Mary F. Kearney (kearneym@mail.nih.gov)

Adam A. Capoferri (adam.capoferri@nih.gov)