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MYELOID TARGETED DISULFIDE LIPID NANOPARTICLE WITH ENHANCED ENDOSOMAL ESCAPE IMPROVES HIV-1 EXCISION

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BACKGROUND

Adenovirus-based CRISPR-Cas9 delivery systems have failed in clinical settings possibly due to the immunogenicity and poor targeting, but lipid nanoparticles (LNPs) offer a safer alternative for targeting viral reservoirs and excising proviral DNA in the infected cells (macrophages and CD4+ T cells). However, poor endosomal escape of LNP remains a major limitation. To overcome this issue, we designed a disulfide-based ionizable lipid library to leverage the reactive oxygen species (ROS) in myeloid phagolysosomes. The ROS-responsive cleavage of disulfide bonds enhances endosomal escape, improving cytosolic delivery of CRISPR-Cas9 guide RNAs (gRNAs) for excision of HIV proviral DNA in infected myeloid cells.

METHODS

A library of disulfide-based ionizable lipids was synthesized and formulated into lipid nanoparticles (LNPs) using microfluidics. The LNPs were characterized for size, charge, and morphology. The efficacy of FLuc reporter mRNA translation in monocyte-derived macrophages (MDMs) guided our selection of the lead ionizable lipid, in comparison with FDA-approved lipids, MC3 and ALC-0315. Biodistribution was evaluated using IVIS imaging, and confocal microscopy confirmed enhanced endosomal escape. The interaction between LNPs and plasma proteins in the bloodstream was analyzed using SDS-PAGE and Western blot. We validated CCR5-targeted LNPs' ability to deliver CRISPR-Cas9 and excise HIV-1 proviral DNA in infected MDMs using PCR, gel electrophoresis, and Sanger sequencing.

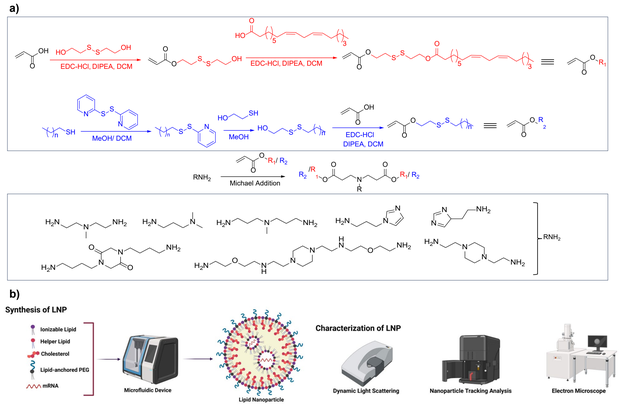

Figure 1. a) Synthetic scheme for the synthesis of disulfide based ionizable lipid, b) Schematic showing LNP preparation via microfluidic device and subsequent characterization of LNP for size, concentration and morphology.

CCR5 decorated disulfide-based LNPs targets latent viral reservoirs and is able to excise proviral DNA from primary macrophages.

RESULTS

Our lead candidate, BA-LNP, has an approximate size of 88 nm and a near-neutral surface charge, demonstrating > 94% mRNA encapsulation efficiency and maintaining > 80% cell viability at therapeutic doses. Compared to MC3 and ALC-0315, BA-LNPs achieved 20-fold higher FLuc mRNA translation in MDMs, indicating superior endosomal escape and cytosolic mRNA delivery. IVIS imaging showed selective mRNA translation in lung and spleen tissues, which are key HIV-1 reservoirs. Confocal microscopy confirmed that BA-LNPs had enhanced endosomal escape compared to MC3-LNPs, supporting their improved mRNA translation efficacy. Western blot analysis identified fibrinogen and β2-glycoprotein as key plasma proteins responsible for organ tropism. Finally, CCR5-targeted BA-LNPs facilitated 80% CRISPR-mediated excision of proviral DNA in infected MDMs, compared to non-targeted LNPs, as confirmed by PCR and Sanger sequencing, highlighting their potential in reservoir-targeted HIV-1 therapeutics.

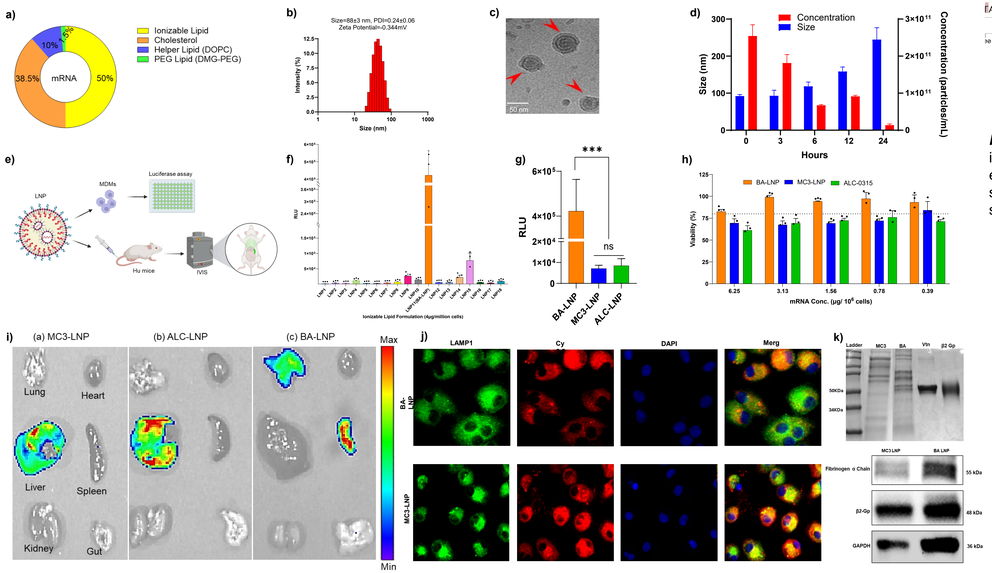

Figure 2. a) Pie chart of LNP composition, b) Size, PDI and zeta potential of LNP measured by dynamic light scattering, c) Cryo-EM image of LNP (particle are designated by red arrowhead), d) plasma stability of LNP over a period of 24 h, e) Schematic showing LNP treatment and transfection efficacy measured using luciferase assay, along with biodistribution of LNPs in humanized mice (hu mice), f) Screening of synthesized ionizable lipid in primary MDMs, g) Protein translation efficiency of lead candidate (BA-LNP), MC3-LNP, and ALC-LNP in primary MDMs, h) MDM cell viability following treatment with BA-LNP, MC3-LNP, and ALC-LNP, i) Biodistribution of BA-LNP in NSG Hu-mice compared to commercial LNP, captured under IVIS, after 6hr of intravenous injection, j) Confocal Microscope image to check endosomal escape efficacy of BA-LNP against MC3-LNP after 12hrs where LNPs were dyed with Cy5.5 (red) and LAMP1 (green) was used as the lysosomal marker, k) SDS-PAGE and Western Blot confirming the interaction of LNP with specific plasma protein responsible for organ tropism.

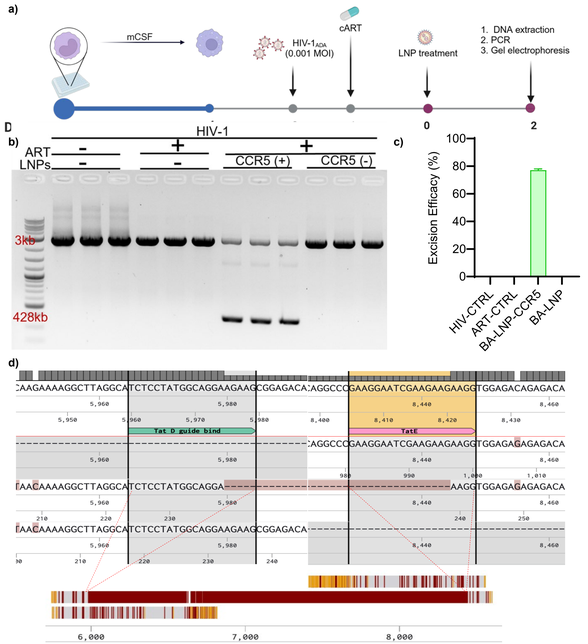

Figure 3. a) Schematic illustrating the workflow for culturing MDMs and were infected with HIV-1ADA and treated with ART and LNPs, b-d) Agarose gel electrophoresis blot shows excised proviral DNA, with CCR5 targeted BA-LNP showing almost 80% excision of proviral DNA which was then confirmed via Sanger sequencing

CONCLUSIONS

- BA-LNPs exhibited 20-fold higher mRNA translation in MDMs compared to MC3 and ALC-0315.
- Biodistribution studies revealed selective organ tropism in the lungs and spleen, key HIV reservoirs.
- Western blot identified fibrinogen and β2-glycoprotein as key drivers of organ tropism.
- CCR5-targeted BA-LNPs enabled 80% HIV-1 proviral DNA excision in infected MDMs, confirmed by PCR and Sanger sequencing.
- These findings establish BA-LNPs as a potential non-viral CRISPR delivery platform for HIV-1 reservoir targeting.

Acknowledgements
NIH Funding sources: R01MH121402, R01MH115860
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