CD4-Targeted mRNA Delivery of Tat Reverses HIV-1 Latency

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Background and Methods

Interventions that reverse viral latency may be needed for an HIV cure. The HIV-1 protein Tat drives viral RNA expression but is cytotoxic and difficult to deliver to cells harboring the latent reservoir. Here, we tested whether CD4-targeted delivery of mRNA expressing a noncytotoxic Tat could antagonize viral latency.

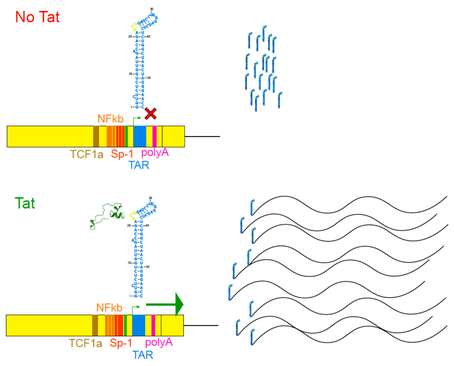


Figure 1: Tat regulation of HIV-1 transcription. Viral transcription is regulated by expression the viral protein Tat. The HIV-1 promoter resides in the 5' Long Terminal Repeat (yellow). In the absence of Tat, trasncription initiates but stalls after ~60 nts. This results in the expression of abortive transcripts. In the presence of Tat elongation is transactivated. This results in expression of viral RNAs

mRNA encoding Tat variants were made and transfected into TZM-bl cells to assess transactivation. Five J-Lat clones were treated with Tat mRNA-LNPs or small molecule agents and evaluated using flow cytometry, live cell imaging, or single cell multiomic (RNA+ATAC) profiling. Ibalizumab (anti-CD4) was conjugated to mRNA-LNPs to generate CD4-targeted mRNA-LNPs. Primary CD4 T cells were treated with Ibalizumab-targeted Tat or GFP mRNA-LNPs to assess mRNA expression, toxicity, and activation. PBMCs from people living with HIV (PLWH) on ART were treated with CD4-targeted Tat mRNA-LNPs and assayed for p24 expression in both the culture supernatant and cellular pellets.

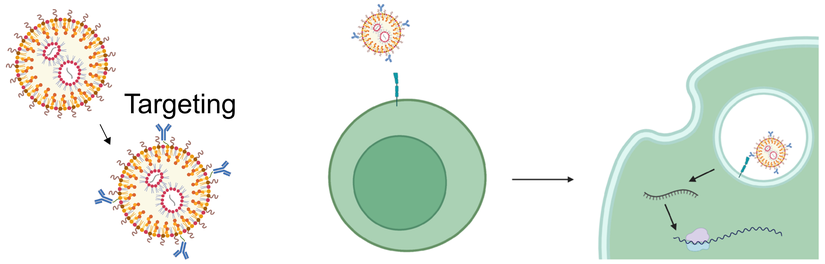


Figure 2: Antibody Targeting of Lipid Nanoparticles. Lipid nanoparticles can be covalently modified to display monoclonal antibodies on their exterior surface. This permits mRNA delivery to cells that would not normally take up LNPs, like CD4+ T cells, through receptor-mediated endocytosis and endosomal escape of the mRNA into the cytosol. In this study, the FDA approved mAb ibalizumab was conjugated to LNPs to deliver mRNA to CD4+ cells

Results

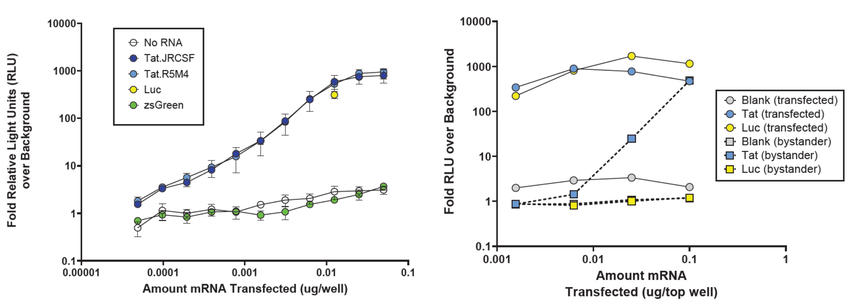
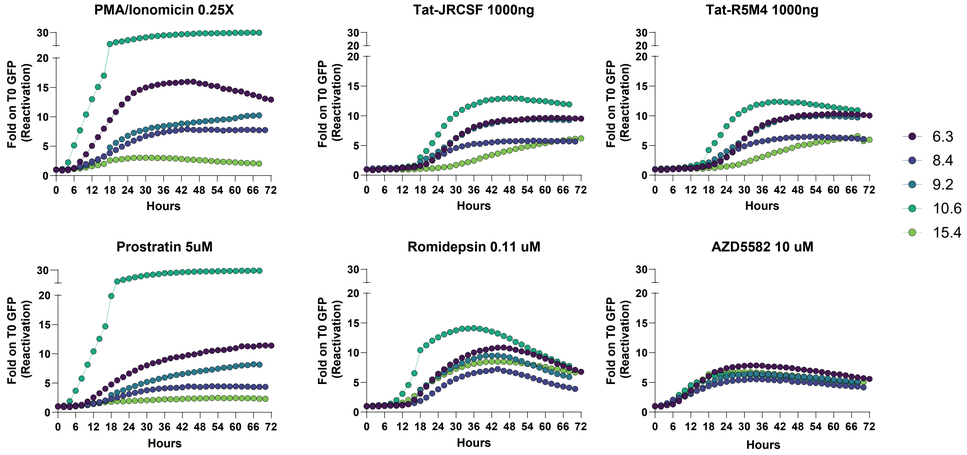


Figure 3: Potent transactivation of the HIV-1 promoter by mRNA-encoded Tat. Tat mRNA was assess for transactivation of HIV-1 expression in the TZM-bl cell line and compared to transfection of luciferase and GFP mRNA. Transfection of cells on one side of a semipermable membrane resulted in transactivation of cells on the opposite side (right).

CD4-targted Tat mRNA-LNPs selectively drive viral gene expression without evidence of cell toxicity. These findings show that viral transcriptional programs, including latency, can be modified using CD4-targeted gene therapy



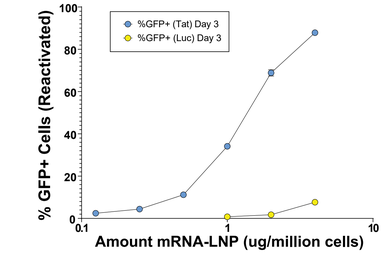


Figure 4: Tat mRNA-LNP Latency Reactivation in J-Lat Cells. (Top) Five J-Lat clones were treated with various LRAs and monitored for GFP signal with live cell imaging. (Left) Tat mRNA-LNPs and assessed for GFP expression 3 days later in J-Lat 10.6 using flow cytometry. % GFP cells, which are reactivated, is plotted on the ordinate relative to dose of mRNA transfected on the abscissa

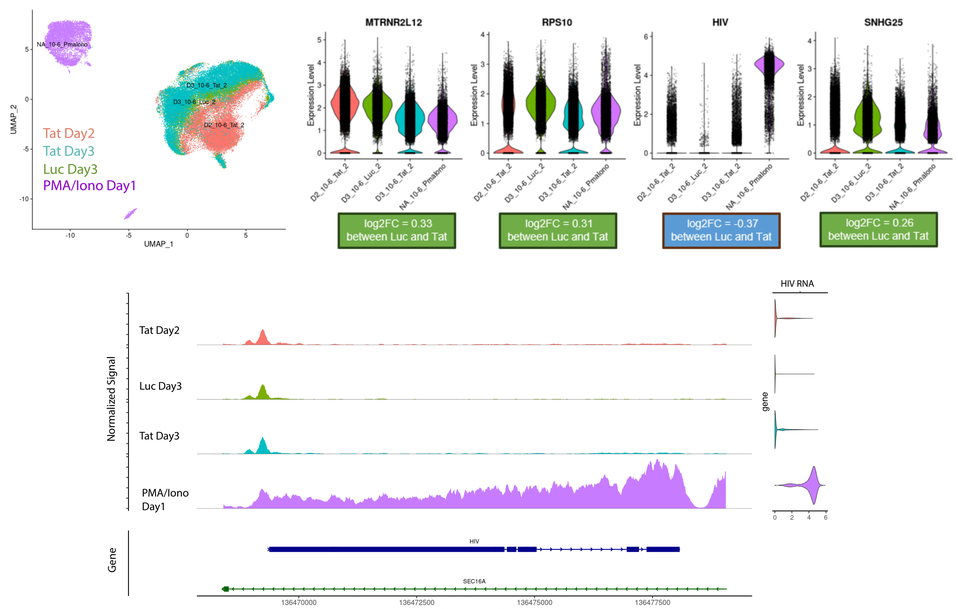
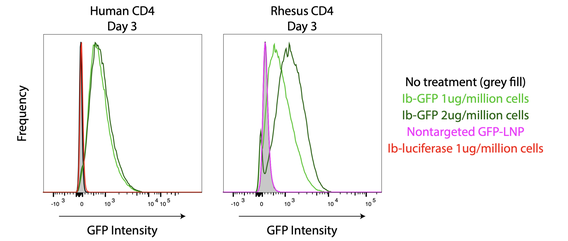


Figure 5: Multi-omic analysis of Tat-treated J-Lat 10.6 Cells. J-Lat 10.6 cells were treated with Tat or Luciferase mRNA-LNPs at 2mg/million cells (~60% reactivation, Figure 4) or PMA/Ionomycin. They were then subjecteed to mutiomic profiling with RNAseq (principle components analysis of RNAsequencing top left, differentially expressed genes top right) and ATAC sequencing (bottom, HIV-1 genome accessibility depicted) 16-72 hours later.



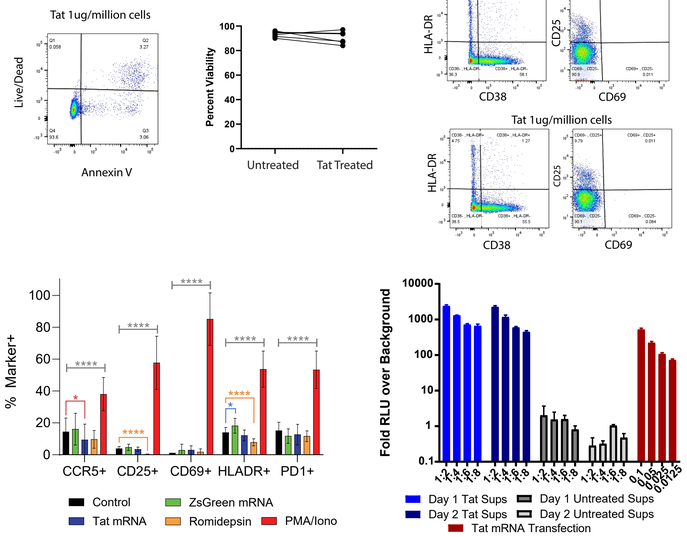


Figure 6: Toxicity and expression of CD4-Targeted mRNA-LNPS. Top: efficient mRNA delivery to human and NHP CD4-T cells using ibalizumab-targeted mRNA-LNPs. Bottom: No impact of CD4-Targeted Tat delivery on cell viability (6 donors, n.s.) or activation markers (representative and summary shown), despite evidence of Tat expression in culture supernatants.

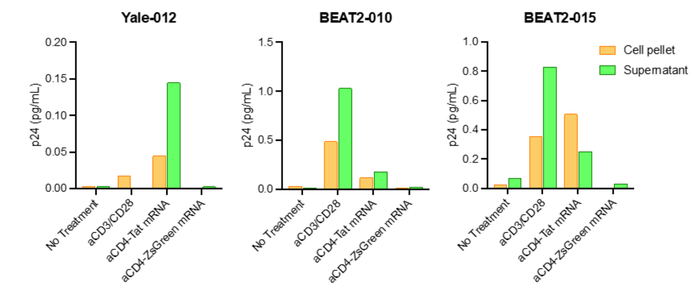


Figure 7: Reactivation of viral protein expression following CD4-Targeted Tat delivery. PBMCs from PWH on cART were left untreated, activated with anti-CD3/CD28, or treated with CD4-target mRNA-LNPs. p24 levels from cells and supernatants are plotted.

Conclusions

CD4-targeted Tat mRNA delivery may represent a new method of latency reactivation in HIV Cure strategies.

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