CXCR4 Targeted CRISPR/Cas9 Lipid Nanoparticles for Excision of Latent Proviral DNA

534

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BACKGROUND

A key challenge in curing HIV lies in identifying and removing integrated proviral DNA from viral target cells, such as CD4+ T and myeloid cells. Recently, the creation of a viral vector designed to deliver CRISPR guide RNAs (gRNAs) demonstrated the potential for eliminating HIV-1. Building on these findings, we have advanced the approach by developing: (1) Multiple gRNAs targeting viral exons,(2) Lipid nanoparticles (LNPs) designed to target viral reservoirs,(3) LNPs modified with a CXC chemokine receptor-4 (CXCR4) ligand. We propose that this targeted delivery of gRNAs using LNPs could successfully remove viral DNA from the viral reservoirs.

METHODS

A library of gRNAs was screened across over 4000 HIV strains to derive a dual gRNA system to disrupt five HIV-1 exons (tat1-2/rev1-2/gp41). CXCR4-targeting peptide decorated LNP (T-LNP) was formulated by using microfluidic mixing. The receptor specificity was shown by CXCR4 inhibition assay. IVIS was performed to demonstrate lymphoid tissue targeting on humanized mice (hu-mice). The intact proviral DNA assay (IPDA) assay was utilized to test CRISPR gRNA delivery and viral elimination efficiency in latently HIV-infected lymphocytic cell lines (JLat 8.4, Jurkat E6), human peripheral blood lymphocytes (PBLs), and lymphoid tissue from HIV-infected, ART-treated hu-mice.

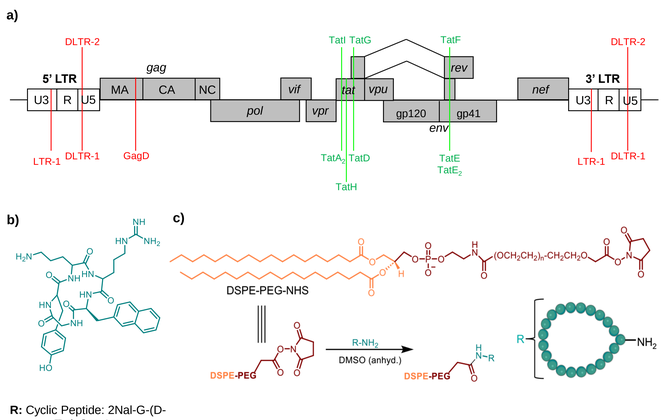


Figure 1. *a) In comparison with LTR-gag gRNAs, TatDE mosaic gRNA targets multiple exons in the HIV-1 genome. b) Chemical structure of CXCR4 targeting cyclic peptide c) Reaction scheme for the synthesis of DSPE-PEG conjugated cyclic peptide.*

CXCR4-targeted lipid nanoparticles selectively removed Proviral DNA from the HIV-1 reservoir

RESULT

T-LNP and a control LNP without CXCR4 targeting (C-LNP) were tested in primary CD4+ T cells, cell lines, and hu-mice. Unlike C-LNP, T-LNP in humanized mice showed a significant shift in tissue targeting from the liver to the spleen, a primary HIV reservoir. T-LNP showed significantly higher viral excision efficiency in latently infected cells whose specificity was confirmed by a receptor inhibition assay through increasing concentrations of a CXCR4 inhibitor. T-LNP achieved nearly 80% viral DNA excision in HIV-1-infected PBLs, compared to ~7% with C-LNP. In HIV-1-infected, ART-treated humanized mice, T-LNP showed ~60% CRISPR efficacy in splenic tissue and blood, while C-LNP showed <5% efficacy, analyzed by the IPDA.

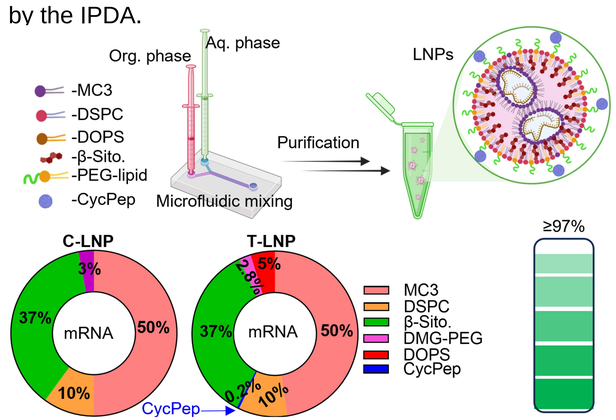


Figure 2. *(A) Schematic representation of LNP formulation via microfluidic mixing and subsequent purification. (B) Donut charts showing the molar composition of the lipids used to formulate C-LNP and T-LNP. (C) mRNA encapsulation efficiency of LNPs, evaluated using the RiboGreen assay, indicates >97% encapsulation for both formulations.*

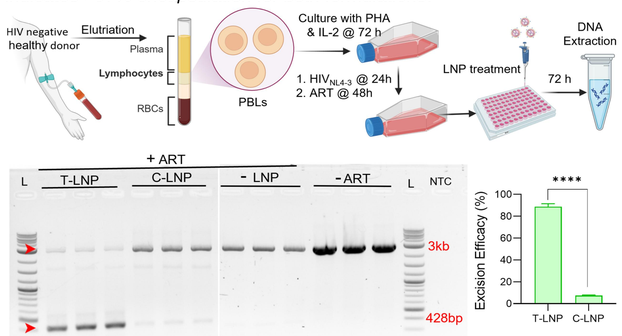


Figure 3. *(A) Timeline of elutriation, HIV infection, ART, LNP treatment, and DNA extraction of PBLs collected from healthy HIV-seronegative donors. (B and C) PCR gel showing near-complete HIV-1 DNA excision with T-LNP. (C) Excision efficiency quantification using ImageJ and densitometric analysis.*

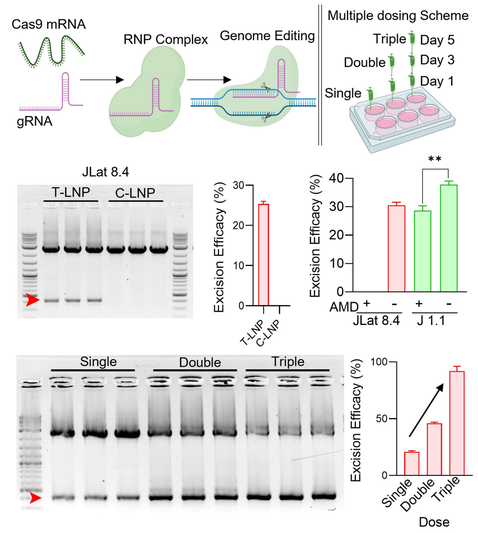


Figure 4. *(A) Schematic of CRISPR-Cas9 gene editing. (B) Multiple LNP doses. (C, D) DNA excision efficacy of C-LNP and T-LNP in JLat 8.4 cells. (E) Effect after AMD070 treatment. (F, G) PCR gel and quantification of HIV-1 DNA excision after multiple LNP doses in JLat 8.4 cells.*

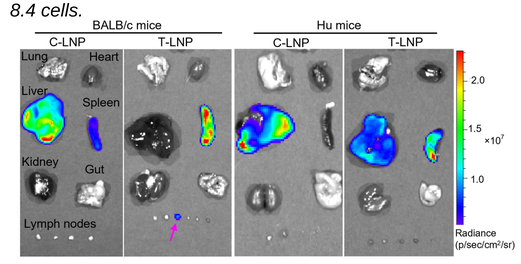


Figure 5. *Tissue selective mRNA translation of LNP in the BALB/c and humanized mice (Hu mice).*

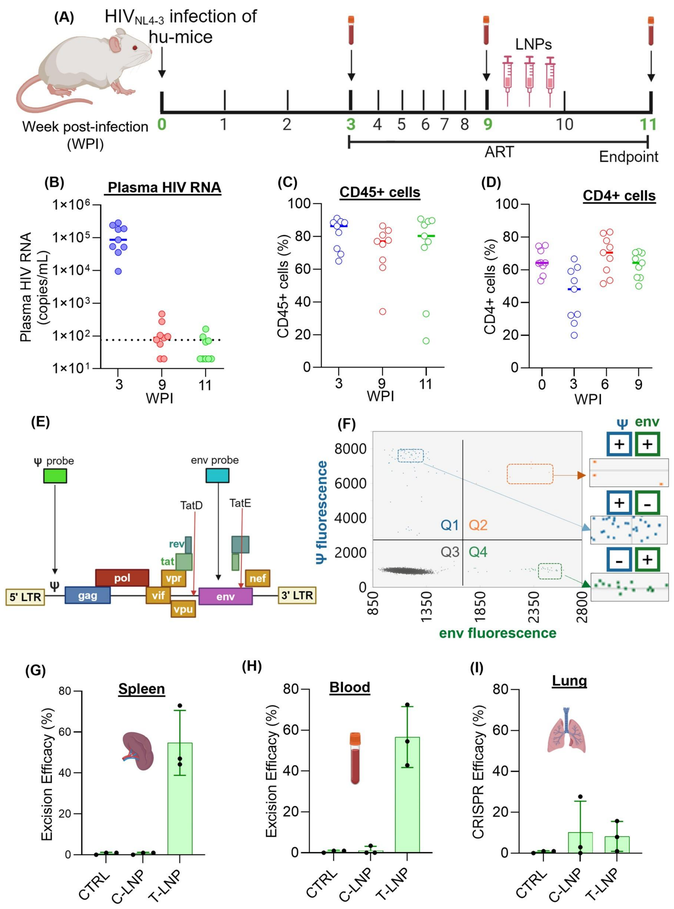


Figure 6. *(A) Study timeline for blood collection for immune cell profiling, HIV infection, ART and LNP treatments, and endpoint with sacrifice of humanized mice, followed by collection of blood and major organs. (B) Plasma HIV-1 RNA copies. (C, D) Levels of CD45+ and CD4+ cells in the mice blood. (E) Schematic representation of the HIV-1NL4-3 genome. (F) Representative illustration of the intact proviral DNA assay (IPDA) 2D duplexed droplet digital PCR plot. (G, H, I) HIV-1 DNA excision efficacy of LNPs in spleen, blood, and lung, analyzed by IPDA assay.*

CONCLUSIONS

T-LNP demonstrated lymphoid tissue and cell-targeted CRISPR/Cas9 delivery. This delivery system was effective in eliminating HIV-1 DNA from infected CD4+ T cells. Furthermore, T-LNPs have shown superior excision efficiency compared to C-LNP in eliminating HIV-1 DNA from hu-mice infectious reservoirs. Comparisons between Cas9 and 12s are underway based on the latter targeting of the T-rich protospacer adjacent motif.

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