Targeting the HIV-1 Latent Reservoir with Tat-CRISPR Lipid Nanoparticles

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BACKGROUND

Human immunodeficiency virus (HIV) infection remains incurable due to latently infected cells resistant to anti-retroviral therapy (ART). One HIV cure strategy is to permanently silence the latent reservoir. The HIV Tat, trans-activator of transcription, protein promotes productive transcriptional elongation, enabling the rapid production of viral proteins and virions. Therefore, we sought to mutate the Tat gene to reduce Tat expression and prevent HIV re-activation in latent cells. .

METHODS

In this study, we used CRISPR/Cas9 delivered by lipid nanoparticles (LNPs) to target the viral Tat gene (Tat-CR LNPs). We first optimized a T cell specific LNP formulation for RNA delivery and then delivered Cas9 mRNA and guide RNAs (gRNAs) against Tat to HIV infected cells.

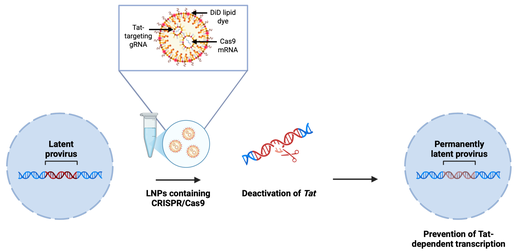


Figure 1. Targeting the HIV Tat gene with CRISPSR/Cas9 LNPs. T cell optimized LNPs contain Cas9 mRNA and a Tat-targeting gRNA. The LNPs are labeled with a lipophilic tracer dye, DiD.

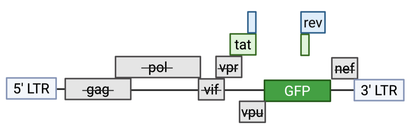


Figure 2. HIV-GFP reporter virus gene schematic. A destabilized GFP is inserted in the *Env* ORF and all viral genes, besides Tat and Rev, have silencing mutations.

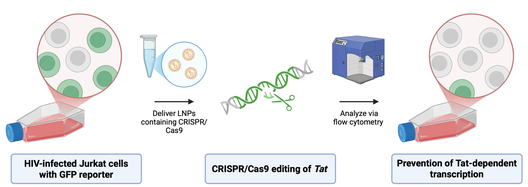


Figure 3. HIV-GFP Jurkat model and CRISPR/Cas9 approach. Tat-CR LNPs are delivered to HIV-GFP Jurkats and GFP expression is quantified via flow cytometry.

CRISPR/Cas9 can induce mutations in HIV DNA to prevent viral reactivation. Permanently silencing viral gene expression in the latent reservoir could lead to an HIV cure. We observe reduced HIV expression after CRISPR/Cas9 editing of the viral Tat gene in three T cell models.

RESULTS

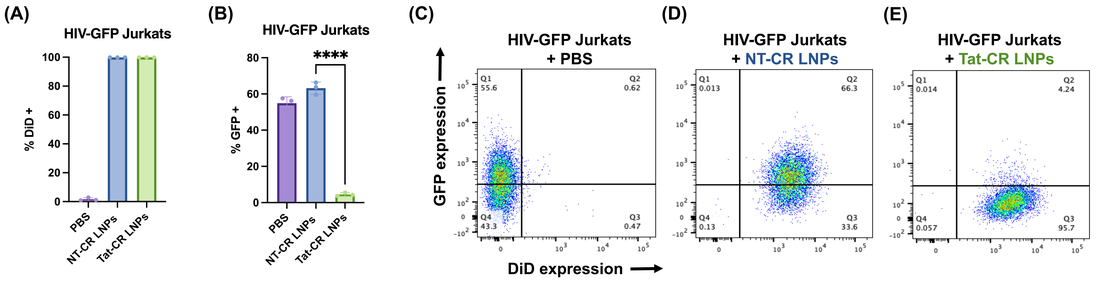


Figure 4. Tat-CR LNPs potently reduce HIV expression in HIV-GFP Jurkats. HIV-GFP Jurkats were analyzed via flow cytometry for **(A)** DiD and **(B)** GFP expression between NT-CR LNPs and Tat-CR LNPs. Representative flow plots for HIV-GFP Jurkats treated with PBS **(C)**, NT-CR LNPs **(D)**, or Tat-CR LNPs **(E)**. A single dose of Tat-CR LNPs reduced the % of GFP+ Jurkat cells from 63% to 4.5% (****, p <. 0.0001).



Figure 5. Inference of CRISPR Edits (ICE) sequence analysis. ICE analysis determined that 81% of the sequences in the Tat-CR LNP treated sample contained insertion or deletion mutations around the gRNA cut site (R^2 = 94.0).

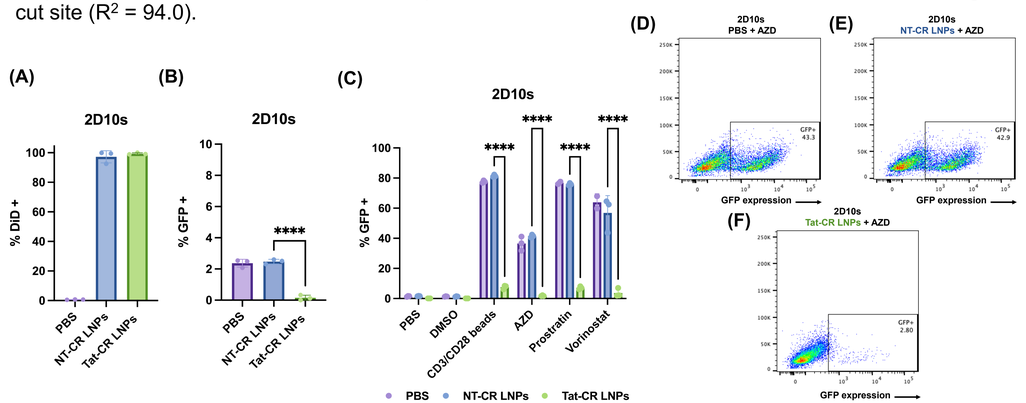


Figure 6. Tat-CR LNPs prevent reactivation of inducible Jurkat-derived latency model (2D10s). 2D10s were analyzed via flow cytometry for **(A)** DiD and **(B)** GFP before induction with latency reversal agents and **(C)** afterwards. Representative flow plots for 2D10s treated with AZD and **(D)** PBS, **(E)** NT-CR LNPs, and **(F)** Tat-CR LNPs.

RESULTS

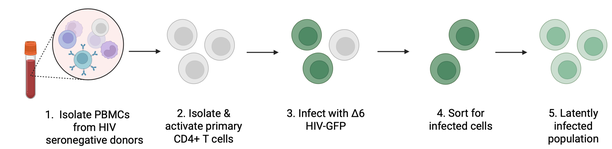


Figure 7. Primary CD4+ T cell model using HIV-GFP.

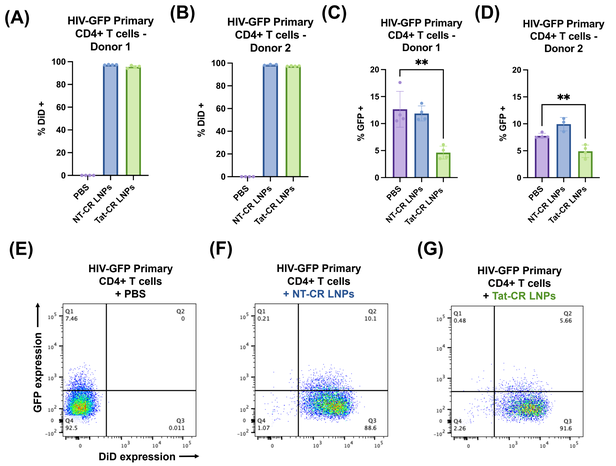


Figure 8. Tat-CR LNPs partially knockdown (~50%) HIV expression in primary CD4+ T cells. Primary CD4+ T cells from two donors were analyzed via flow cytometry for **(A, B)** DiD and **(C, D)** GFP. Representative flow plots for donor 2 treated with **(E)** PBS, **(F)** NT-CR LNPs, and **(G)** Tat-CR LNPs.

CONCLUSIONS

Tat-CR LNPs effectively deliver CRISPR/Cas9 components to immortalized and primary T cells. Sequence analysis indicates that most of the population is edited after a single dose of Tat-CR LNPs. Further, our results validate that genetically editing the HIV Tat gene significantly reduces HIV expression in three infected T cell models, including primary cells. Overall, this study suggests that our **Tat-CR LNPs can target infected T cells, attenuate Tat expression, and prevent HIV reactivation *in vitro*.**

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