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# Abortive HIV-1 RNA transcripts are detected in serum of people living with HIV during long-term effective antiretroviral therapy

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## Introduction

A major focus of HIV-cure research is to find novel methods to reduce or elimination the viral reservoir, and therefore it is important to monitor the dynamics of the viral reservoir in people living with HIV (PLWH). Established assays to measure the viral reservoir use cells from PLWH, which requires intricate handling and labor intensive procedures. The identification of suitable targets and markers of the HIV-reservoir are therefore a main topic of HIV-cure research. The transactivation-response element (TAR) RNA has been proposed as potential biomarker of the latent HIV-reservoir. TAR-RNA is a short abortive HIV-1 RNA transcript and is expressed in latently infected cells and can be detected in serum. However, the dynamics of abortive HIV-1 RNA expression during long term therapy remains largely unknown. Here, we established a qPCR assay to monitor abortive HIV-1 RNA transcripts in serum of PLWH and investigated the dynamics before and during effective antiretroviral therapy.

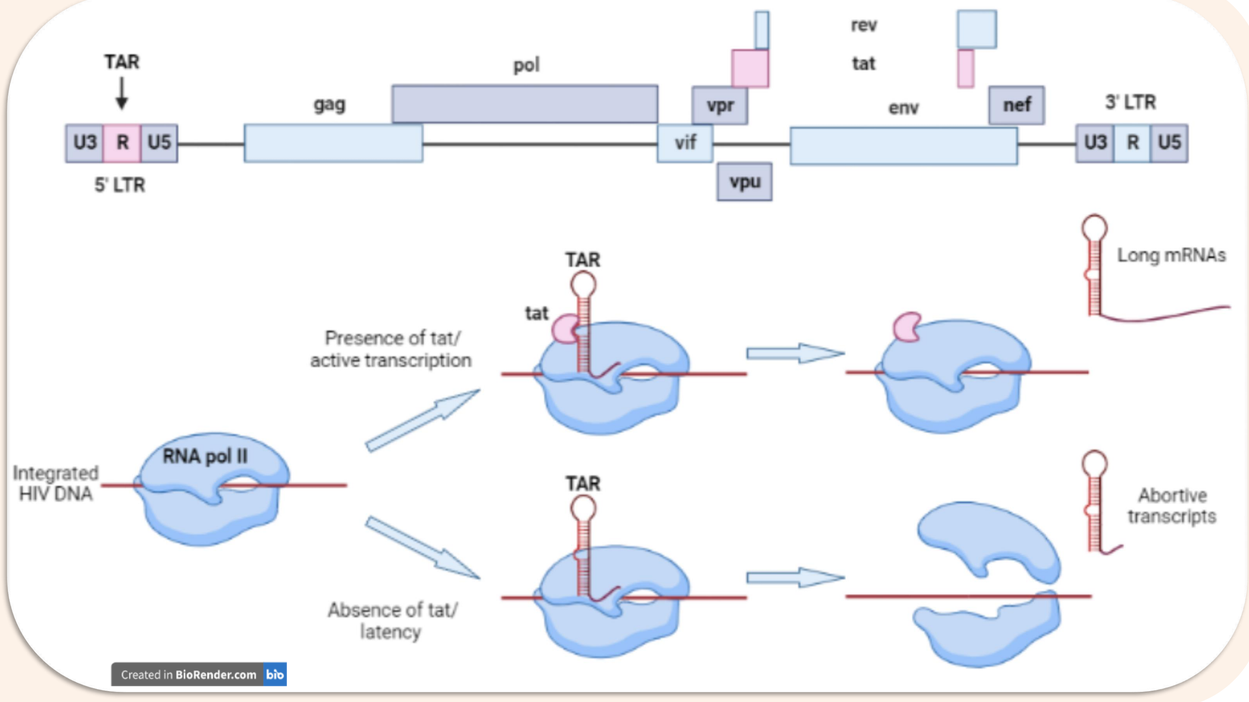
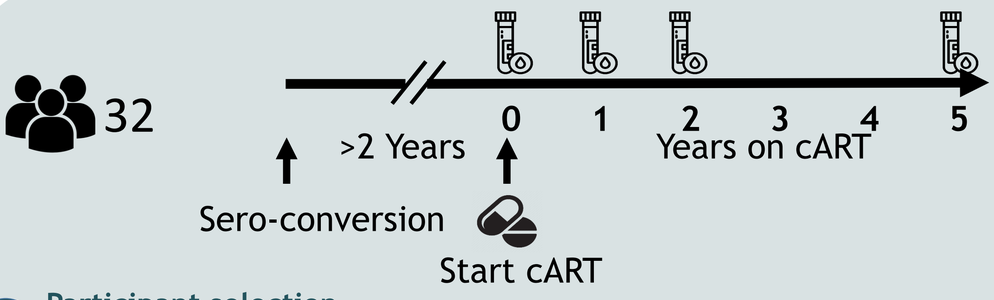


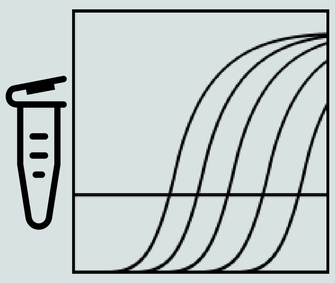

Figure 1. Shows the location of the transactivation-response element (TAR) in the HIV-genome, and the processing of TAR-RNA during active transcription and during latency

## Methods




**1 Participant selection**

Pre-cART (0), and 1, 2, and 5 years on-cART serum samples from thirty-two participants enrolled in the Amsterdam Cohort Studies who had been living with HIV for at least 2 years prior to starting cART, and had an undetectable viral load after 1 year of cART and maintained and undetectable viral load thereafter, were selected.



**Laboratory methods**

RNA was isolated from serum samples, cDNA was synthesized and a qPCR was used to measure abortive HIV-1 RNA.

**Statistical analysis**

**2** Repeated measures ANOVA with post-hoc pairwise comparison was used to investigate the time-course effect.

**3** Univariable linear regression analysis was used to investigate associations of abortive HIV-1 RNA at the different timepoints and pre-ART variables: CD4+ T-cell count, CD8+ T-cell count, CD4/CD8 ratio and viral load.

## Results

### 1 Participant characteristics

| | |
|---|---|
| Age (median-IQR) | 43 (36-49) |
| Start cART '96-'98 | 30 (94%) |
| Baseline CD4+ T-cell count | 354 (246 - 460) |
| Baseline CD8+ T-cell count | 950 (765 - 1186) |
| CD4/CD8 ratio | 0.34 (0.22-0.48) |
| Baseline viral load ($log_{10}$ copies/mL) | 4.5 (4.1 - 4.8) |

Table 1. Participant characteristics. Summary statistics are presented as median (IQR) or n (%).

### 2 Dynamics of abortive HIV-1 RNA transcripts

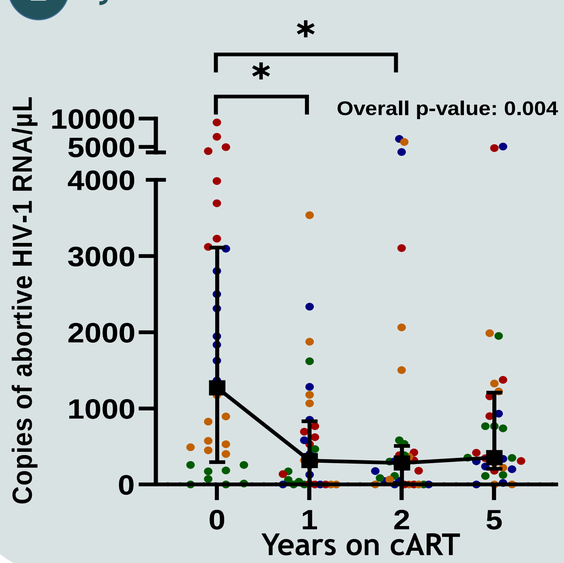



Figure 2. Dynamics of abortive HIV-1 RNA transcripts during the first five years of cART. A repeated measures ANOVA with post-hoc pairwise comparisons was used to investigate the time-course effect. Squares indicate the median and whiskers the interquartile range per time point. Individual data points are presented in colors corresponding to the pre-cART (0) quantiles.

### 3 Associations of baseline variables and abortive HIV-1 transcripts

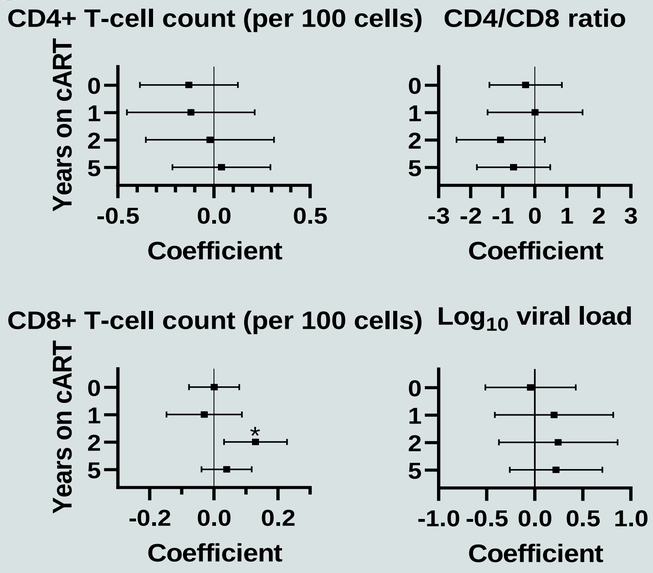



Figure 3. The results of the univariable regression analysis to investigate the association between pre-cART variables and $log_{10}$ abortive HIV-1 RNA transcripts pre-cART and at 1, 2 and 5 years of cART.

## Conclusions

Here we show that abortive HIV-1 RNA is detected in serum during long term therapy and represents a potential biomarker for monitoring latent viral reservoir. Future studies will determine whether serum-derived abortive HIV-1 RNA levels are associated with cellular viral reservoir measurements.

## Funding & contact

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amsterdam cohort studies

Target2Cure consortium

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