Chemical and Structural Optimization of Lipid Nanoparticles for Pulmonary and Other Delivery

by

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ABSTRACT

Messenger ribonucleic acid (mRNA) therapeutics are a promising new class of pharmaceutical, with unprecedented versatility to address a wide range of conditions. When, in 2020, Moderna and Pfizer/BioNTech transformed oncogenic treatments into SARS-CoV-2 vaccines in just months, they demonstrated the flexibility and potency of lipid nanoparticles (LNPs) as a platform with broad applications. Moreover, beyond immunizations, mRNA LNPs also have the potential to dose proteins intracellularly for trafficking to the cell surface or nucleus that are extremely difficult to administer with conventional strategies. Unfortunately, this new modality is limited by production costs, storage logistics, dose-associated toxicity, and few routes of administration. In my thesis research, I pursued a number of projects intended to address these shortcomings, hoping to facilitate the broader application of mRNA therapeutics.

First, I designed and optimized a process by which mRNA vaccine formulations could be prepared into inhalable dry powders for local administration to the lung epithelium. Due to the large surface area of the lung, cellular surface protein deficiencies such as cystic fibrosis are extremely debilitating and virtually impossible to treat with conventional protein replacement. By employing dioleoyl-3-trimethylammonium propane (DOTAP) as a helper lipid, tuning poly(ethylene glycol) content, and including mannitol as an excipient, I consistently obtained LNP powders with in vivo transfection comparable to that of conventional liquid formulations. However, unlike liquid formulations, these powders possess excellent aerosolization characteristics and do not require ultracold storage, making them promising candidates for at-home administration. The key results of this chapter have been published in Advanced Healthcare Materials (DOI: 10.1002/adhm.202400509).

Next, I explored two novel ionizable lipid libraries for mRNA delivery, both of which employed the Ugi four-component reaction. The goal of these projects was to identify more potent ionizable lipids which could facilitate smaller, cheaper, and safer doses for gene therapies. First, I screened hundreds of ionizable lipids in vitro across a variety of structural features, generating the training data set for a machine learning algorithm. The machine learning algorithm identified the chemical components most commonly predicted to form

successful ionizable lipids, which were then used to synthesize a much smaller, optimized library of likely hits. I screened this new library in vivo and identified lipid 119-23, which outperforms commercial lipids DLin-MC3-DMA (Alnylam) and SM102 (Moderna) in both intravenous lung and intramuscular calf transfection. The key results of this chapter have been published in Nature Materials (DOI: 10.1038/s41563-024-01867-3).

As a follow-on to this library, I explored the design space of higher-molecular weight Ugi lipids, iteratively screening new headgroups, linkers and tails in vivo to rationally design even more potent ionizable lipids. I found that, in line with previously reported structure-function relationships, the most potent lipids contained a mixture of long, unsaturated alkene tails and short saturated branched tails. Although further exploration of this library is planned, the most potent lipids identified to-date already show in vivo liver transfection comparable to that of best-in-class non-biodegradable lipids such as cKK-E12 and C12-200. Additionally, these lipids consistently produce high quality inhalable powders, with strong in vivo activity.

Finally, to further optimize LNP transfection activity, I explored the inclusion of small amine excipients at formulation. We found that these excipients consistently improved activity, both through direct modulation of key quality attributes such as encapsulation efficiency and surface charge, as well as aiding in endosomal escape. These excipients also facilitated the use of LNPs to deliver DNA, improving transfection by roughly three-fold for over a month of expression. Although this work is also incomplete, and concerns remain about the retention of these small molecules through normal LNP processing steps such as dialysis, the inclusion of these fifth components holds great promise for more-efficient mRNA therapies.

Overall, my thesis research encompasses several contributions to the field of mRNA lipid nanoparticles, including chemical and formulation strategies to increase potency, as well as a novel process to facilitate potent dosing to the lung epithelium. I hope that as the field grows and evolves, these projects will contribute to the development of new treatments for genetic conditions, new cures for intractable diseases, and cheaper, safer, and more-effective immunizations for the world.

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