Potent HIV Latency Reversal by Lipid Nanoparticles Encapsulating Tat mRNA

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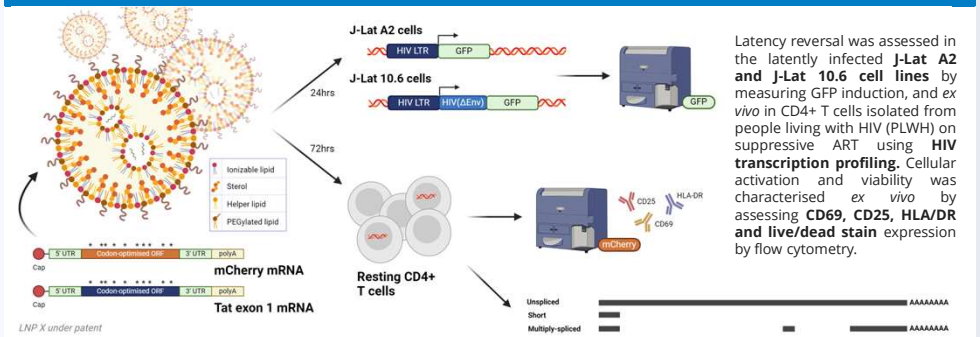
Background

Current Latency Reversal Agents (LRAs) do not potently upregulate **transcriptional elongation** and **splicing,** so have been **unable to reduce the size of the HIV reservoir** [1]. They are also **not HIV-specific,** so exhibit various **off-target effects** by targeting host transcription pathways [2-3].

The **HIV Tat protein** is known to be a highly **potent, HIV-specific LRA** *in vitro* through the promotion of transcriptional elongation [4]**.** When delivered by **mRNA and Lipid nanoparticles (LNPs)** *ex vivo*, Tat upregulates multiply spliced HIV RNA and p24 production [5-6]. However, there **remains a need for a T-cell tropic LNP** to optimally deliver mRNA to resting T cells to enhance potency, reduce total dose and minimise off-target effects.

We developed an **LNP containing mRNA encoding Tat exon 1 (Tat-LNP-X)** and characterised its ability to potently reverse HIV latency.

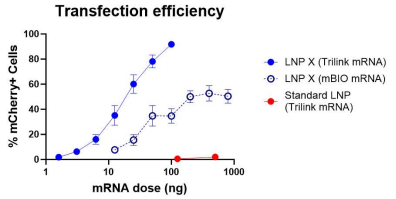
Methods



Latency reversal was assessed in the latently infected **J-Lat A2 and J-Lat 10.6 cell lines** by measuring GFP induction, and *ex vivo* in CD4+ T cells isolated from people living with HIV (PLWH) on suppressive ART using **HIV transcription profiling.** Cellular activation and viability was characterised *ex vivo* by assessing **CD69, CD25, HLA/DR and live/dead stain** expression by flow cytometry.

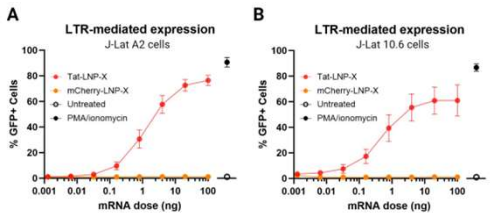
A range of LNPs with **varying lipids** and **codon-optimised mRNAs** were evaluated. LNPs encapsulating mRNA encoding either **Tat exon 1 (Tat-LNP-X)** or mCherry reporter protein **(mCherry-LNP-X)** were formulated through microfluidic mixing. **Transfection efficiency** was assessed in **resting CD4+ T cells** from HIV- donors.

LNP X potently transfects resting CD4+ T cells



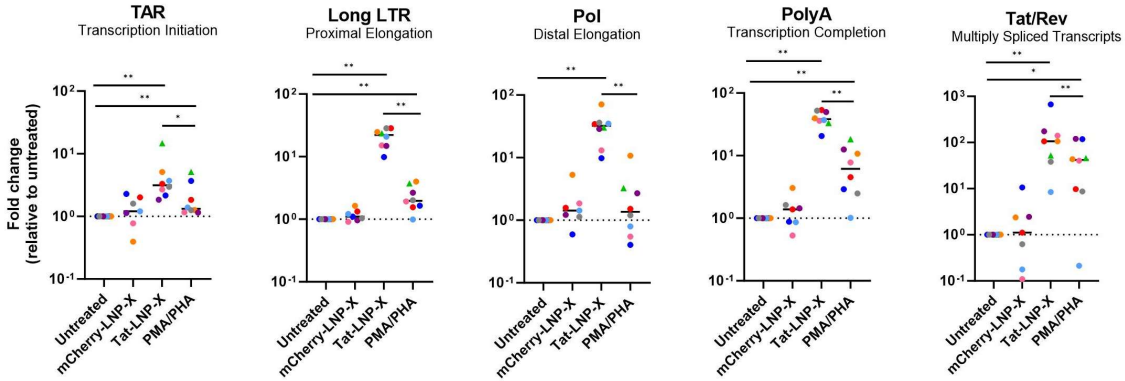
Poor transfection efficiency is observed when resting CD4+ T cells are transfected with Standard LNPs. **Potent transfection is achieved using LNP X** encapsulating mRNA encoding mCherry. Each mRNA is provided by different suppliers (Trilink or mBIO) and therefore has **unique, proprietary codon-optimisation**. n=2-11; Means±SEM.

Tat-LNP-X potently reverses HIV latency in cell line models



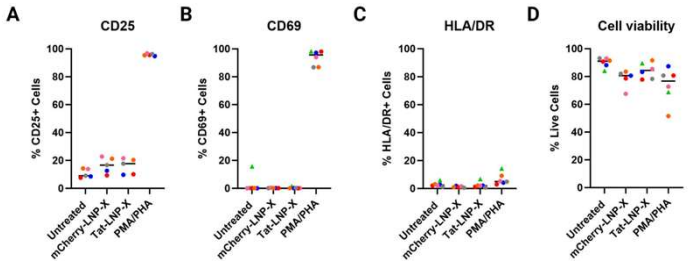
Tat-LNP-X **potently upregulated LTR-mediated expression** in J-Lat A2 cells (Panel A) and J-Lat 10.6 cells (Panel B), with minimal reactivation observed in cells treated with control LNPs. n=3 (A) or 4 (B); Means±SEM

Tat-LNP-X upregulates HIV transcription *ex vivo*



CD4+ T cells from PLWH on suppressive ART were treated with **2µg of Tat-LNP-X** for 48 (▲) or 72 (●) hours *ex vivo,* then analysed for their expression of various HIV transcripts by **digital RT-PCR.** This allows us to assess whether our LRA can overcome commonly observed blocks in transcription. Tat-LNP-X **overcame transcriptional blocks to elongation, completion and splicing**, resulting in a **105.7-fold upregulation of multiply spliced** transcripts compared to untreated. Tat-LNP-X also **outperformed the PMA/PHA positive control** for elongation, polyadenylation and splicing. Transcript expression has been normalised to RNA input and plotted as fold change of transcript expression relative to untreated control (indicated by dotted line). Bars represent median of n=8 donors; **p<0.01, *p<0.1 paired Wilcoxon signed rank test.

Tat-LNP-X has no impact on global cell activation or viability



CD4+ T cells from PLWH on suppressive ART were treated with **2µg of Tat-LNP-X** for 48 (▲) or 72 (●) hours *ex vivo,* then analysed for their expression **of cellular activation markers** CD25 (A), CD69 (B), HLA-DR (C) **and live/dead stain** (D) by flow cytometry.

Expression of early and late activation markers was **comparable between untreated** cells and cells treated with LNPs. **Cell viability was also unaffected** by Tat-LNP-X treatment. Bars represent median of n=5-6 donors.

Conclusions

Our novel LNP X formulation **potently transfects resting CD4+ T cells,** which are generally considered very hard to transfect. The delivery of **Tat exon 1 mRNA** by LNP X results in the **potent reversal of HIV latency** both *in vitro* and *ex vivo,* thereby overcoming the limitations of current LRAs which infrequently upregulate multiply-spliced transcripts and exhibit toxicities.

Ongoing work includes the assessment of whether the potent upregulation of multiply-spliced transcripts observed results in a **reduction in HIV DNA** *ex vivo*, thereby **eliminating the HIV reservoir.** Further characterisation of whether Tat-LNP-X reverses latency in an HIV-specific manner will also be undertaken, by assessing off-target effects from either the LNP or the secretion of Tat protein following delivery. The **distribution of LNP X** and latency reversal *in vivo* will also be determined in a **humanised mouse model.**

Acknowledgements
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References
[1] Zerbato et al., Curr Opin Virol (2019) [2] Pace et al., PLoS Pathogens (2016) [3] Ke at al., PLoS Pathology (2015) [4] Donahue et al., J Virol (2012) [5] Van Gulck et al., Antimicrob Agents Chemother (2023) [6] Pardons et al., Nat Commun (2023)