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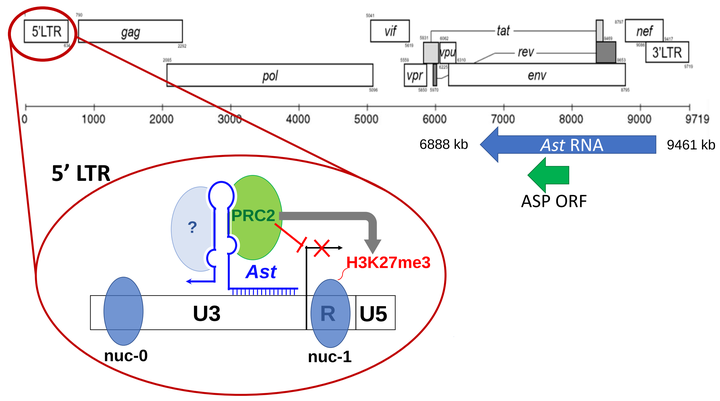
EXPRESSION OF HIV-1 ANTISENSE TRANSCRIPTS IN DONORS ON ART

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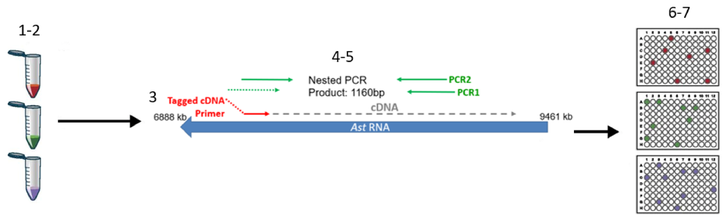
BACKGROUND

Natural antisense transcripts (NATs) encoded by viruses, such as Hbz in HTLV-1, have been shown to regulate viral expression. In HIV-1 M group, in vitro experiments have shown that expression of a NAT called *Ast* promotes the establishment and maintenance of latency through PRC2-induced epigenetic regulation of viral expression. We investigated levels of *Ast* expression in single infected cells isolated from donors on ART.



METHODS

The cell-associated RNA and DNA single-genome sequencing (CARD-SGS) assay was adapted to measure the fraction of infected cells with *Ast* RNA. Antisense RNA in two regions of *gag* and *pol* (nt764-2283 and nt1826-3529) were also evaluated. Additionally, a digital PCR approach was used to quantify both sense and antisense expression in *env*.



1. Isolate PBMC
2. Divide into aliquots at endpoint and extract RNA
3. Synthesize cDNA
4. Spread across 96 well plates
5. Perform Nested PCR
6. Detection with Gel Red
7. Sequence Positives

Antisense HIV RNA can be detected in donors on long-term suppressive ART

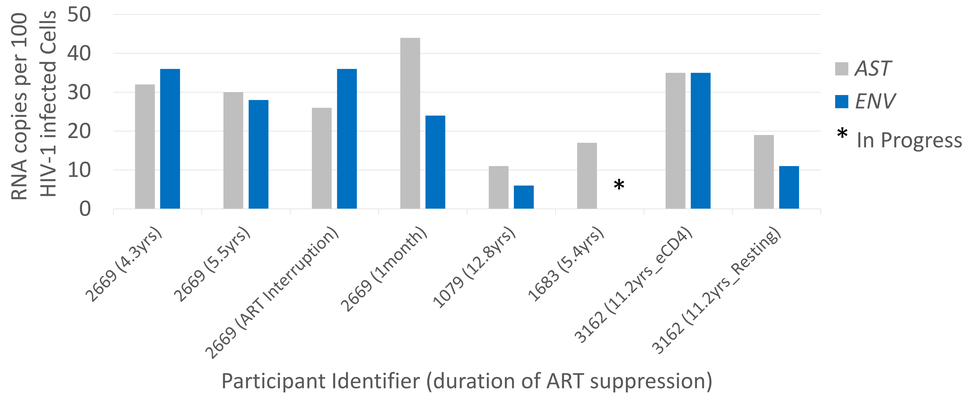
RESULTS

Ast RNA was detected in all donor PBMC samples by CARD-SGS

PID	Sample Type	Duration of Suppression on ART (yrs)	Number of infected cells per million	Estimated # infected cells assayed	Estimated # infected cells with *Ast* RNA*	Estimated percent of infected cells with HIV *Ast* RNA	Average *Ast* RNA copies per cell (range)
2669	PBMC	4.3	180	1440	63	**4.4%**	**1.1 (1-3)**
	PBMC	5.5	130	1040	52	**5.0%**	**1.0 (1-2)**
	PBMC	~2 weeks (ART Interruption; Not suppressed)	250	2000	74	**3.7%**	**1.2 (1-3)**
	PBMC	1 month (suppressed)	90	720	40	**5.6%**	**1.0 (1-2)**
1079	PBMC	12.8	80	720	12	**1.7%**	**3.6 (1-30)**
1683	PBMC	5.4	188	1880	20	**1.1%**	**1.1(1-2)**
3162	CD4	11.2	3100	496	31	**6.3%**	**1.1(1-2)**
	Resting CD4	11.2	2700	4050	37	**0.01%**	**1.0(1-2)**

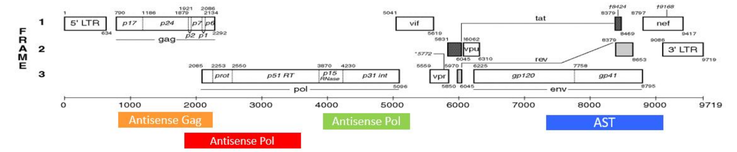
* Assuming identical sequences are from single cell

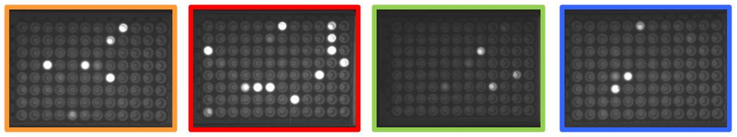
Endpoint PCR with primers and probes was used to detect levels of Ast and Env RNA in donor samples



RESULTS

CARD-SGS detected antisense transcript expression across the HIV-1 genome in donor samples





Donor 2669; 1 month suppressed on ART; 1e6 PBMCs

CONCLUSIONS

- Our findings confirm expression of HIV-1 antisense RNA in donors on ART and indicate that antisense transcripts may span the entire length of the HIV-1 provirus, consistent with in vitro studies.
- Detection of *Ast* RNA in unstimulated cells from donors on ART warrants the investigation of its bifunctional role as a coding and a regulatory RNA in vivo.
- Altering in vivo levels of *Ast* may represent a new HIV-specific approach to reverse or induce viral latency.

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