Dual Receptor Targeted LNPs Enhance HIV-1 Proviral DNA Excision

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BACKGROUND

Since its inception, HIV-1 retrovirus has caused more than 40 million deaths worldwide, exhibiting a tremendous epidemiological burden. ART has been employed as a gold standard treatment for HIV-1 for decades; however, therapeutic interruption results in viral rebound, and the reasons underlying this rebound viremia are the existence of latent viral reservoirs in several cellular and tissue compartments of the body. Latency development in HIV-1 infection is associated with an exclusive increase in CCR5 receptor expression in the latently infected cells; Hence, we formulated CCR5-targeted Lipid Nanoparticles containing an FDA-approved ionizable lipid MC3. Encapsulating CRISPR-Cas9 mRNA in LNPs to excise the latent Proviral DNA resulted in selective targeting and elimination of HIV-1 from both T-lymphocyte and myeloid cellular compartments. The in vivo mRNA translation studies demonstrated exclusive expression in the spleen and lymph nodes that harbor a considerable viral reservoir.

METHODS

CCR5-targeted lipid nanoparticles (LNPs) constructs encapsulating CRISPR-Cas9 and guide mRNA (gRNA) were formulated using microfluidics. Ribogreen assay was performed to determine the mRNA encapsulation efficiency. Nanoparticle size and zeta potential were analyzed by Dynamic Light Scattering (DLS). Luciferase and cell viability assay were conducted to analyze the nanoparticle's toxicity profile and differential uptake efficiency in human macrophages. CCR5-targeted CRISPR-Cas9 encapsulated LNPs were tested for their HIV-1 excision efficiency in primary human monocyte-derived macrophages (MDMS), T-lymphocytic, and monocytic cell lines. Protein corona assessment studies were conducted to obtain mechanistic insights into the endogenous targeting abilities of the LNP. In vivo analysis of mRNA translation was performed by the incorporation of helper lipid DOPS.

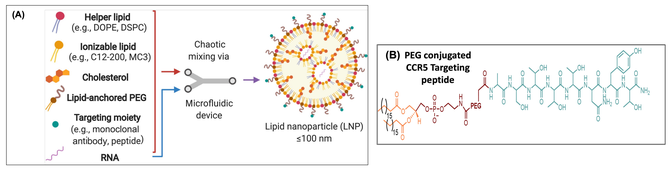


Figure 1. (A) Formulation scheme for the CCR5 peptide conjugated Lipid Nanoparticles using microfluidics. **(B)** Chemical structure of the PEG-conjugated CCR5 targeting peptide incorporated in the targeted LNP formulation.

CCR5 Targeted LNPs demonstrate superior HIV-1 excision efficiency in both lymphoid and monocytic cellular compartments

RESULTS

The formulated CCR5 receptor-targeted LNPs (M5-LNPs) had an mRNA encapsulation efficiency of more than 90% and a particle size of less than 100nm. The developed LNPs were non-toxic at doses ranging from 16µg to 1µg per million cells in primary macrophages. The protein translation efficacy was higher in M5-LNPs compared to the control LNPs in a dose-dependent manner, as determined by the expression of reporter luciferase mRNA. M5-LNPs encapsulating CRISPR-Cas9 mRNA targeted towards the latent Proviral HIV-1 DNA achieved higher HIV-1 excision efficiency in both monocytic and lymphocytic cell lines relative to the control LNPs. M5-LNPs also exhibited superior HIV-1 excision efficiency in HIV-1-infected MDMs relative to the control. SDS page analysis revealed the spectrum of proteins associated with the unique protein corona of MC3, containing LNPs with and without the presence of helper lipid in the LNP composition. In *vivo* mRNA translation resulted in protein expression localized to the spleen and lymph nodes, which harbor latently infected cells.

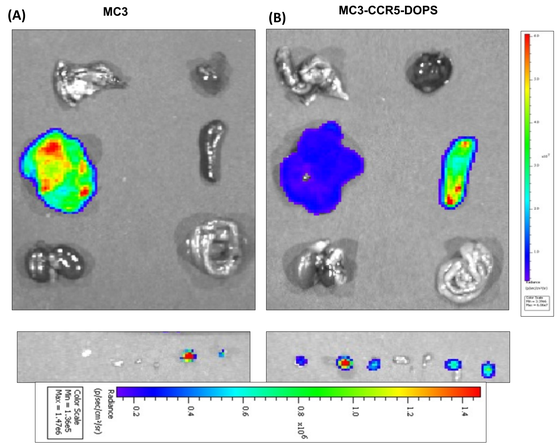
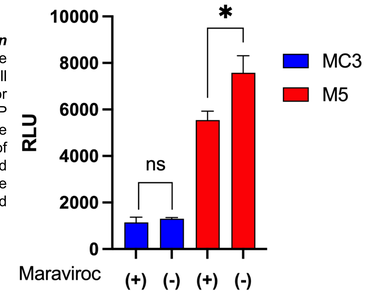


***Figure 3. Ex vivo* mRNA translation *of MC3 LNPs*. (A, B)** A representative mRNA translation image of MC3 LNPs in ex vivo organs dissected from a BALB/c mouse captured under IVIS. LNPs with a Fluc-mRNA dose of 0.5mg/kg were injected via the tail vein, and biodistribution was observed at 6h post-injection. LNPs were injected with and without the presence of helper lipid DOPS in the formulation composition, and the luminescence intensity in the form of total flux in mice was quantified

CCR5 Inhibition in Primary Macrophages

Figure 4. CCR5 Receptor Inhibition Analysis: Primary macrophages were treated with 0.25ug/million cell concentration of a CCR5 receptor antagonist maraviroc before LNP treatment. Subsequently, cells were treated with 2ug/million cell dose of Fluc mRNA in both the targeted and untargeted LNPs. Their relative luminescence intensities were plotted and compared.



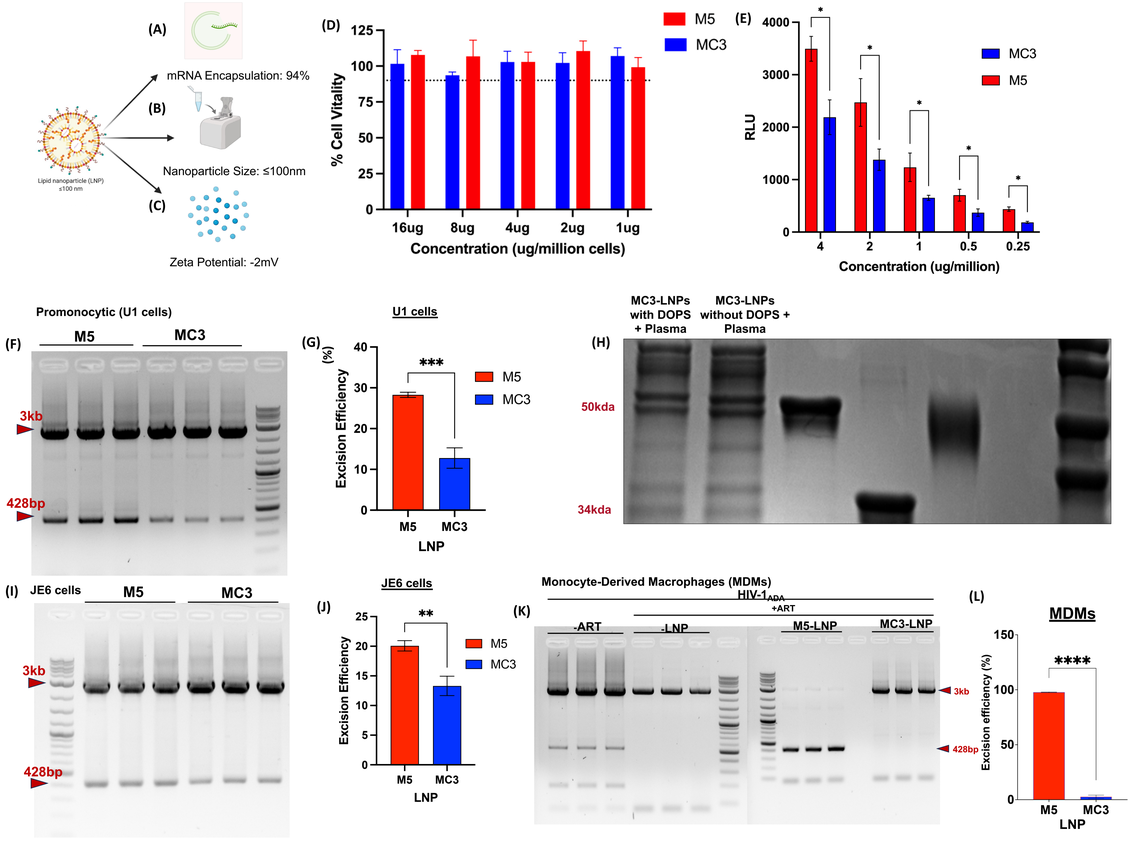


Figure 2. Formulation and in vitro validation: **(A, B, C)** Schematic illustrating the determination of mRNA encapsulation efficiency, zeta potential, and particle size of LNPs. **(D)** Assessment of cell viability via the CTB assay in MDMs at different mRNA dose concentrations. **(E)** Luciferase mRNA translation efficacy of CCR5-targeted and control LNPs in MDMs; data were recorded as RLU in a dose-dependent manner. **(F, I)** PCR gel electrophoresis blots showing excision of HIV-1 DNA in latently infected monocytic (U1) and lymphocytic (JE6) cells treated with CRISPR-Cas9 encapsulating M5 and MC3-LNPs at a concentration of 2 µg/million cells. **(K)** *Ex vivo* evaluation of HIV-1 excision efficacy of CCR5-targeted and control LNPs in MDMs infected with HIV-1_{ADA}. **(G, J, L)** Quantification of HIV-1 DNA excision efficacy of LNPs using ImageJ and densitometric analysis. **(H)** SDS Page analysis of the LNP adsorbed plasma proteins relative to the proteins responsible for specific organ Tropism.

CONCLUSIONS

- CCR5-targeted LNPs demonstrated more than 90% mRNA encapsulation efficiency.
- CCR5-targeted LNPs resulted in higher luciferase mRNA translation efficacy than untargeted LNPs in MDMs.
- CRISPR-Cas9 encapsulating CCR5-targeted LNPs exhibited greater HIV-1 excision in both monocytic and lymphocytic cells, as well as in HIV-1-infected MDMs, relative to control LNPs.
- SDS page analysis revealed the protein corona composition adsorbed onto the LNPs.
- In vivo mRNA translation resulted in protein expression localized to the spleen and lymph nodes, which harbor latently infected cells.
- Dual-targeted LNPs could enable concurrent CRISPR delivery to CD4+ T cells and MDMs, strengthening the viral elimination strategy.

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