# Persistent HIV-1 Viremia Despite Intensive Antiviral Therapy Due to Non-responsive Clonal Viral Lineages

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## Background

After initiation of antiviral therapy (ART), plasma HIV-1 RNA is usually undetectable after one month. In rare cases, viral suppression may not be achieved despite good adherence and with virus susceptible to the therapy. We used ultra-deep Primer ID next gen sequencing (NGS) to study the viral population and evolution of HIV-1 in two patients with persistent viremia on intensive antiviral therapy.

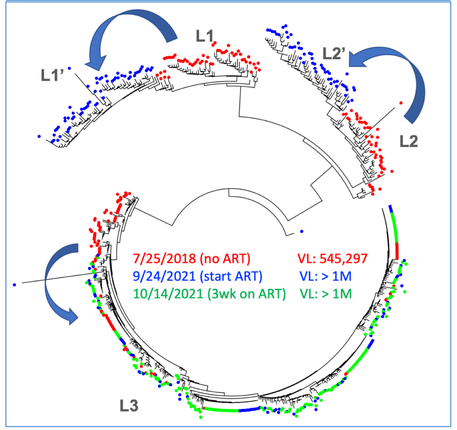
## Methods

We extracted viral RNA from plasma samples collected at multiple timepoints over the duration of the treatment from two patients (**VEX1** and **VEX2**). We constructed Primer ID NGS libraries covering part of the *pol* gene and the *env* V1/V3 region and sequenced them on the Illumina MiSeq platform. We used the 'tcs' pipeline to construct template consensus sequences (TCS) for each region, and searched for drug resistance mutations (DRMs). The Geno2pheno pipeline was used to predict co-receptor tropisms.

## Results

- Patient **VEX1** was followed for two years on ART. **VEX2** restarted ART in the hospital and received directly observed therapy for nearly 6 months.
- Both had over 1 million viral copies/mL at the initiation of the therapy, and the CD4 cell counts were extremely low. Their viral loads slowly declined to approx. 10,000 copies/mL at the end of follow-up but complete viral suppression was not achieved for either patient despite hospitalization and appropriate ART.
- DRMs were not detected in either patient throughout the treatment with detection sensitivity as low as 0.1%.
- The sequencing results for **VEX1** showed that there were both X4- and R5-tropic viruses at all timepoints and R5 viruses decayed much more slowly than X4 viruses, up to 35-fold more slowly in the initial 3 months of ART. Phylogenetic analysis revealed that the persistent R5 viruses were largely clonal while little clonality was found in the persistent X4 virus.
- In **VEX2**, all viruses were R5-tropic. There were three major distinct lineages in the viral population, and two of them completely disappeared after the initiation of ART while the other lineage persisted throughout therapy (Fig 1). Of note, the persistent lineage in **VEX2** was highly clonal (Fig 2).

**Fig. 1. Pooled maximum likelihood tree at *env* V1V3 region of 3 time points from patient VEX2.**



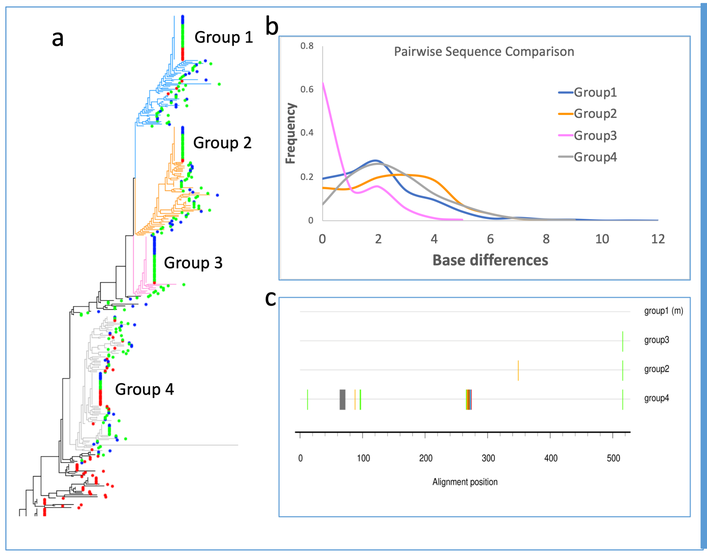




**Fig. 2. Persistent lineage in VEX2 was highly clonal.** a) Maximum likelihood tree of the persistent lineage at the *env* V1V3 region of three time points of patient VEX2. Sequence nodes were color coded as in Fig. 1. The colors of the branches indict the groups for the clonal sequences. b) Pairwise sequence comparison of the 4 clonal groups. All the 4 groups are highly clonal. c) Highlighter plot of the group consensus sequences, using group 1 as the reference sequence. Groups 1, 2 and 3 are close to each other while group 4 is substantially different from group 1-3.

## Conclusions

- **Persistent viremia on ART can come from clonal viral lineages that carry no DRMs.**
- **Clonally expanded and infected host cells might contribute to the phenomenon.**
- **Further study is needed to explore the origin of these clonal viral genomes.**

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