Lipid nanoparticle delivery of promoter-targeted siRNA for a functional HIV cure

Ellen Bowden-Reid [1], Ernest Moles Meler [2], Maria Kavallaris [2], Anthony Kelleher [1,3], Chantelle Ahlenstiel [1,3]

[1] The Kirby Institute, UNSW, [2] Children's Cancer Institute Australia, [3] The RNA Institute, UNSW

INTRODUCTION

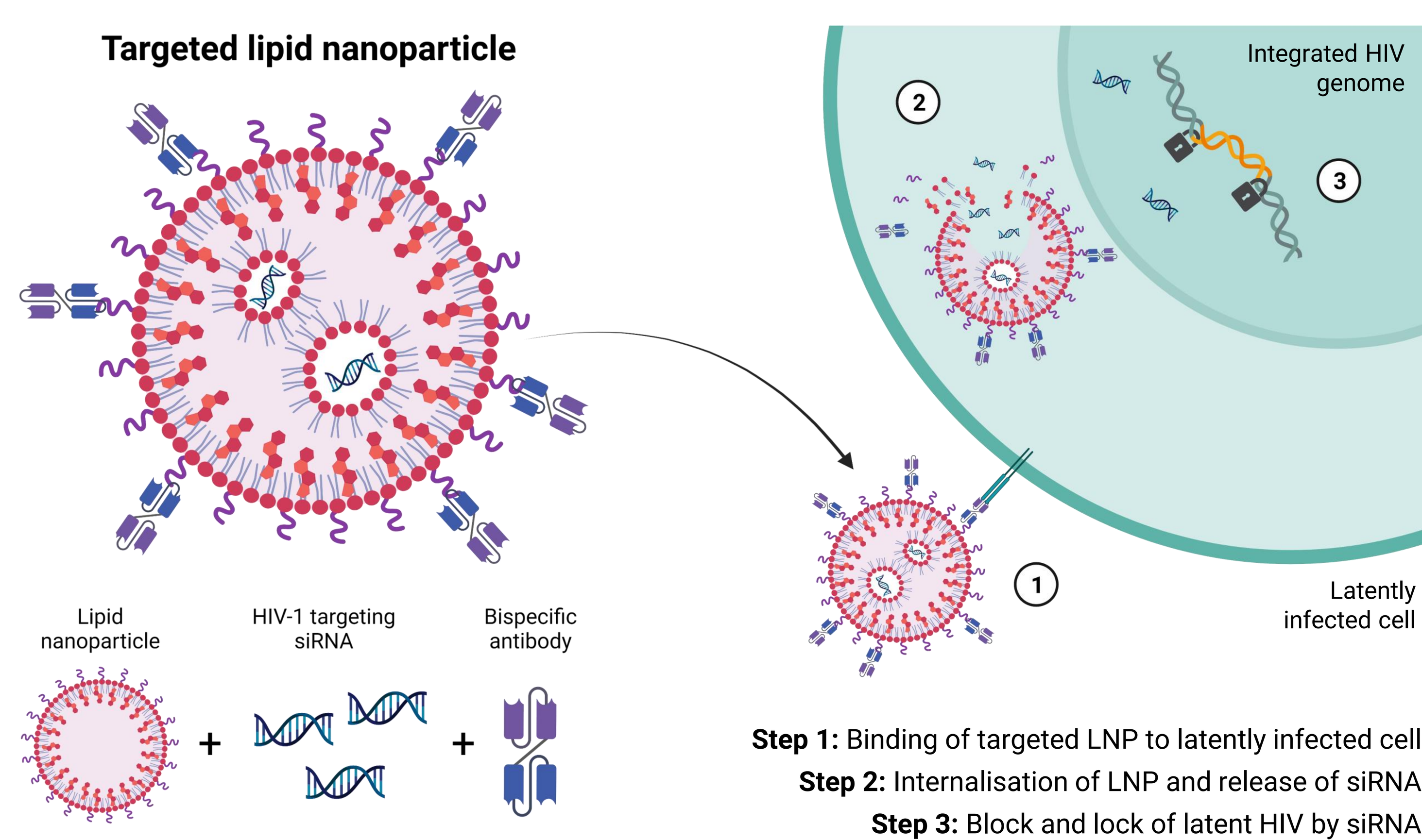
- Globally, there are 39 million people estimated to be living with HIV. During early infection, a virus reservoir of latently infected cells is established and hides from the immune system to persist life-long.
- The latent reservoir is resistant to antiretroviral treatment and can reactivate as the source of viral rebound if treatment is ever stopped. It is one of the major barriers to achieving a HIV cure.
- Short interfering RNAs (siRNAs) can be used to permanently silence HIV, which 'block' virus transcription of the reservoir and 'lock' it in a latent state (Ahlenstiel et al. 2015, Mendez et al. 2018). They are designed to target conserved regions of the viral genome and offer a broad-spectrum cure strategy.
- siRNAs can be combined with existing lipid nanoparticle (LNP) technologies (which can also be tailored with targeting moieties) for effective delivery to cells of the latent reservoir.

AIMS

- **Identify effective LNP formulations for the delivery of functional HIV targeting siRNA therapeutics in cell lines and primary cells**
- **Optimise targeting of LNPs using bispecific antibodies (BsAb) specific for HIV latent cell types**
- **Develop a multiplexed siRNA therapeutic delivered via targeted LNPs for the delivery of a functional HIV cure**

METHODS

- 19 bp siRNAs were designed to target conserved regions of the HIV promoter (5'LTR)
- siRNAs were encapsulated in a LNP using a NanoAssemblr® Ignite™. For targeted LNPs, BsAbs were added at a ratio of 1 molecule per 25 PEG molecules. Moderna- and Onpattro-based LNP formulations were trialed for efficacy
- HIV pseudovirus (NL4.3 ΔEnv EGFP) infection was measured via flow cytometry and HIV live virus (NL4.3) by RT-qPCR for gag mRNA
- Data is mean±SEM of 3 replicates. Significance shown is: *P<0.05, **P<0.01, ****P<0.0001. Statistical comparisons were made between HIV infected control populations using an unpaired t test



CONCLUSIONS & FUTURE DIRECTIONS

- LNPs offer an attractive delivery strategy that can effectively deliver functional siRNA to suppress HIV infection
- LNP formulation is an important consideration for efficient delivery
- Our siRNAs are designed to conserved regions of the HIV promoter and can be multiplexed together to offer a breadth of protection against the diverse global subtypes of HIV
- Continue optimising delivery of single and multiplexed siRNAs to primary cells via LNPs

RESULTS

Characterisation of Moderna-based LNPs with novel siRNA targeting the HIV 5'LTR

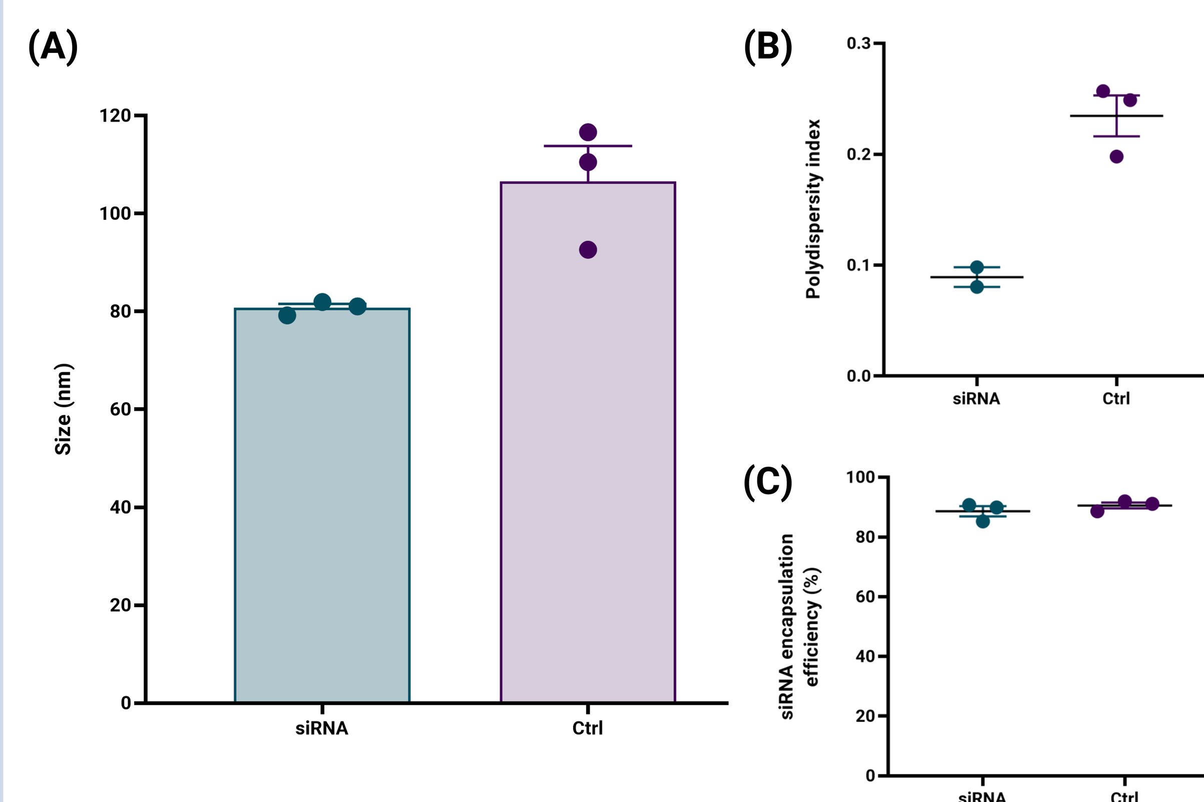


Figure 1. Characteristics of Moderna-based LNPs. LNP-siRNA (siRNA) and LNP-scrambled (ctrl) were assembled with NxGen microfluidic mixing using a NanoAssemblr® Ignite™ with a flow rate of 12mL/min. The molar lipid ratios used were based off the Moderna LNP formulation. **(A)** Size and **(B)** polydispersity index were determined with Dynamic Light Scattering using a Malvern Zetasizer Ultra, and **(C)** siRNA encapsulation efficiency was quantified using the RiboGreen assay.

HeLa T4+ cells internalise LNPs complexed with novel siRNA

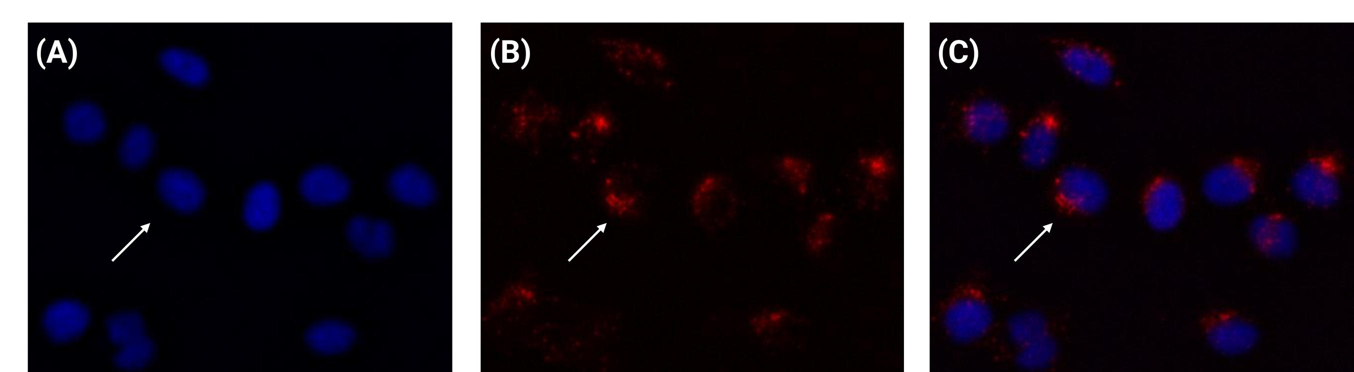


Figure 2. Uptake of LNPs in HeLa T4+ cells. HeLa T4+ cells were treated with LNPs for 3h at 37°C prior to fixing with 0.5% PFA. Fluorescence was imaged using a Leica Thunder Imaging System. **(A)** DAPI stained nuclei (blue), **(B)** Rhodamine B tagged LNPs (red), **(C)** merged fluorescent images.

LNP formulation plays important role in efficient delivery of functional siRNA

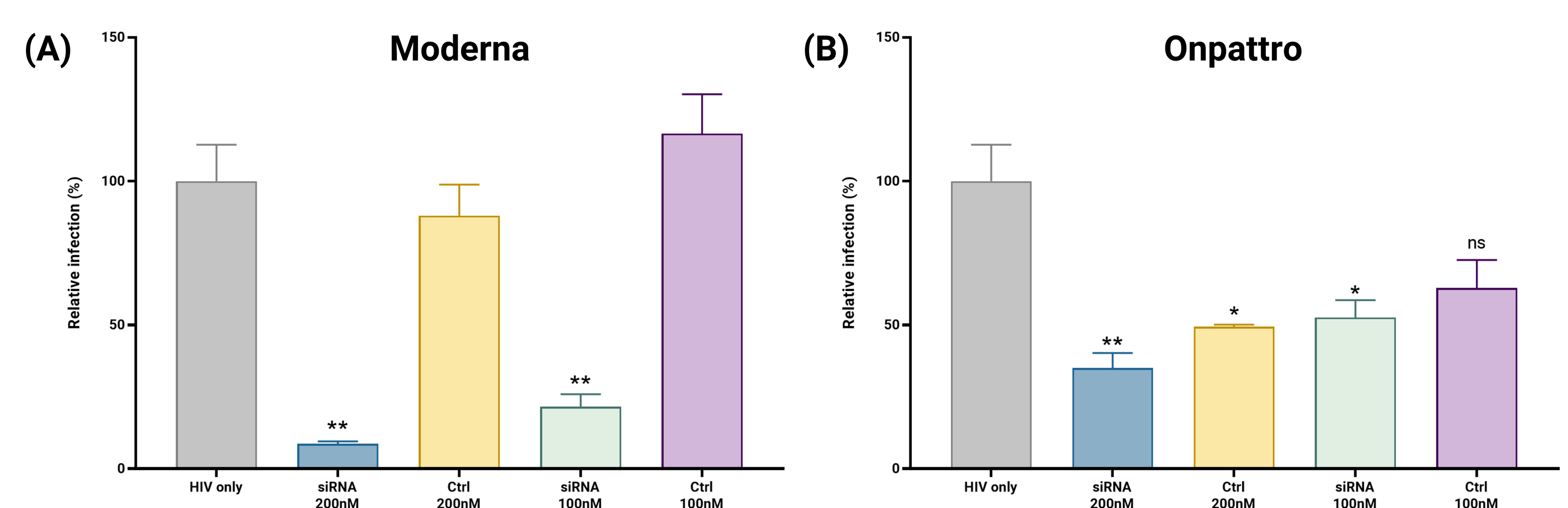


Figure 3. Comparison of Onpattro- and Moderna-based LNP formulations on delivery efficiency of functional siRNA in HeLa T4+ cells. HeLa T4+ cells were infected with HIV NL4.3 ΔEnv EGFP (Zhang et al. 2004) prior to **(A)** Moderna-based or **(B)** Onpattro-based LNP transfection with 100 or 200nM siRNA or control. EGFP expression was measured 3 days post-infection using a BD LSRFortessa SORP. *P<0.05, **P<0.01.

siRNA delivered via LNPs reduce HIV mRNA by 80%

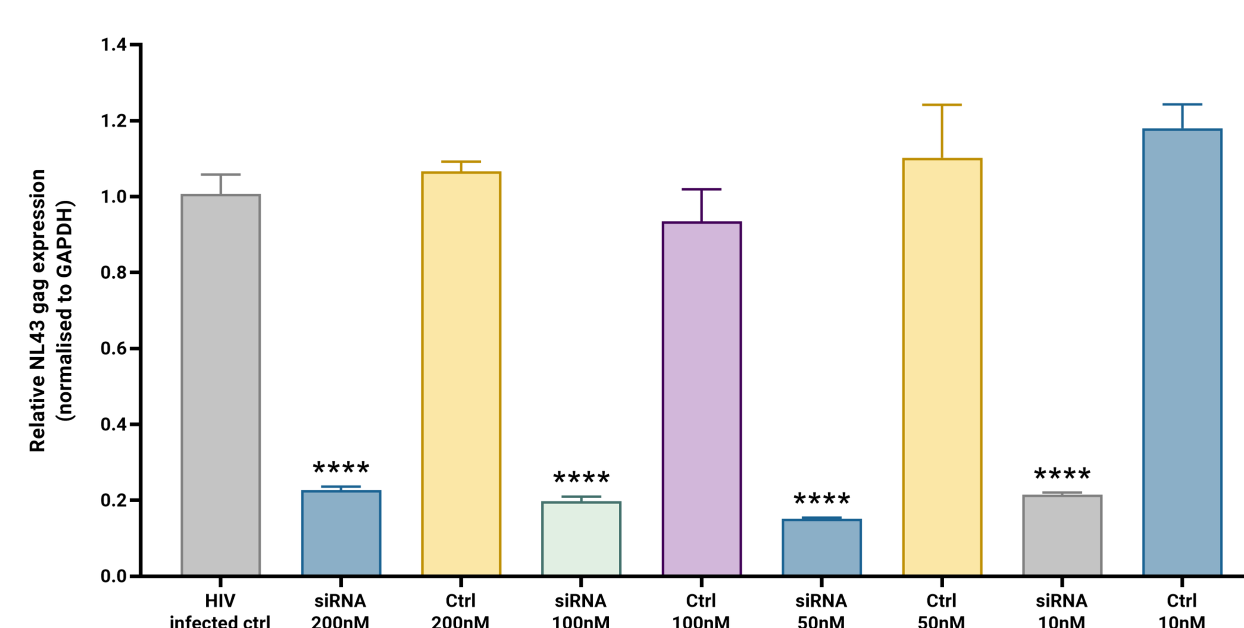
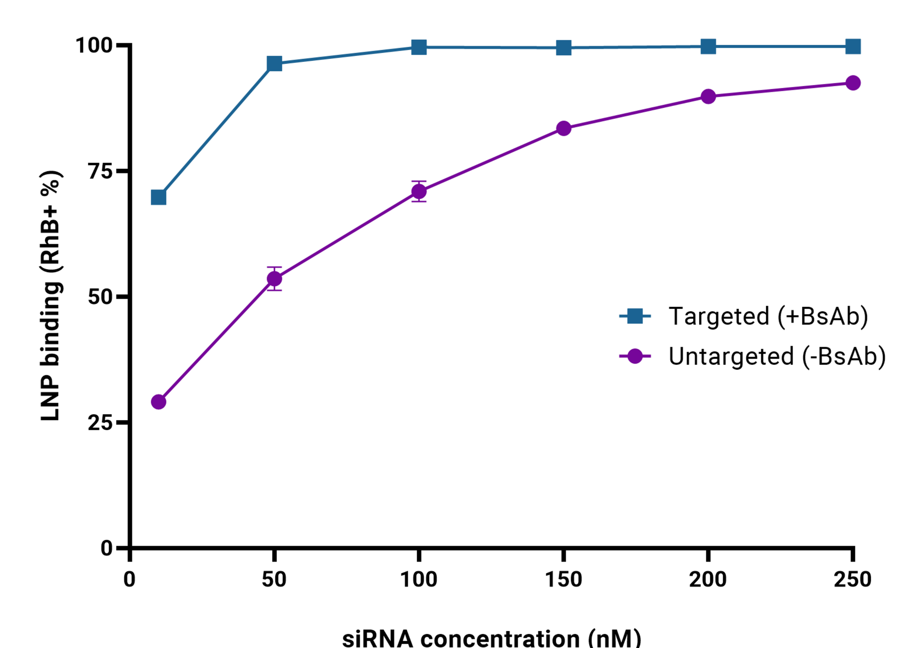


Figure 4. Assessing Moderna-based LNP delivery efficiency of functional siRNA in HeLa T4+ cells. HeLa T4+ cells were infected with HIV NL4.3 1 day prior to LNP transfection with 10 to 200nM siRNA or control. Viral gag mRNA was measured 7 days post-infection via RT-qPCR and normalised to GAPDH. ****P<0.0001.

Targeted LNPs associate with more than 99% primary CD4+ T cells at 50nM and above

Figure 5. Comparison of BsAb targeted and untargeted LNP binding to activated primary human CD4+ T cells. Primary CD4+ T cells isolated from whole blood were activated and incubated with 10 to 250nM LNP-siRNA (± BsAb, Moderna-based) for 3h at 37°C. A ratio of 1 BsAb molecule per 25 PEG molecules was used for targeted LNPs. Cells were fixed in 0.5% PFA, and the percentage of Rhodamine B fluorescent positive (RhB+) cells was analysed using a BD LSRFortessa SORP.



ebowdenreid@kirby.unsw.edu.au
@EBowdenReid

UNSW RNA Institute
UNSW Kirby Institute
UNSW Sydney
Children's Cancer Institute

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