# LTR Variations Between Clades Drive Differences in HIV-1 Expression and Reactivation


Chobanian & Avedisian School of Medicine
Virology, Immunology & Microbiology

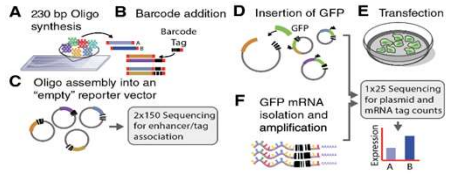
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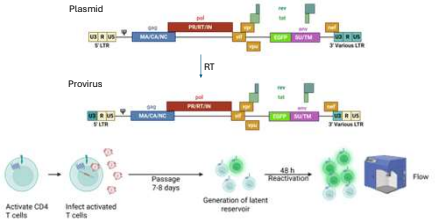

## BACKGROUND

- ART cessation for PLWH leads to reactivation of the latent viral reservoir.
- This latent reservoir is thought to be a major barrier to curing HIV infection.
- HIV transcription is regulated by the 5' long terminal repeat (LTR).
- The LTR is controlled by combinatorial mechanisms which influence latency and reactivation.
- **We hypothesize that variations of the 5' LTR contribute to differences in proviral gene expression and the establishment, maintenance, and reactivation of the latent reservoir.**

## METHODS




- MPRA: 6000 LTRs examined using this method in Jurkat T-cells.
- The U3 region of five LTRs across clades A, B, and C replaced the U3 region of NL43-Δenv-EGFP (**NL43-LTRs**).
  - These viruses were used to infect CD4+ T-cells and Jurkat cells, and used to eventually create latent cellular models.
  - Differences in infection, latency, and reactivation were measured using flow cytometry and qRT-PCR.

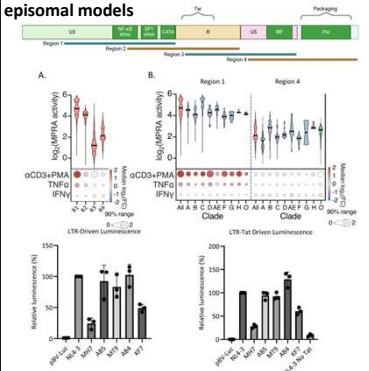




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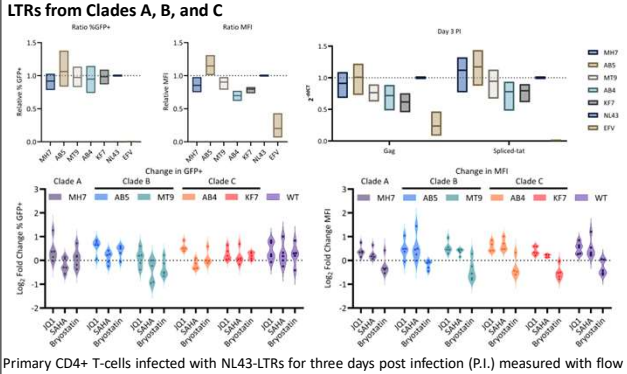

## RESULTS

### Tiled and Full-length LTRs show differences in episomal models



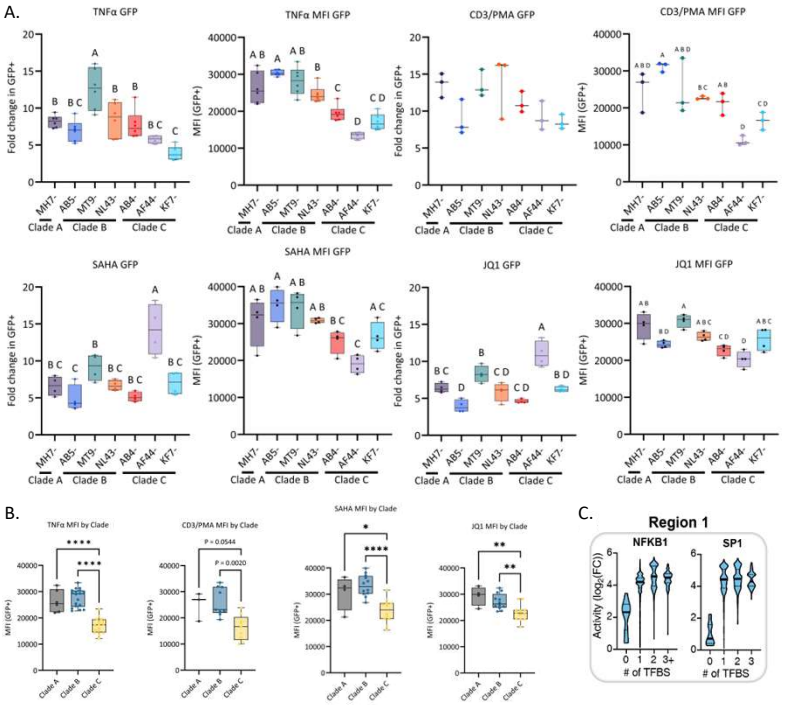

Tiled (top) and full-length (bottom) LTRs transfected into Jurkat or HEK 293 T cells respectively

### Infection and reactivation in primary CD4+ T-cells show differences between LTRs from Clades A, B, and C




Primary CD4+ T-cells infected with NL43-LTRs for three days post infection (P.I.) measured with flow cytometry or qRT-PCR (top). Latently infected primary CD4+ T-cells reactivated with three different LRAs for 48 h measured by flow cytometry (bottom)

### Variation in Reactivation of LTRs from different clades

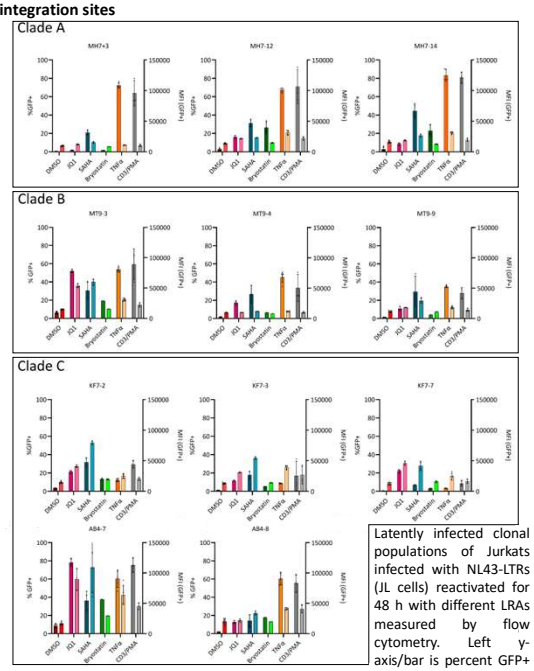



A. Jurkat T-cells infected with NL43-LTRs for 48 h were sorted for GFP+ and GFP- cells. GFP- cells were kept and expanded for 7 days P.I. then reactivated for 48 h with various LRAs. Levels of reactivation were measured using flow cytometry. Statistical significance designated by compact letter display from a one-way ANOVA with a Tukey's post-hoc analysis. B. Mean fluorescent intensity (MFI) of GFP+ signal binned by clade of LTR. Statistical differences measured by a qtr. one-way ANOVA with a Tukey's post-hoc analysis. C. Transcriptional activity of LTRs binned by number of NF-κB sites. D. Number of NF-κB sites in tested LTRs

| LTR | NF-κB |
|---|---|
| MH7 –A | 2 |
| AB5 –B | 2 |
| MT9 –B | 2 |
| NL43 –B | 2 |
| AB4 –C | 3* |
| AF44 –C | 2* |
| KF7 –C | 2* |

NF-κB – consensus sequence: 5'-GGGRNWYYCC-3' *NF-κB – (Clade C):5'-GGGGCGTTCC-3'

### Variation in responses to latency reversal agents across clades and integration sites




Latently infected clonal populations of Jurkats infected with NL43-LTRs (JL cells) reactivated for 48 h with different LRAs measured by flow cytometry. Left y-axis/bar is percent GFP+ cells. Right y-axis/bar is arithmetic MFI. Integration site was determined by inverse PCR.

Integration Sites of JL cells

| LTR | Clade | Integration site | Intron/Exon | Fwd/Rev | Chromosome | About 4-5 bases of overlap Location |
|---|---|---|---|---|---|---|
| MH7+3 | A | DNAJC10 | Intron | Fwd | 2 | Chr2: 182756504-509 |
| MH7-12 | A | CDK13 | Intron | Rev | 7 | Chr7: 39983114-117 |
| MH7-14 | A | DLG1 | Intron | Rev | 3 | Chr3: 197219168-173 |
| MT9-9 | B | SLC9A6 | Intron | Rev | X | ChrX: 136096447-452 |
| KF7-2 | C | XPO7 | Intron | Fwd | 8 | Chr8: 21957476-481 |
| KF7-3 | C | Catalase | Intron | Fwd | 11 | Chr11: 34469610-615 |

## CONCLUSIONS

- The MPRA allowed for 6000+ LTRs to be examined for transcriptional activity across multiple clades
- When in a near full length virus, differences in LTR can be observed.
- Clade C LTRs tested have statistically less transcriptional activity possibly due to mutations in NF-kB sites.
- 15 Jurkat lines containing latent LTR-driven GFP reporter viruses (JL cells) were established.
- Integration site, chromatin dynamics, and other intrinsic cellular properties may contribute to differences.

### PLAIN LANGUAGE SUMMARY

There are many variations/clades of HIV-1. Our research aims to understand how clade-associated sequence differences in the LTR may drive latency and reactivation.