Potent, HIV-specific latency reversal through CRISPR activation delivered by lipid nanoparticles

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A joint venture between The University of Melbourne and The Royal Melbourne Hospital

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Background

One approach towards **HIV remission** is the **reactivation of viral transcription** and subsequent **elimination of infected cells** ('shock and kill').

Latency-reversing agents (LRAs) alone tested in clinical trials to date have **failed** to reduce the size of the HIV reservoir[1].

Potential explanations include their **lack of potency** to induce transcriptional elongation and splicing. Furthermore, current-generation LRAs are **non-HIV-specific**, resulting in **toxicity** due to activation of host gene transcription[2,3].

CRISPR activation (CRISPRa) has been proposed as a **more potent, HIV-specific LRA**[4-7]. However, assessment *ex vivo* and *in vivo* has been hampered by a **lack of a delivery vehicle** for the CRISPRa machinery.

The recent advances in **mRNA-lipid nanoparticle (LNP) technology** provide rationale for assessing their potential in advancing CRISPRa as an LRA.

We hypothesize that **rationally designed mRNA-LNPs** can be used for **targeted** delivery of the **CRISPRa** system to T cells that constitute the **latent HIV reservoir**

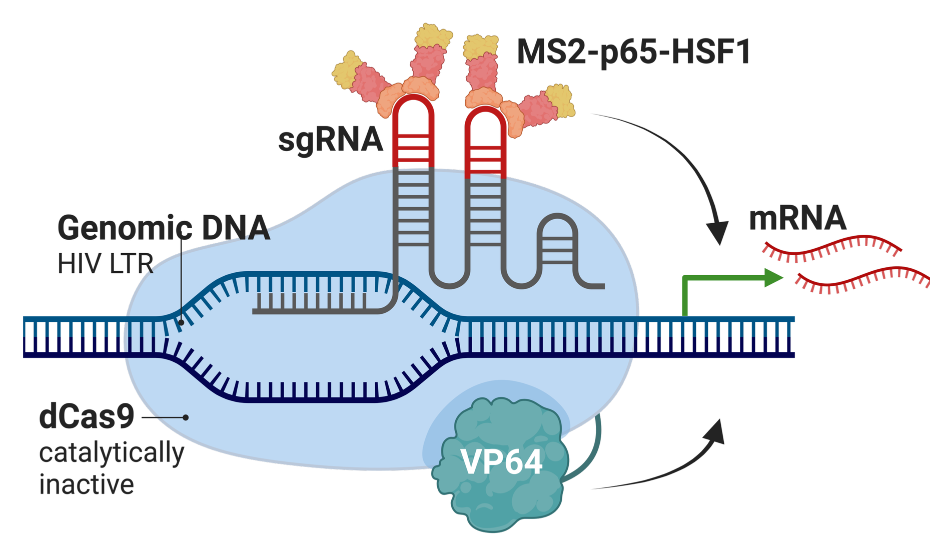

Figure 1. CRISPRa system. CRISPR can be repurposed to induce sequence-specific activation of transcription without inducing DNA cleavage of the target site. The dCas9-SAM system uses a catalytically inactive Cas9 (dCas9) combined with transcriptional activator domains (VP64, p65 and HSF1) and can be targeted to the HIV LTR to induce transcription initiation and elongation.

Poor transfection of CD4⁺ T cells using standard LNPs

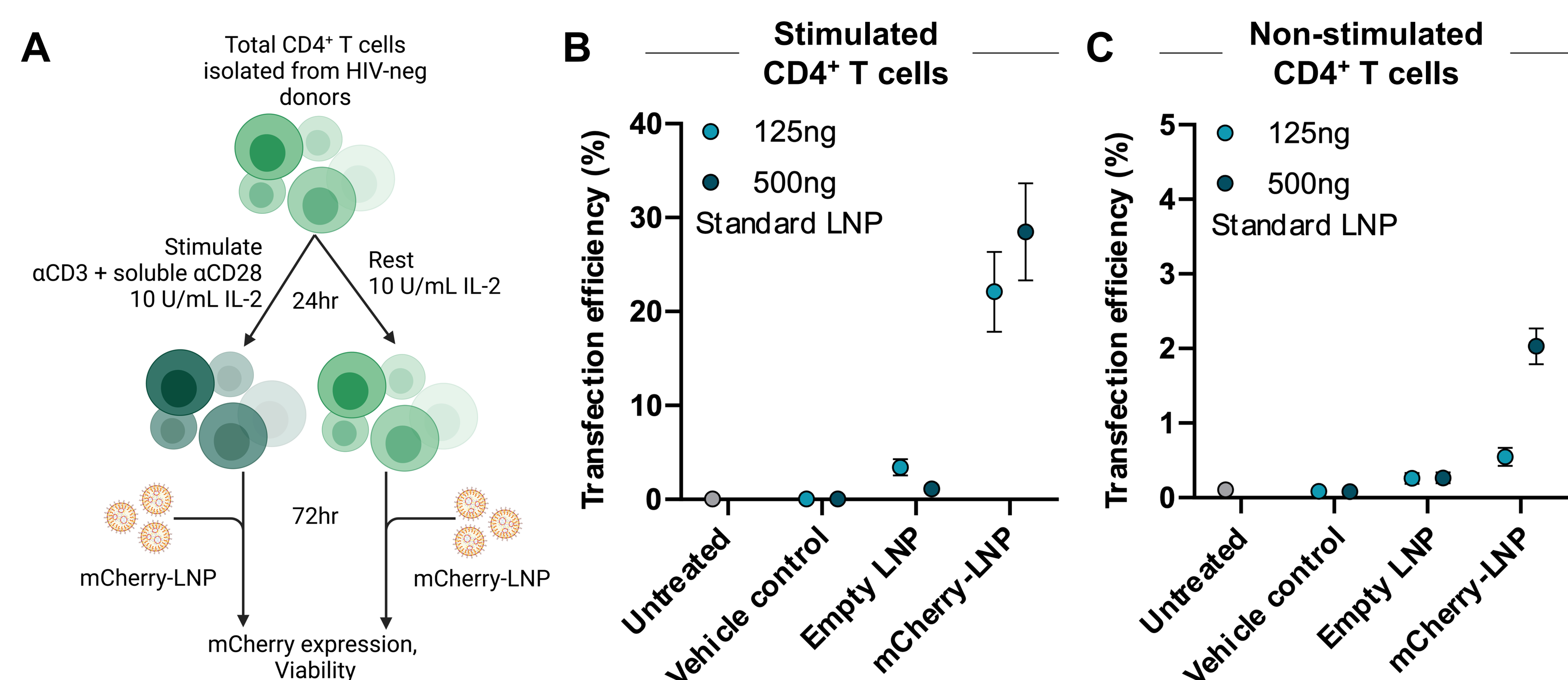

Figure 2. Pre-stimulation of CD4⁺ T cells is a pre-requisite for LNP transfection when using standard LNPs. (A) CD4⁺ T cells were isolated from HIV-negative donors and either stimulated (B) or rested (C), then treated for 72hr with standard LNPs encapsulating mCherry reporter mRNA. Standard LNPs follow the Onpattro® formulation. Transfection efficiency was measured using flow cytometry. Means±SEM of n=4 donors.

Highly potent transfection of non-stimulated CD4⁺ T cells using a novel, modified LNP formulation

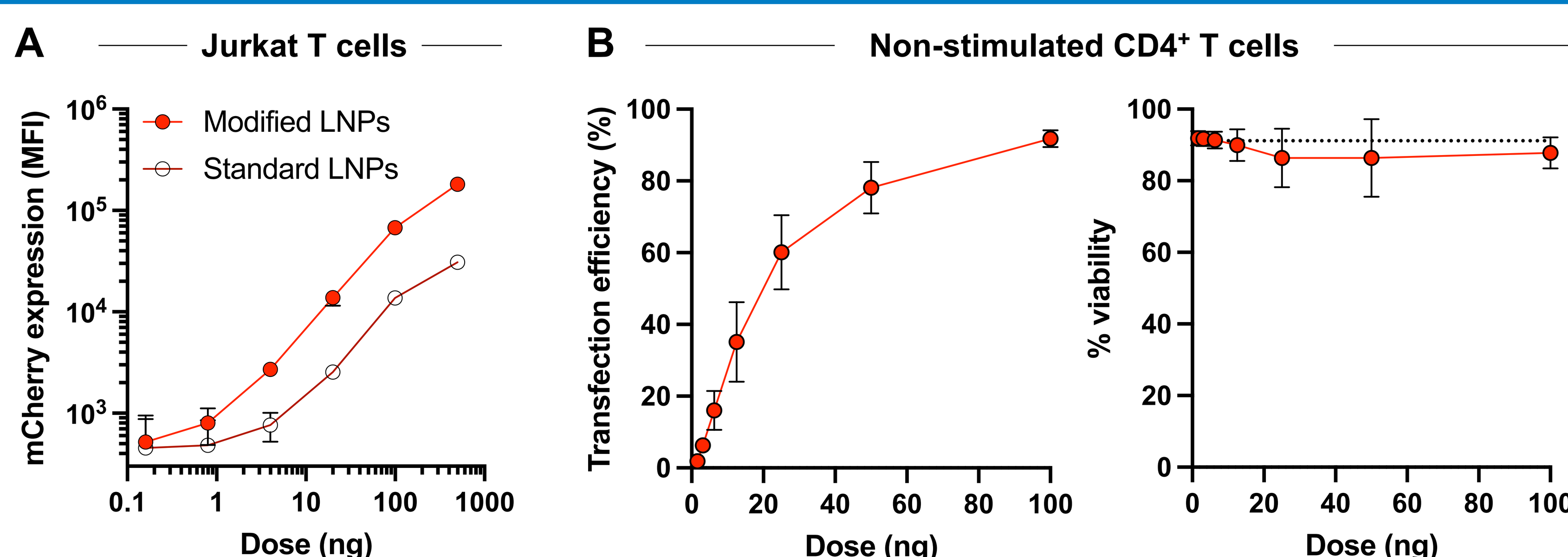

Figure 3. Modified LNPs induce superior protein expression over standard LNPs and are capable of potent transfection of non-stimulated primary CD4⁺ T cells. (A) Jurkat T cells were treated with equal doses of standard or modified* mCherry-LNPs for 24hr, followed by quantification of mCherry expression using flow cytometry. (B) Non-stimulated CD4⁺ T cells were treated and analyzed as in Fig 2. Dotted line indicates baseline viability. Means±SEM of n=2 experiments (A) or donors (B, C). *Lipid mixture proprietary

Targeted LNPs exhibit enhanced potency and specificity towards T cells in mixed cultures

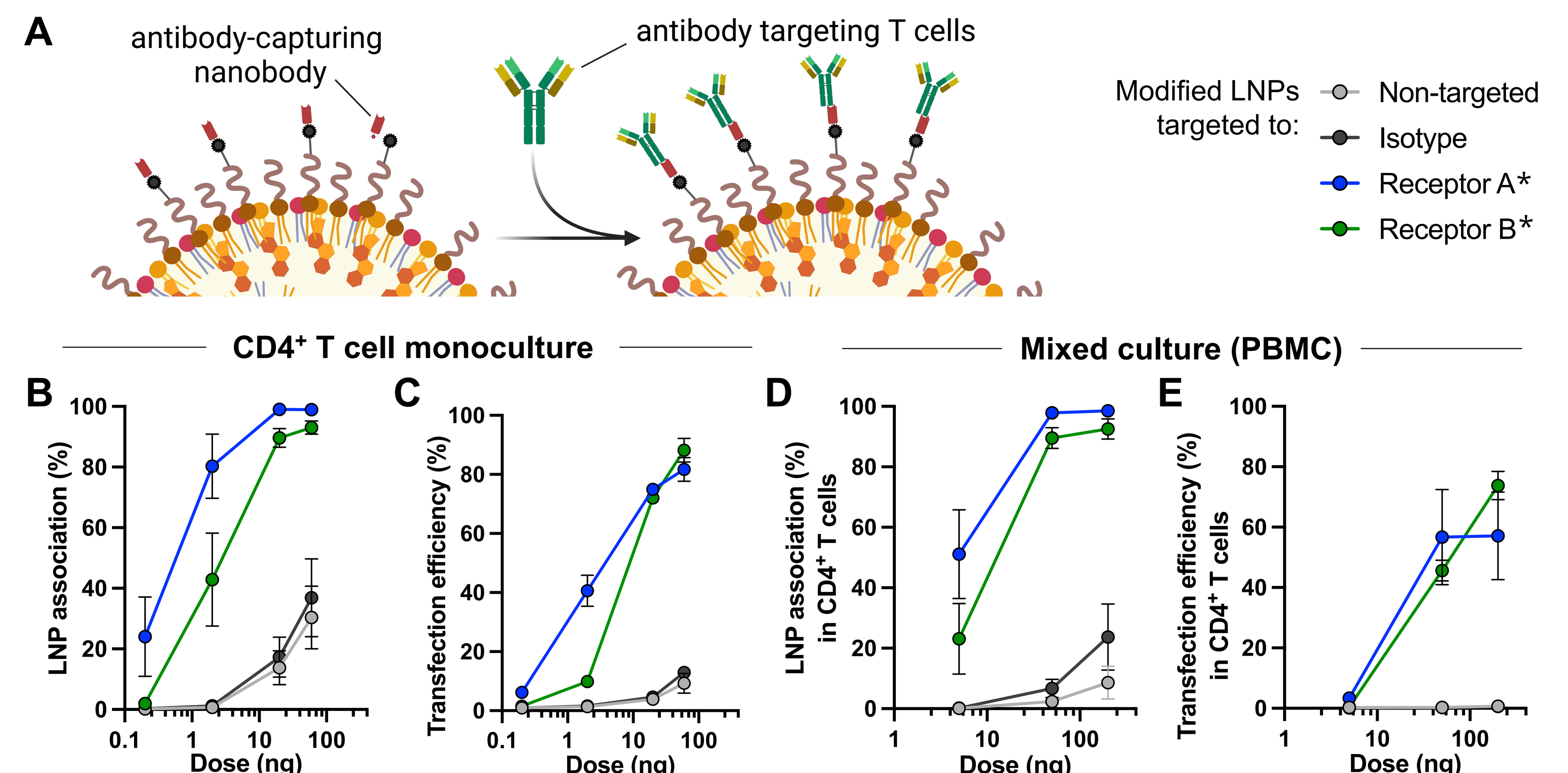

Figure 4. Antibody-conjugated LNPs exhibit enhanced association with and mRNA delivery to CD4⁺ T cells. (A) Method of antibody conjugation onto LNP surface in upright orientation. (B-E) Non-stimulated CD4⁺ T cells or PBMC were treated with targeted or control modified mScarlet mRNA-LNPs for 1hr, washed, then cultured for another 23hr. LNP association (B,D) and transfection efficiency (C,E) were measured in the CD4⁺ T cell compartment. Means±SEM of n=2-4 donors. *Proprietary information

CRISPRa-LNP treatment potently reactivates LTR-mediated transcription in cell line models of latency

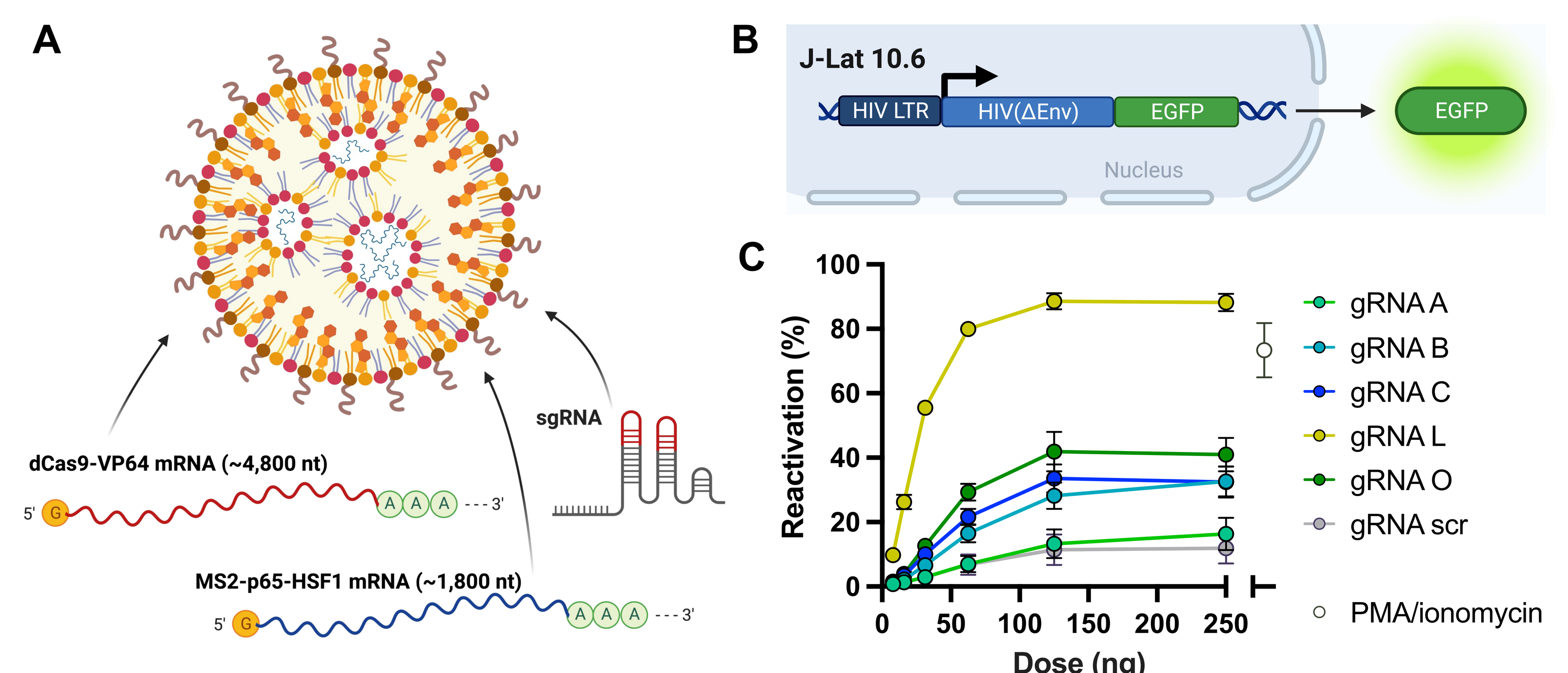

Figure 5. Modified LNPs co-encapsulating the three-component dCas9-SAM CRISPRa system potently reverse latency in J-Lat 10.6. (A) mRNAs encoding dCas9-VP64, MS2-p65-HSF1 and one of five HIV LTR-targeting[4,8] or scrambled gRNAs are co-packaged into one LNP. (B,C) J-Lat 10.6 cells were treated with modified CRISPRa-LNPs for 24hr. GFP expression was determined as a measure of reactivation. Means±SEM of n=3 experiments.

CRISPRa-LNP treatment is minimally toxic in CD4⁺ T cells from HIV-negative donors

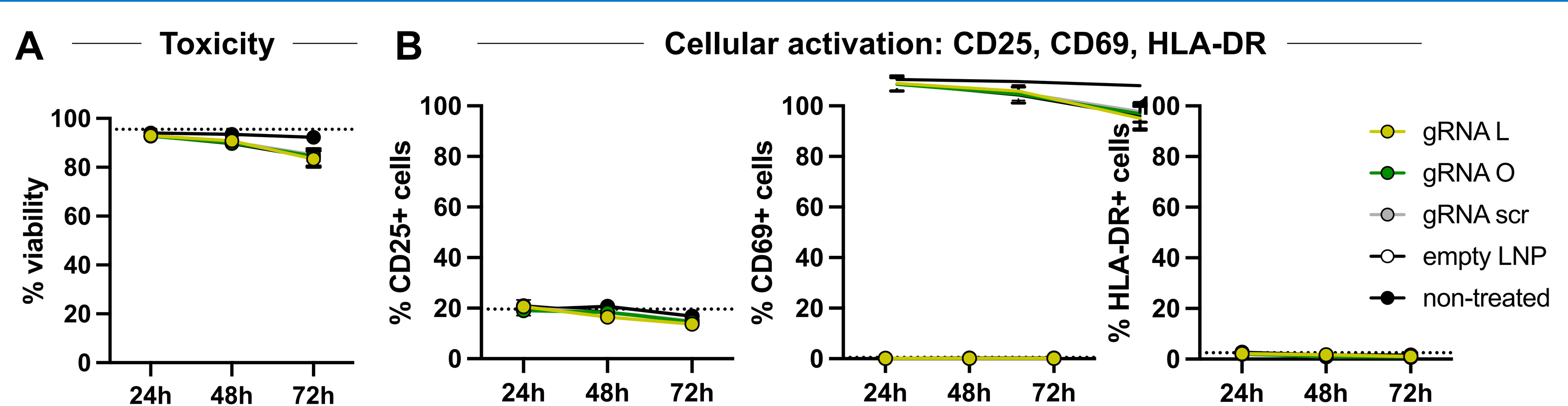

Figure 6. CRISPRa-LNP treatment minimally affects CD4+ T cells from HIV-negative donors. Non-stimulated CD4⁺ T cells were treated with 200 ng modified CRISPRa-LNPs for 24-72hr, after which viability (A) and cellular activation (B) were measured using flow cytometry. Means±SEM of n=4 donors. Dotted lines indicate baseline expression levels.

Conclusions

Targeted, modified LNPs provide a **potent delivery vehicle** for mRNA-based therapeutics to primary CD4⁺ T cells, including **CRISPR activation as a next-generation LRA.**

Ongoing work includes the assessment of targeted CRISPRa-LNPs in ***ex vivo*** CD4⁺ T cells from ART-suppressed individuals, as well as ***in vivo*** assessment of mRNA delivery to circulating and tissue-resident CD4⁺ T cells.

References

[1] Zerbato et al., Curr Opin Virol (2019). [2] Pace et al., PLoS Pathogens (2016). [3] Jones et al., PLoS Pathogens (2014). [4] Zhang et al., Sci Rep (2015). [5] Bialek et al., PLoS One (2016). [6] Limsirichai et al., Mol Ther (2016). [7] Saayman et al., Mol Ther (2016) [8] Tantale et al. Nat Commun (2021).

Acknowledgements

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