CD4 T cell-Targeting Lipid Nanoparticles in the Search for a Genetic HIV Cure

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BACKGROUND

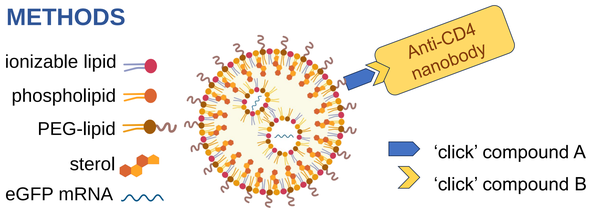
Successful HIV treatments show that a cure requires clearing the HIV reservoir and making CD4 cells resistant to reinfection. **CRISPR-Cas9** offers potential for **excising proviral DNA** and **mutating CCR5**, but **targeted delivery** remains a challenge. Here, we introduce CD4-targeted lipid nanoparticles (LNPs) for precise, efficient, and safe CRISPR-Cas9 delivery.



HIV provirus excision

CCR5 mutation

METHODS

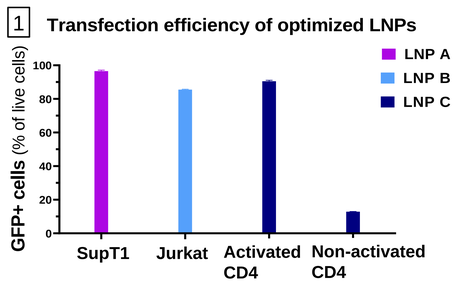


We first screened a **LNP library** to identify a lipid formulation with optimal particle size, surface charge and eGFP mRNA encapsulation efficacy. We selected LNPs with optimal binding and transfection potential in various primary and immortalized **T cells.**

Next, we packaged single guide RNA (**sgRNA**) and **Cas9 mRNA** into our optimal LNP formulation and delivered it to T cells. We tested sgRNAs for ***CCR5*** in T cells, and sgRNAs for HIV **Tat** and **LTR** in J-Lat 10.6 cells, to evaluate the efficiency of **co-receptor gene editing** and **proviral excision**, respectively.

Finally, we applied **click chemistry** to conjugate the optimal LNP to **anti-CD4 nanobodies (Nbs),** targeting them towards CD4+ cells. We labeled the CD4 Nb-LNP conjugates with DiD dye and evaluated the binding to T cells both *in vitro* on primary CD4 cells as *in vivo* in **humanized mice** (NSG-SGM3 model), using flow cytometry.

RESULTS: optimized LNPs efficiently transfect and edit *CCR5* in CD4 T cells



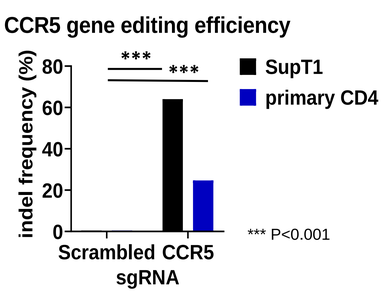


Figure 1 Our LNP screening identified optimized eGFP mRNA LNP formulations for human CD4 T cell transfection, with transfection efficiencies up to 95%.

Figure 2 CRISPR LNPs containing CCR5 sgRNA successfully mutated CCR5 in primary and immortalized T cells. Sanger sequencing of PCR amplicons showed insertion and deletion (indel) frequency up to 65% (*** P<0.001).

RESULTS: anti-HIV CRISPR LNPs hamper HIV infection

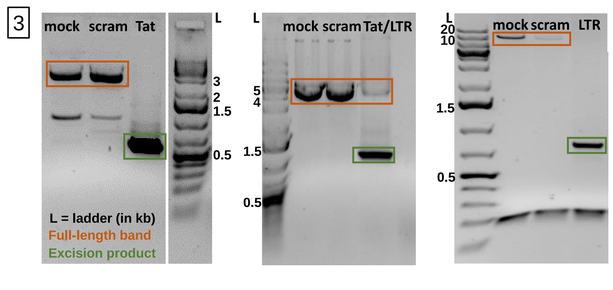
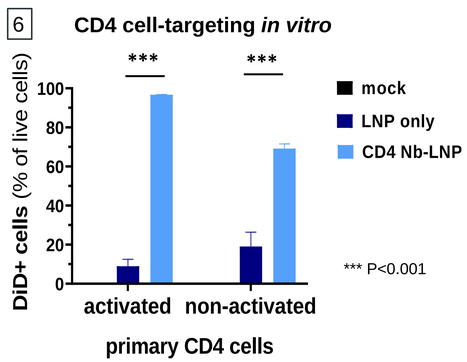


Figure 3-4 Delivery of Tat or Tat/LTR CRISPR LNPs to J-Lat 10.6 cells resulted in the expected cleavage of HIV proviral DNA, as confirmed by nested PCR and agarose gel electrophoresis. Lastly, LTR CRISPR LNPs resulted in up to 100% proviral excision and up to 80% indels in the HIV LTR (***P<0.001).

RESULTS: CD4 cell-targeting LNPs for precise delivery to CD4+ cells



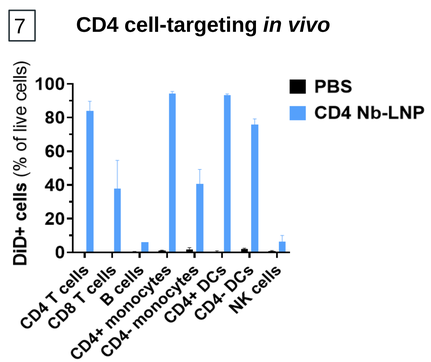


Figure 6 Conjugation of the optimal LNP to anti-CD4 nanobodies (Nbs) enhances the binding to primary human CD4 cells significantly, as indicated by the increase in DiD+ cells for both activated as non-activated CD4 cells compared to LNP alone.
Figure 7 *In vivo* administration of DiD-labeled CD4 Nb-LNPs to humanized mice shows preferential binding to circulating CD4+ cells, compared to CD4- cells.

Optimized lipid nanoparticles enable efficient *CCR5* gene editing and HIV proviral excision

LTR CRISPR LNPs inhibit HIV infection and virion production

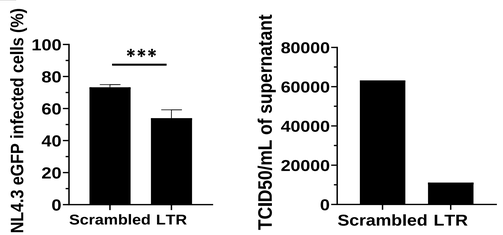


Figure 5 Anti-HIV CRISPR LNPs were able to inhibit infection of SupT1 cells with replication competent HIV*NL4.3 eGFP*, (left)(*** P<0.001). Also, we observed reduced virion production in the supernatant of the cells, as determined by TZMbl assay (right).

CONCLUSIONS

With successful gene editing of the CCR5 co-receptor and full-length HIV proviral excision, we provide proof of concept for **CRISPR-Cas9 LNPs** as a genetic **cure for HIV**. Moreover, coupling with a CD4-nanobody targets LNPs to CD4+ cells *in vitro* and *in vivo*, supporting more **specific** and **safer delivery to HIV-target cells**.
We will now validate CD4-targeted CRISPR LNPs co-encapsulating CCR5 and anti-HIV sgRNAs in a **humanized mouse model of HIV infection.**

ADDITIONAL KEY INFORMATION

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