# Nonsuppressible HIV Viremia may Harbor Replication-Incompetent Viruses with 5' Leader Deletions

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## BACKGROUND

- Individuals with non-suppressible viremia (NSV) have persistent low-level viremia despite ART adherence and no drug resistance
- We identified three NSV individuals(LV4,LV7,LV8) with plasma variants containing deletions in the 5′ leader region.

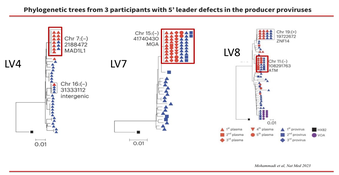
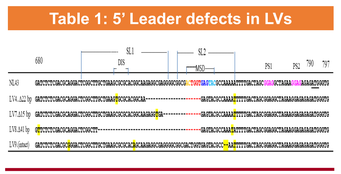



**Table 1: 5' Leader defects in LVs**

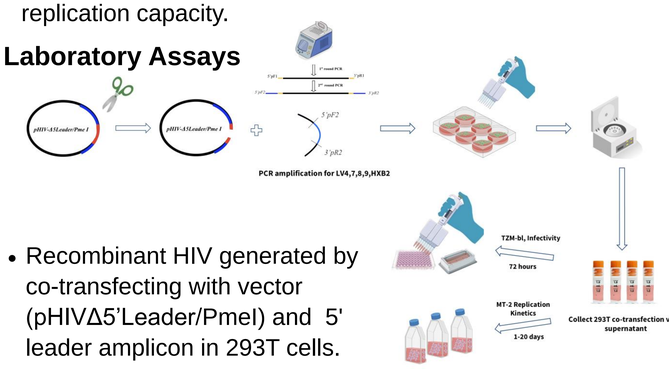



- We evaluated the replication competence of these variants by using a recombinant HIV system to assess viral infectivity and replication capacity.

## STUDY STRATEGY/METHODS

- Investigated 5' leader defects in NSV participants (LV4, LV7, LV8) with LV9 (intact 5' leader) as a control.
- Synthesized 5' leader defective sequences of varying sizes.
- Constructed an HIV-1 vector with a 5' leader (653-780) deletion and a PmeI site using the NL43 backbone.
- Generated recombinant HIV with 5' leader sequences from NSV participants or synthesized amplicons.
- Assessed their impact on virion production, infectivity, and replication capacity.

### Laboratory Assays




- Recombinant HIV generated by co-transfecting with vector (pHIVΔ5'Leader/PmeI) and 5' leader amplicon in 293T cells.
- HIV infectivity was quantified using TZM-bl cells (72 hours post-infection) through β-galactosidase assay.
- HIV replication capacity was assessed by monitoring kinetics in MT2 cell infections by β-galactosidase assay over 23 days.

## RESULTS

### Absence of viral replication for viruses harboring 5' leader deletions

**Figure 1.** 5' leader deletions lead to replication-defective viruses in TZM-bl cells infectivity assays for HIV-1 recombinants carrying 5′ leader deletions (LV4, LV7, LV8) and no deletions (LV9). Y-axis: HIV infectivity, X-axis: virus input. Data are mean ± S.D. from two independent experiments.

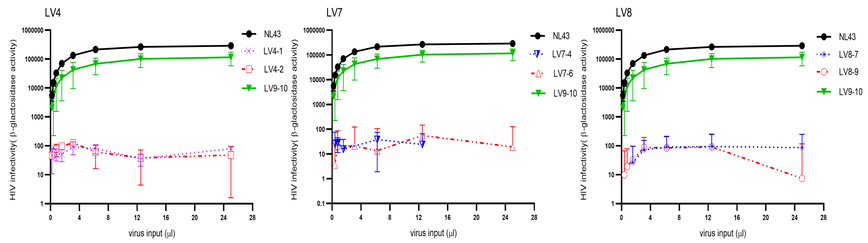
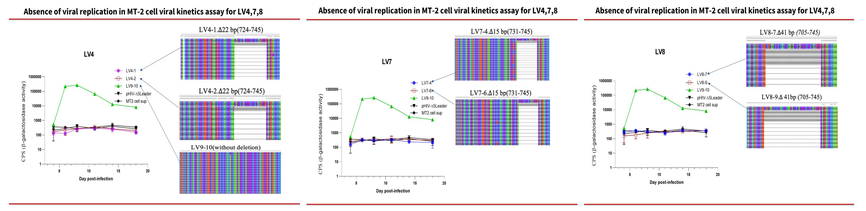
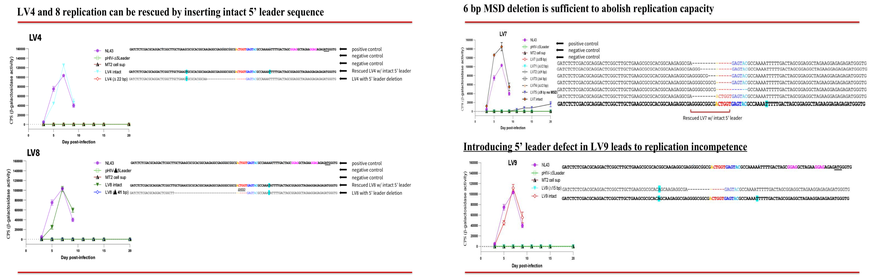



**Figure 2.** Replication kinetics analysis of HIV-1 recombinants carrying 5′ Leader deletion region of NSV participants (LV4, LV7, LV8) and LV9 in MT2 cells. Controls included pHIV-Δ5Leader vector and MT2 cells. The sequence integrity of the 5′ leader was confirmed using NGS sequencing of culture supernatants.



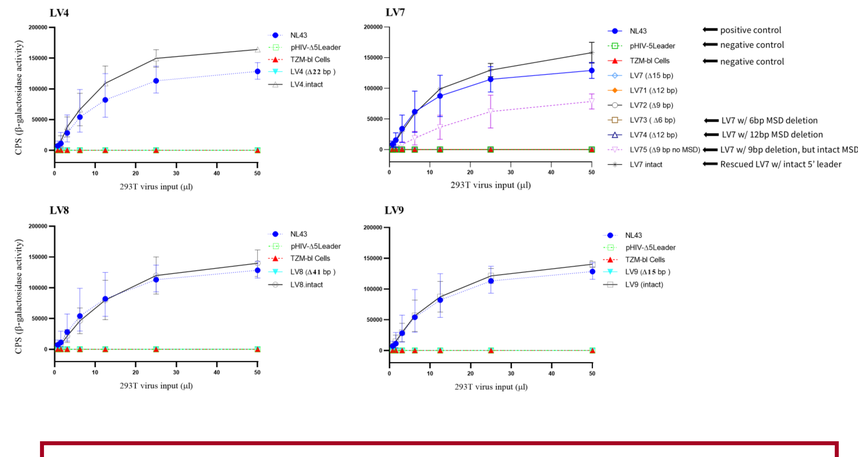

### Investigating 5' Leader defects in synthesized amplicons

**Figure 3.** Replication kinetics of HIV-1 recombinants carrying synthesized 5′ leader variants in MT2 cells. Replication can be rescued with an intact 5' leader and partially restored with an intact MSD. Data are mean ± S.D. from two independent experiments.




### Investigating 5' Leader defects in synthesized amplicons

**Figure 4.** Infectivity assays in TZM-bl cells for HIV-1 recombinants with synthesized 5′ leader variants. The y-axis represents HIV infectivity, and the x-axis represents virus input. Data are shown as mean ± S.D. from two independent experiments.




## CONCLUSIONS

- Recombinant HIV-1 viruses with 5′ leader deletions (15-41bp) from NSV participants were found to be replication-incompetent using two assays (TZM-bl infectivity and MT-2 replication kinetics).
- Synthesized viruses showed that the main defect was localized to deletion in the major splice donor site (MSD), where even a small (6nt) deletion resulted in replication-incompetence
- Replication was restored with an intact 5′ leader and partially restored with an intact MSD.
- Persistent low-level viremia with 5′Leader defects may involve mostly replication-defective virions, posing minimal transmission risk.

## ACKNOWLEDGEMENTS

We thank the participants and the study staff. This work was funded in part by NIH grants AI169768 (to Dr. Li) and the DARE Delaney Collaboratory (AI164560).

2025 CROI Conference on Retroviruses and Opportunistic Infections