Optimization and Analysis of Lipid Nanoparticles for *in vivo* mRNA Delivery

By

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ABSTRACT

Messenger RNA (mRNA) therapeutics have the potential to treat a diverse array of diseases requiring protein expression, with applications in protein replacement therapies, immunotherapies, and genome engineering. However, the intracellular delivery of mRNA is challenging and necessitates a safe and effective delivery vector. Lipid nanoparticles (LNPs) have shown considerable promise for the delivery of small interfering RNAs (siRNA) to the liver but their utility as agents for mRNA delivery have only been recently investigated. New delivery materials for mRNA delivery are also being developed which have the potential to transfect non-liver targets, but the screening of these vectors *in vivo* is low-throughput and it is difficult to determine transfected cell types. There is a need both for efficacious, well-characterized mRNA delivery materials and for methods to facilitate *in vivo* screening of novel materials.

We first developed a generalized strategy to optimize LNP formulations for mRNA delivery to the liver using Design of Experiment methodologies. By simultaneously varying lipid ratios and structures, we developed an optimized formulation which increased the potency of erythropoietin-mRNA-loaded LNPs *in vivo* 7-fold relative to formulations previously used for siRNA delivery. Next, we explored the immune response and activity of base-modified LNP-formulated mRNA administered systemically *in vivo*. We observed indications of a previously-uncharacterized transient, extracellular innate immune response to mRNA-LNPs, including neutrophilia, myeloid cell activation, and up-regulation of four serum cytokines.

Although we have developed a more efficacious liver-targeting LNP, many mRNA therapies will require delivery to non-liver tissues. Using trial-and-error approaches, we discover novel formulations capable of inducing mRNA expression *in vivo* in the spleen, lung, and fat. To increase the throughput of *in vivo* screening, we report a new barcoding-based approach capable of evaluating the biodistribution and pharmacokinetics of many LNP formulations in a single mouse. Then, we develop a method that can identify mRNA expression delivered from LNPs both in bulk tissues and with single cell resolution.

Together, the work reported here contributes to the development of mRNA therapeutics by increasing mRNA-LNP potency and characterizing their immunogenicity *in vivo*. Furthermore, we hope the multiple *in vivo* screening methods described in this Thesis will accelerate the discovery of new mRNA delivery vectors capable of transfecting desired tissues and cell types.

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