Lipid Composition and CXCR4 Decoration Facilitate HIV-1 CRISPR Delivery and Viral Excision

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BACKGROUND

A principal limitation in achieving an HIV cure rests in finding, and then eliminating integrated proviral DNA in viral target cells (CD4+ T and myeloid cells). Recently, the development of a viral vector to deliver clustered regularly interspaced short palindromic repeats (CRISPR) guide RNAs (gRNAs) provided proof of concept that HIV-1 elimination could be achieved. We now extend those results by creating:

(1) Multiple viral exons targeting gRNAs
(2) Viral reservoir-targeted lipid nanoparticles (LNPs)
(3) Decorating LNPs with CXC chemokine receptor-4 (CXCR4) ligand

We posit that targeted LNP-based delivery of gRNA can eliminate 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 cyclic-peptide (Cyc) conjugated LNP was formulated by microfluidic mixing. The CXCR4 receptor specificity of the LNP was shown by blocking to demonstrate lymphoid targeting. The delivery of gRNA and subsequent CRISPR excision were tested in lymphocytic (JLat 8.4 & Jurkat E6) and promonocytic (U1) cell lines and also confirmed in infected primary human lymphoblasts.

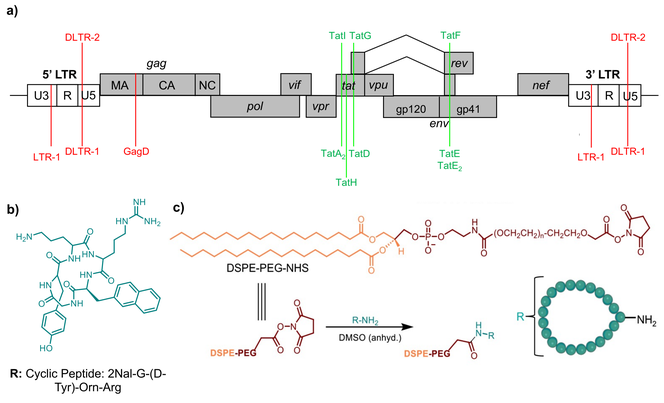


Figure 1. a) In comparison with LTR-gag gRNAs, TatDE mosaic gRNA targets multiple exons in the HIV-1 genome. b) Chemical structure of CXCR4 targeting cyclic peptide. c) Reaction scheme for the synthesis of DSPE-PEG conjugated cyclic peptide.

Compositionally unique, CXCR4-targeted lipid nanoparticles can specifically eliminate viral DNA from HIV reservoir.

RESULTS

CXCR4 targeted LNP (T-LNP) was formulated with CRISPR gRNA-Cas9, D-Lin-MC3-DMA, DOPE, DOPS, DMG-PEG and β-sitosterol. In hu-mice, T-LNP showed a shift in tropism from the liver to spleen; one of the major HIV reservoirs. Further analysis revealed a substantially high mRNA expression of T-LNP in CD4+ T cells compared to nontargeted C-LNP. T-LNP demonstrated nearly 60% excision of viral DNA in lymphocytic cell lines; however, it was only 15% in monocytic cells. Moreover, in the presence of the CXCR4 blocker, T-LNP demonstrated a decrease in DNA excision efficacy, confirming their CXCR4-specificity.

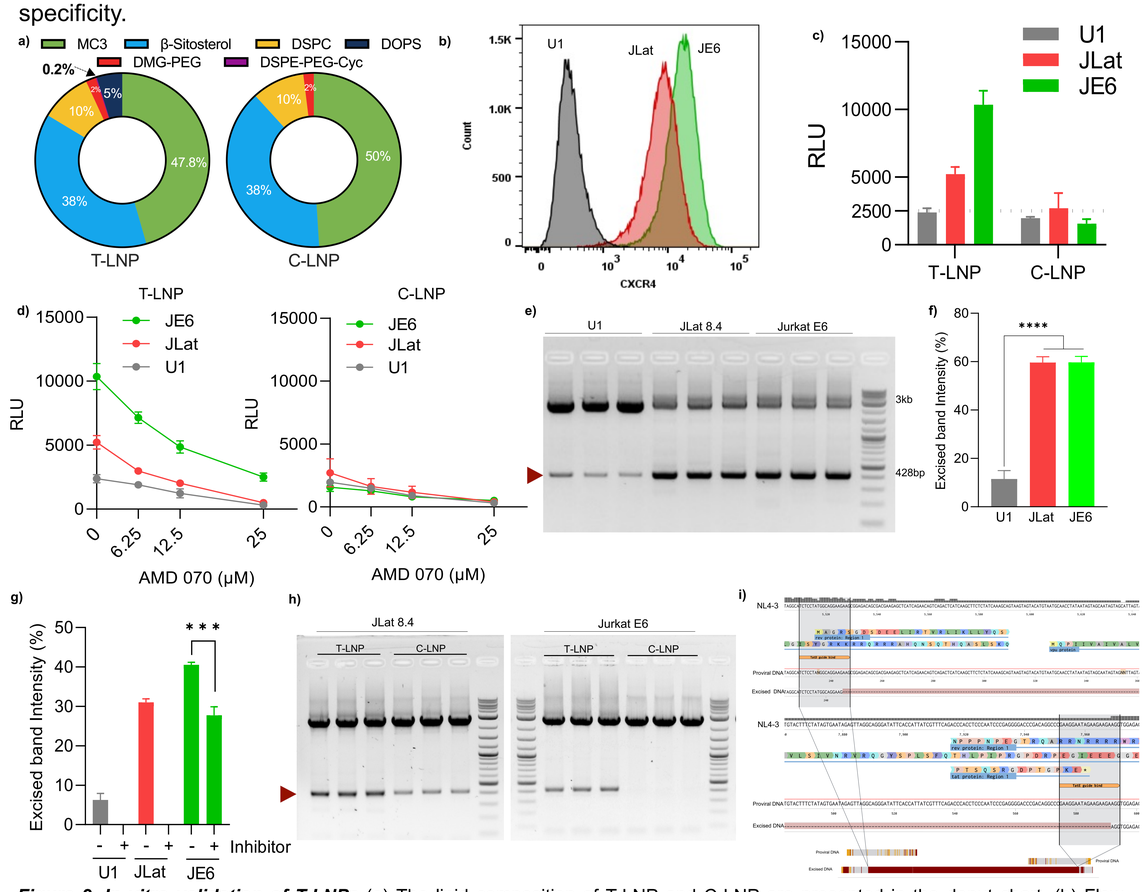


Figure 2. In vitro validation of T-LNPs (a) The lipid composition of T-LNP and C-LNP are presented in the donut chart. (b) Flow cytometry analysis revealed higher CXCR4 expression on T-lymphocytic JLat 8.4, Jurkat E6 cell lines as compared to monocytic U1 cell lines. (c,d) CXCR4-receptor induced mRNA expression of LNPs. (e to h) Cell-specific proviral DNA elimination efficacy of LNPs with TatDE CRISPR gRNAs. i) Sanger sequencing on the excised amplicon confirms CRISPR edit.

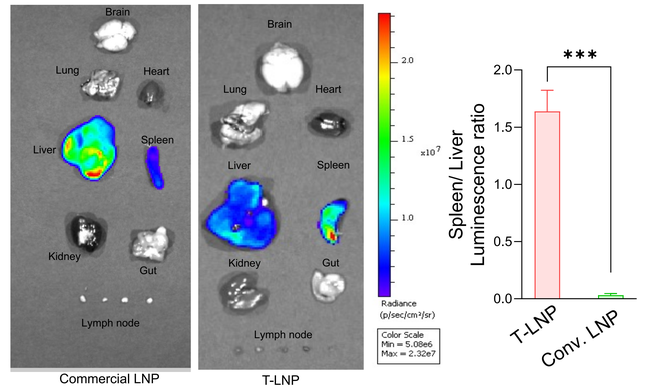


Figure 3. In vivo biodistribution of LNPs. a) Representative biodistribution of LNPs in NSG Hu-mice, captured under IVIS, after 6 h of intravenous injection. b) T-LNP showed ~1.8 times higher spleen/liver luminescence ratio compared to conventional LNP.

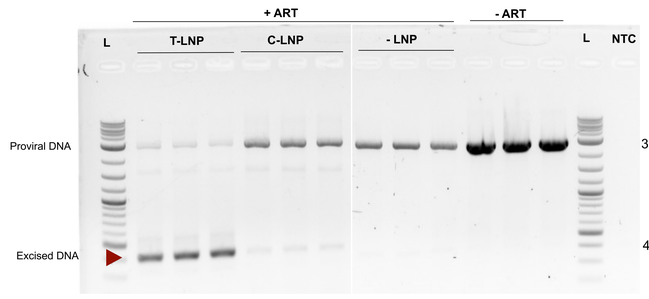


Figure 4. Ex vivo viral elimination efficacy of T-LNP in primary peripheral blood lymphocytes (PBLs). Agarose gel electrophoresis blot shows excised proviral DNA after treatment with CRISPR gRNAs containing T-LNPs.

CONCLUSIONS

- ❑ CXCR4 targeting ligand conjugated T-LNP was successfully synthesized by microfluidic mixing.
- ❑ T-LNP shows CD4+ T-cell specific mRNA expression and demonstrated substantially higher viral elimination from the lymphocytes than in monocytes.
- ❑ T-LNP with unique lipid composition shifts the tropism from the liver to the spleen, one of the major HIV-1 reservoirs.
- ❑ T-LNP shows ~90% viral elimination efficacy from the HIV-infected PBLs.
- ❑ This work provides significant insight into the development of a non-viral delivery vector for HIV-1 reservoirs targeted gene therapy.

Acknowledgements

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