CD4-targeted Lipid Nanoparticle Delivery of Tat mRNA Induces HIV-1 Transcription in Hu-Mice on ART

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BACKGROUND

Many HIV cure strategies seek to expose and eliminate persistently infected cells by inducing proviral gene expression under antiretroviral therapy (ART). Because HIV-1 Tat potently amplifies proviral transcription, we developed CD4-targeting lipid nanoparticles (LNPs) encapsulating a tat variant mRNA, Tat86.R5M4, with attenuated cytotoxicity (CD4-LNP-Tat) to drive gene expression in human CD4+ T cells harboring provirus *in vivo*. To enable real-time visualization of proviral transcription dynamics and infected cell localization *in vivo*, we used a replication-complement transmitted/founder HIV-1 reporter virus expressing nanoLuciferase, enabling temporal non-invasive bioluminescent imaging (BLI).

METHODS

CD4-targeted LNPs were generated by surface-conjugation of the non-depleting anti-CD4 antibody ibalizumab. Hu-mice received intravenous CD4-LNPs encapsulating mRuby or firefly luciferase mRNA to assess cell-specific delivery. For latency reversal studies, mice infected with nanoLuciferase-expressing HIV-1_{CH040} were suppressed on combination ART and treated intravenously with CD4-LNP-Tat. Proviral transcription was monitored longitudinally by bioluminescence imaging (BLI) using an IVIS Spectrum imaging system (PerkinElmer). Other analyses included serial measurements of plasma viral loads by qPCR for viral RNA and analyses of cell-associated viral RNA as well as proviral DNA in CD4+ T cells isolated from peripheral blood and tissues.

CD4-targeted LNP delivery of HIV Tat mRNA induces proviral transcription *in vivo*

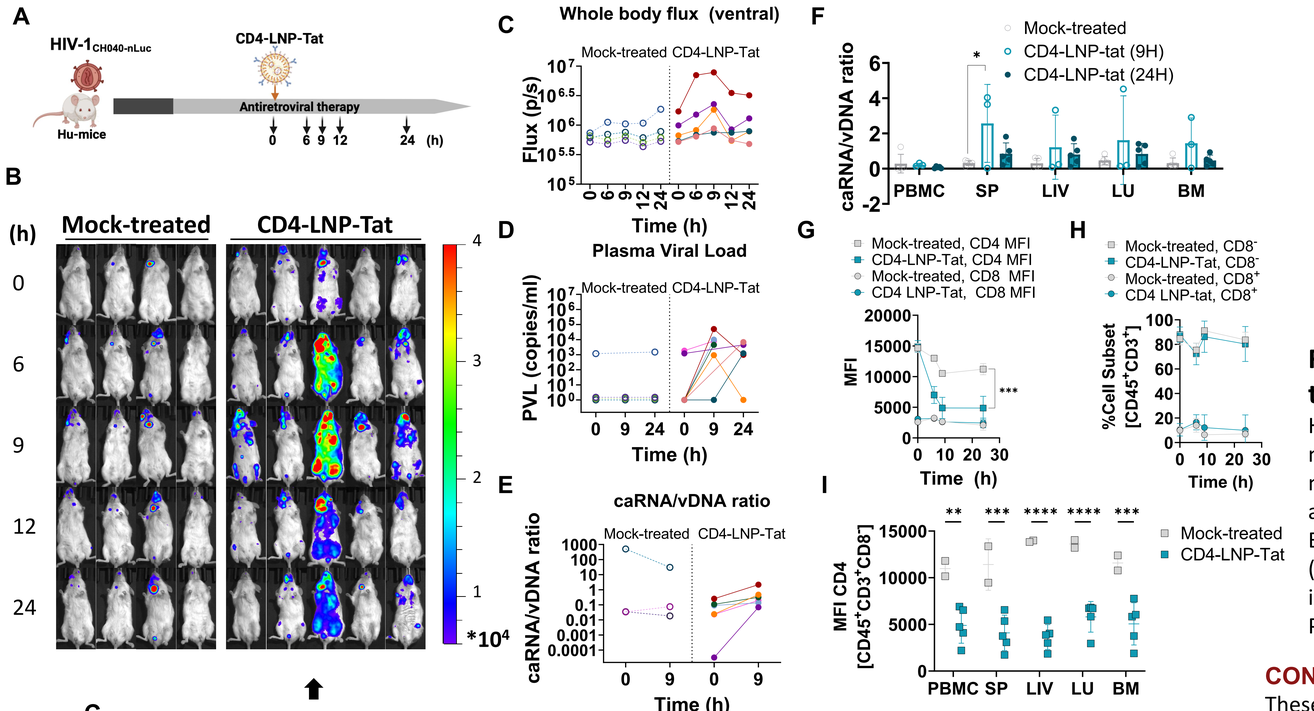


Fig 2: CD4-LNP-Tat induces HIV-1 transcription in ART-suppressed humanized mice

Hu-mice were infected with HIV-$1_{CH040-nLuc}$(30,000 PUF) and maintained on ART (TDF/FTC/DTG) for viral suppression. After 4 weeks, mice received CD4-LNP-Tat (10 µg/mouse) IV, RO administration and were monitored longitudinally by noninvasive BLI and peripheral blood sampling. Mock-treated mice received vehicle (PBS) alone. (A) Experimental outline. (B) Representative bioluminescence images. (C) Longitudinal quantification of nLuc signal from mice in (B), expressed as total flux per animal. (D–F) qRT-PCR measurements at indicated times post-administration: PVL (D), caRNA/vDNA ratio in PBMC (E), and tissues (F). Average MFI (G) and frequencies (H) for CD4- and CD8-expressing cells gated on $CD45^+CD3^+CD8^-$ and $CD45^+CD3^+CD8^+$ populations, respectively, in peripheral blood. (I) CD4 MFI on $CD45^+CD3^+CD8^-$ cells in tissues at 24 h post-administration.

RESULTS

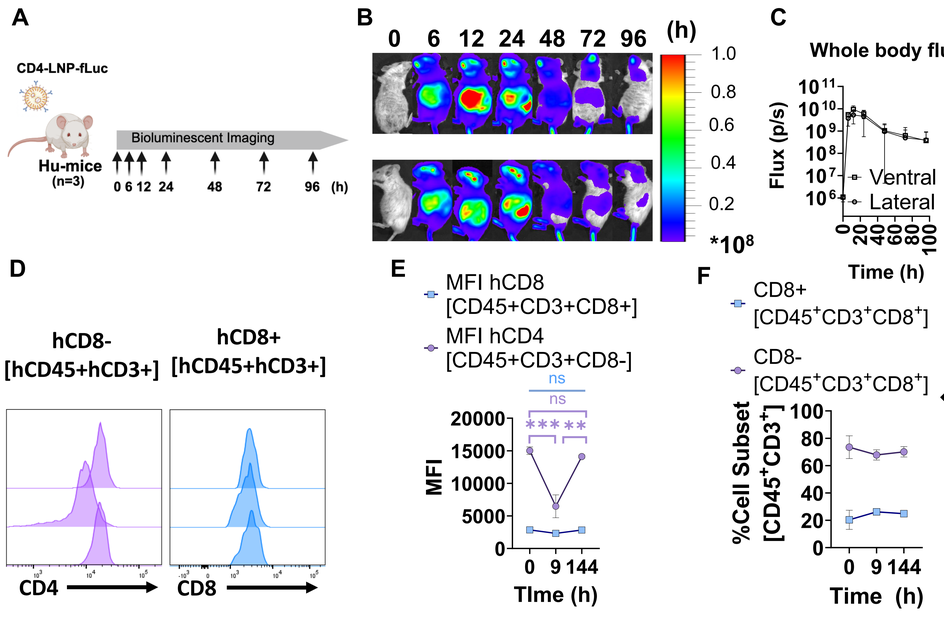


Fig 1: CD4-LNP-mediated mRNA delivery and expression kinetics in hu-mice

Hu-mice received CD4-LNP-fLuc (10 µg/mouse) via IV, RO administration and were followed longitudinally by noninvasive BLI and peripheral blood sampling. (A) Experimental outline. (B) Representative BLI images. (C) Longitudinal quantification of fLuc signal shown as average total flux per animal (n=3). (D) Representative flow cytometry histograms showing surface CD4 and CD8 expression on $CD8^-$ and $CD8^+$ T cells in peripheral blood. (E–F) Mean fluorescence intensity (MFI, E) and frequencies (F) of the populations shown in (D).

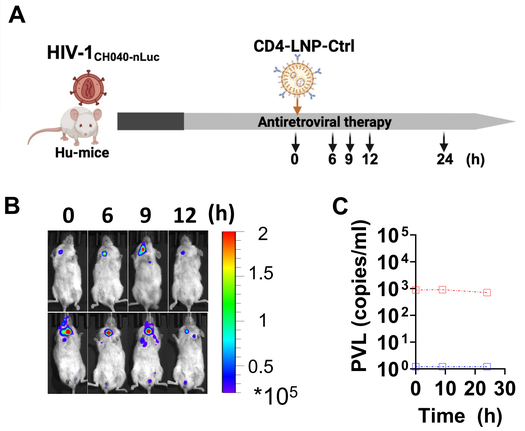


Fig 3: Control LNPs do not induce HIV-1 transcription

Hu-mice were infected with HIV-$1_{CH040-nLuc}$ and maintained on ART for viral suppression. After 4 weeks, mice received CD4-LNP-control (10 µg/mouse) IV, RO administration and were monitored longitudinally by BLI and peripheral blood sampling.
(A) Experimental outline. (B) Representative ventral BLI images (C) Plasma viral load (PVL) measured by qRT-PCR at indicated time points.

CONCLUSIONS

These findings establish CD4-targeted LNP-mediated Tat mRNA delivery as a focused approach to induce HIV-1 proviral transcription *in vivo* during ART enabling targeted interrogation of latency and informing the development of controlled latency-reversal strategies.

ACKNOWLEDGEMENTS

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Figures were generated with Biorender.com and GraphPad Prism

PLAIN LANGUAGE SUMMARY

CD4-targeted lipid nanoparticles carrying Tat mRNA can briefly awaken HIV that remains hidden during antiretroviral therapy, making infected cells visible and potentially enabling their detection and selective clearance from the body.