

Characterizing HIV-1 transcriptional activity during frequent longitudinal sampling in aviremic patients on ART: Implications for HIV cure research

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Introduction

Reversal of latency is currently being pursued in clinical trials as a component of HIV eradication efforts. Reversal of latency has been determined by increases in cell-associated unspliced HIV-1 RNA (CA-US HIV-RNA), but the natural longitudinal dynamics of HIV-1 transcriptional activity has not been fully characterized.

Materials and Methods

We conducted a longitudinal, observational cohort study enrolling 26 aviremic, HIV-1 patients with CD4+ T-cell count >200/ μ L and >2 year on ART.

Table 1.

Monthly blood samples were collected over six consecutive months. HIV-1 transcription as measured by CA-US HIV-RNA and proviral load (total HIV-1 DNA) were quantified in unfractionated CD4+ T cells using digital droplet PCR. Data were analyzed by a linear regression model with random-effects to allow for repeated measurements for individuals.

Results

Data on CA-US HIV RNA were available on 26 patients and Total HIV DNA on 22 patients. **Fig 1a, b, c.**

Three outcome estimates were computed (**Table 2**):

1. Intra-individual contribution to the overall cohort variation in CA-US HIV RNA, Total HIV-DNA and RNA/DNA ratios. This indicates that the majority of the observed cohort variation was attributed to inter-individual differences.
2. 95% prediction intervals for the cohorts absolute level of CA-US HIV RNA and Total HIV-DNA. This estimates the range in which a new random sample or a median in absolute values would fall, if additional sampling from an individual from this cohort, or an individual matching this cohort, were performed.
3. 95% variation interval, estimates the variation around the median absolute level of CA-US HIV RNA, Total HIV-DNA and RNA/DNA ratios. For a single patient this was expressed as multiplication factors (fold change) over the course of 6 months. Hence, within a single individual we observed a 0.360 to 2.776 fold variation in the levels of CA-US HIV RNA measured over the course of 6 months.

Table 2

| | CA-US HIV RNA | Total HIV DNA | RNA/DNA ratio |
|--|---------------------------------------|---|------------------|
| 1. Intra-individual contribution | 15.8% | 5.3% | 30.2% |
| 2. 95% prediction intervals | (0.8; 93 copies/ 10^6 CD4+ T cells) | (183; 11,701 copies/ 10^6 CD4+ T cells) | (0.0018; 0.0257) |
| 3. 95% variation interval (fold changes) | (0.360; 2.776) | (0.612; 1.634) | (0.420; 2.383) |

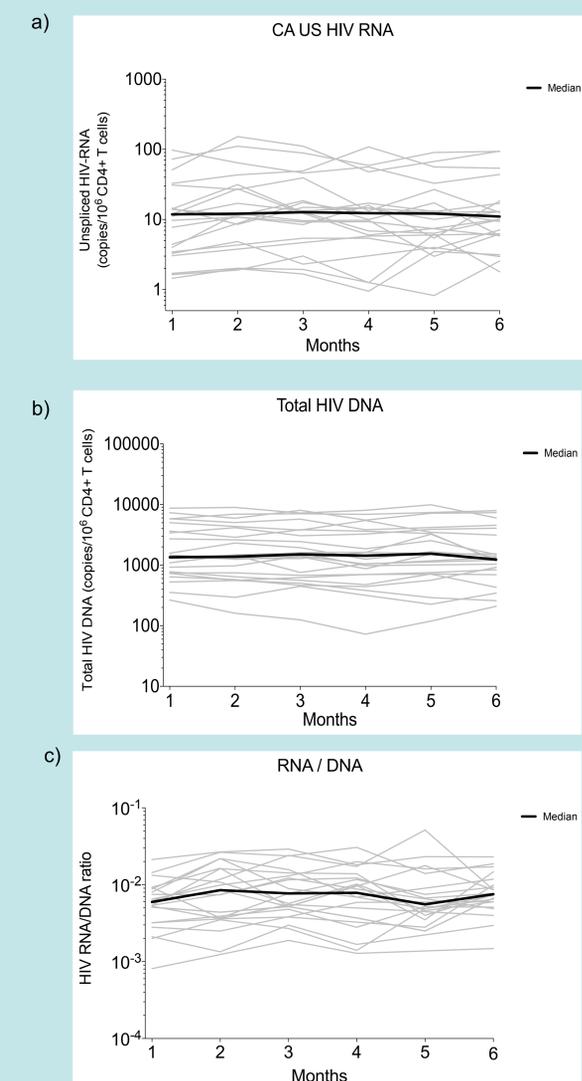
Conclusion

HIV-1 transcriptional activity showed minor fluctuations during frequent sampling over a period of 6 months in aviremic, HIV-1 infected individuals on ART. These data provide the first insights into the natural occurring longitudinal variation of CA-US HIV-RNA, a primary outcome measure in HIV-1 latency reversal trials. These data provide a solid foundation for both design and interpretation of future latency reversal trials.

Table 1

| Baseline characteristics (n=26) | |
|---|------------------|
| Gender | |
| Male, n(%) | 18 (69%) |
| Female, n(%) | 8 (31%) |
| Age (years), median (range) | 49 (31-79) |
| Ethnicity | |
| Caucasian, n (%) | 24 (92%) |
| African Danish, n(%) | 2 (8%) |
| Months since HIV-1 diagnosis, median (range) | 99 (26-346) |
| Months from HIV-1 diagnosis to ART initiation, median (range) | 8.0 (0-186) |
| Months on ART, median (range) | 80 (25-206) |
| Months with HIV RNA <50 copies per mL, median (range) | 71.5 (15-172) |
| Nadir CD4+ count (cells per μ L), median (range) | 208 (0-710) |
| Baseline CD4+ count (cells per μ L), median (range) | 625 (240-1750) |
| Pre ART viral load (copies/ml) log ₁₀ , median (range) | 4.68 (2.76-6.23) |
| ART regimen | |
| 2xNRTI + NNRTI, n(%) | 10 (38.5%) |
| 2xNRTI + protease inhibitor, n(%) | 11 (42%) |
| 1xNRTI + integrase inhibitor + protease inhibitor, n (%) | 1 (3.8%) |
| 2xNRTI + 1xNNRTI + protease inhibitor, n(%) | 1 (3.8%) |
| 2xNRTI + integrase inhibitor, n(%) | 2 (7.6%) |
| 1xNRTI + 1xNNRTI + integrase inhibitor, n(%) | 1 (3.8%) |

Figure 1



Individual absolute values of (1a) CA-US HIV RNA (1b) total HIV DNA and (1c) CA-US HIV RNA/total HIV DNA ratios are shown (grey lines) including group median (black lines).