Dendrimer-based Lipid Nanoparticles Deliver Therapeutic FAH mRNA to Normalize Liver Function and Extend Survival in a Mouse Model of Hepatorenal Tyrosinemia Type I

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Statement of Purpose: mRNA-mediated protein replacement represents a promising concept for the treatment of liver disorders. Children born with fumarylacetoacetate hydrolase (FAH) mutations suffer from hepatorenal tyrosinemia type I resulting in renal dysfunction, liver failure, neurological impairments, and cancer. Protein replacement therapy using FAH mRNA offers tremendous potential to treat Hepatorenal Tyrosinemia Type I (HT-1), but is currently hindered by the development of efficacious mRNA carriers that can function in diseased livers. Structure-guided, rational optimization of 5A2-SC8 mRNA-loaded dendrimer lipid nanoparticles (mDLNPs) increased delivery potency of FAH mRNA, resulting in functional FAH protein and sustained normalization of body weight and liver function in FAH$^{-/-}$ knockout mice. Optimization using luciferase mRNA produced DLNP carriers that were efficacious at mRNA doses as low as 0.05 mg/kg *in vivo*. mDLNPs transfected >44% of all hepatocytes in the liver, yielded high FAH protein levels (0.5 mg/kg mRNA), and were well tolerated in a knockout mouse model with compromised liver function. Genetically engineered FAH$^{-/-}$ mice treated with FAH mRNA mDLNPs had statistically equivalent levels of TBIL, ALT, and AST compared to wild type C57BL/6 mice and maintained normal weight throughout the month-long course of treatment. This study provides a framework for the rational optimization of LNPs to improve delivery of mRNA broadly and introduces a specific and viable DLNP carrier with translational potential to treat genetic diseases of the liver.[1]

Methods: A full description of the various assays and animal models will be provided in the presentation.

Results: To improve therapeutic delivery of FAH mRNA, we reasoned that efficacious carriers would have to be highly tolerated in the livers of mice with compromised liver function, as well as be fundamentally altered in their molar composition to accommodate high loading of long mRNAs with potential for secondary folding and increased electrostatic binding. We selected 5A2-SC8 as the ionizable cationic lipid dendrimer because it has been successful for siRNA delivery to the liver for investigating gene functionality in cancer development and liver regeneration without concern for material toxicity-induced off-target effects.[2-4] We employed a systematic orthogonal matrix design methodology designed to elucidate functional contributions of each lipid within LNPs for efficacious mRNA delivery. Multiple rounds of optimization covered the theoretical space of >500 LNPs. We found that optimal mRNA formulations require significantly decreasing the molar amount of ionizable cationic lipid, increasing the molar amount of zwitterionic phospholipid, and slightly increasing cholesterol and lipid poly(ethylene glycol) to render LNPs with high loading of mRNA and a charge balance that facilitates mRNA release. Optimized DLNPs could deliver mRNA *in vivo* and possess physical attributes amendable to clinical translation. Remarkably, survival of FAH$^{-/-}$ mice treated with FAH mRNA mDLNPs was extended indefinitely without any signs of disease, weight loss, or liver complications (**Figure 1,** see also **Reference #1**).

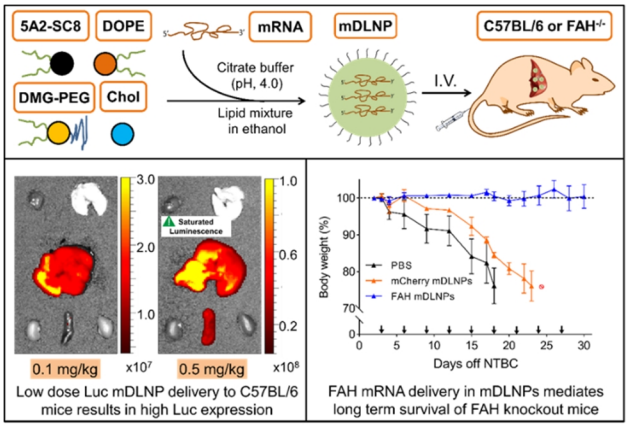

Figure 1. (A) A design of experiments (DOE) methodology was used to reengineer DLNP molar ratios to increase mRNA delivery efficacy. **(B)** IV administration of Luciferase mRNA-loaded DLNPs resulted in robust, dose dependent protein activity in the liver. **(C)** Body weight of FAH$^{-/-}$ mice were monitored in a one month therapy study. Mice were injected with PBS, mCherry mDLNPs, or FAH mDLNP every three days until day 30 (0.35 mg/kg).

Conclusions: 5A2-SC8 mDLNPs delivered FAH mRNA to normalize body mass and liver function and extend survival in FAH$^{-/-}$ knockout mice suffering from HT-1. We found that LNPs optimized for mRNA delivery should contain significantly less ionizable cationic lipid and more zwitterionic phospholipids compared to standard siRNA formulations. This provides a rational design guideline to redevelop other siRNA-delivering LNPs for delivery of mRNA. Moreover, the capability of 5A2-SC8 mDLNPs to deliver FAH mRNA to diseased livers without any carrier toxicity makes this system suitable for treatment of a wide variety of liver diseases.

References:

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