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

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Background:
Development of a HIV cure has remained a significant challenge since the virus emerged ~40 years ago. The block and lock strategy, which aims to permanently silence HIV via short-interfering RNA (siRNA) targeting the virus promoter, is an attractive approach to overcoming this challenge.

Methods:
siRNAs were encapsulated in a lipid nanoparticle (LNP) using a NanoAssemblr® Spark and characterised for size and encapsulation efficiency. HeLa T4+ cells were transfected with LNP-siRNA and the antiviral effectiveness was determined by measuring HIV-1 viral mRNA by RT-qPCR and protein by the Reverse transcriptase assay. LNP-siRNA uptake (as measured by flow cytometry detection of PE in the LNP) was also assessed. A conservation analysis was also performed to determine the potential efficiency of multiplexing two or more siRNA to provide broad-spectrum coverage of diverse global HIV subtypes.

Results:
LNP-siRNA were ~80 nm in size and the encapsulation efficiency was ~90%. More than 98% of cells demonstrated uptake of LNP. Transfection of HeLa T4+ cells with LNP-siRNAs resulted in a significant reduction of both HIV-1 gag mRNA levels and Reverse transcriptase protein *in vitro* ($P<0.01$) compared to controls. Conservation analyses indicated that multiplexing two or more siRNAs could provide broad-spectrum antiviral coverage of >99% sequences across global HIV-1 subtypes.

Conclusion:
Experimental validation confirmed that HeLa T4+ cells have uptake of LNP-siRNAs and these LNPs can successfully deliver functional siRNA for the induction of HIV epigenetic silencing. LNPs, which are already clinically approved for the delivery of other RNA therapeutics, possess enormous potential as a delivery platform for delivering a functional HIV cure. Additionally, the potential to deliver multiplexed siRNAs via LNPs could further develop an antiviral that is effective independent of the HIV subtype.

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