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Anti-HIV Gene Editing Is Inhibited in Active HIV Provirus Through Long-Terminal Repeat Demethylation

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BACKGROUND

HIV latency is a major barrier to HIV cure and is maintained through several mechanisms, including DNA methylation. CRISPR-Cas9 excision of the HIV genome is a promising HIV cure strategy but requires significant improvement prior to clinical application. Here, we studied the impact of proviral methylation on HIV gene editing.

AIMS

- Investigate CRISPR-Cas9-mediated HIV excision efficiency in productively-infected and latently-infected CD4+ T cells.
- Determine the impact of proviral methylation state on CRISPR-Cas9-mediated HIV excision efficiency in latently-infected CD4+ T cells.

METHODS

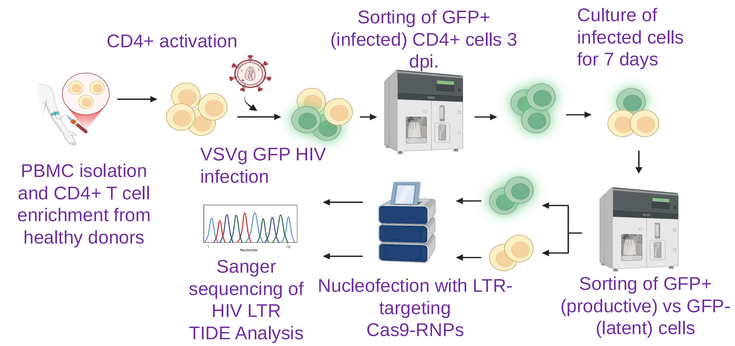


Figure 1. Workflow to evaluate HIV LTR gene editing efficiency in productively-infected and latently-infected CD4+ T cells.

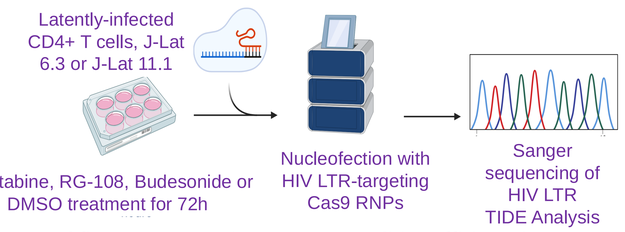


Figure 2. Workflow to assess anti-HIV LTR gene editing efficiency in latently-infected cells treated with DMSO (control), decitabine, RG-108 or budesonide.

REFERENCES

a. Brinkman, E. K., Chen, T., Amendola, M. & van Steensel, B. Easy quantitative assessment of genome editing by sequence trace decomposition. *Nucleic Acids Research* 42, e168–e168 (2014).

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Modulating methylation of the HIV LTR promoter may enhance anti-HIV CRISPR-Cas9 gene editing, accelerating proviral inactivation.

RESULTS

Latently-infected CD4+ T cells are more susceptible to anti-HIV CRISPR-Cas9 editing than productively-infected CD4+ T cells

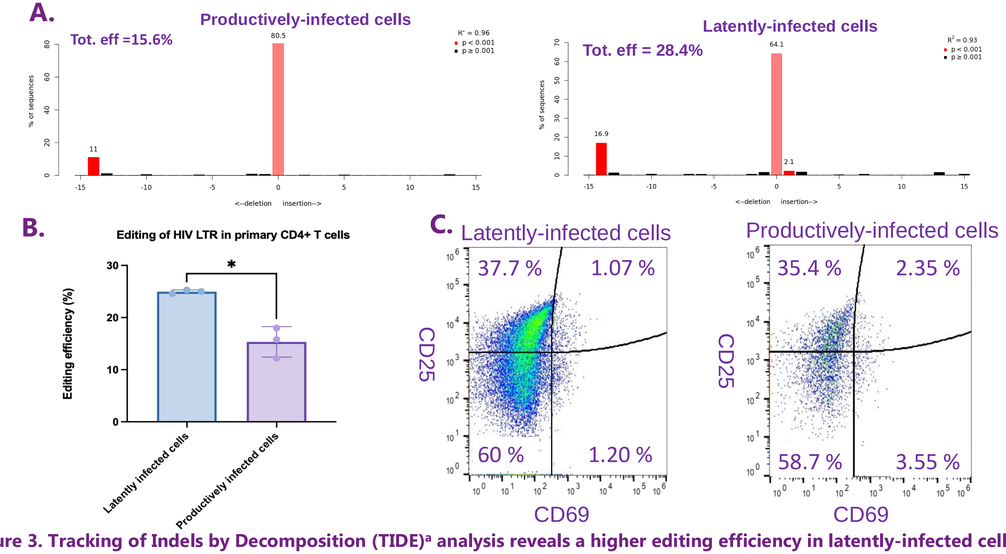


Figure 3. Tracking of Indels by Decomposition (TIDE)[a] analysis reveals a higher editing efficiency in latently-infected cells. Sequences were analyzed using TIDE; one representative donor shown (A) and summarized in a bar graph (B) (n=3). Statistical significance was determined using a t-test (* $p < 0.05$). Flow cytometry was used to detect the level of activation of latently- and productively-infected CD4+ T cells (C)

Demethylation using Decitabine impedes anti-HIV CRISPR-Cas9 editing in latently-infected CD4+ T cell lines

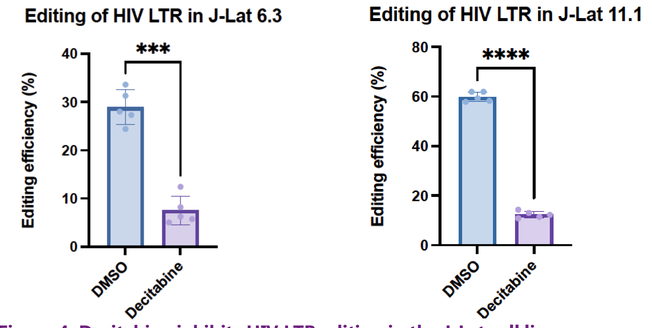


Figure 4. Decitabine inhibits HIV LTR editing in the J-Lat cell line. Statistical significance was determined using a t-test (***$p < 0.0005$; *****$p < 0.0001$).

Decitabine induces reactivation of HIV in latently-infected CD4+ T cell lines

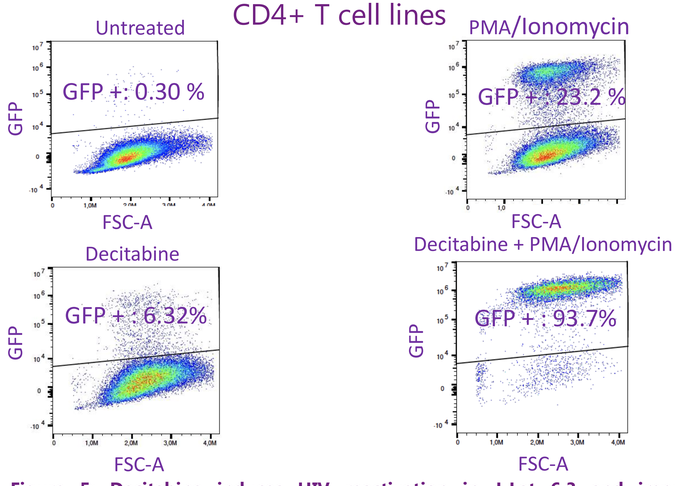


Figure 5: Decitabine induces HIV reactivation in J-Lat 6.3 and improves reactivation when combined with PMA/Ionomycin, a latency reversing agent.

CONCLUSIONS

- HIV latently-infected CD4+ T cells are more susceptible to CRISPR-Cas9-mediated excision than productively-infected cells.
- Decitabine treatment to demethylate the genome impedes CRISPR-Cas9-mediated excision in latently-infected CD4+ T cell lines.
- Decitabine reactivates HIV in latently-infected CD4+ T cell lines
- Epimodulators including pro-methylation agents and chromatin modulators may enhance HIV cure-focused gene therapy strategies.