Engineering Lipid Nanoparticles for *In Utero* mRNA Delivery

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Statement of Purpose: Clinical advances enable the prenatal diagnosis of genetic diseases that are treated with protein and enzyme therapies—such as mRNA-mediated protein replacement—administered after birth. Though this is a promising strategy for some diseases, many genetic diseases feature irreversible pathology that begins during fetal development.[1] Thus, *in utero* therapeutic intervention—occurring prior to or in the early stages of disease progression—could reduce the mortality and morbidity of these diseases.[2] However, the use of drug delivery platforms for *in utero* nucleic acid therapies have only recently emerged.[3] Specifically, lipid nanoparticles (LNPs), which have been extensively studied for treating genetic diseases in adults[4], have not been developed for fetal therapy. Here, we developed and screened a library of new ionizable lipid structures to investigate LNPs for *in utero* mRNA delivery in mice.

Methods: Ionizable lipids were synthesized from polyamine cores and epoxide-terminated alkyl chains using Michael addition chemistry and combined with excipients and mRNA using microfluidic mixing to created LNPs, as previously described.[3] LNPs were characterized using dynamic light scattering (size), Ribogreen assays (encapsulation), and 6-(p-toluidinyl)naphthalene-2-sulphonic acid assays (pKa). Balb/c and C57BL/6 mice were used to assess fetal delivery. A midline laparotomy was performed, and fetuses were injected with LNPs encapsulating luciferase mRNA via the vitelline vein (Fig. 1A). After 4 or 24 hours, fetuses were extracted, dissected, and imaged by IVIS to detect luminescence indicative of functional luciferase delivery. To determine toxicity and survival, fetuses were delivered by cesarean section 4 days post-injection, and live fetuses were counted. Multiplex assays were conducted on livers and blood to assess immunotoxicity.

Results: We prepared a library of 16 LNPs, each with unique ionizable lipids, that ranged in size from 64.6-135.2 nm in diameter with pKa values between 5.57 and 7.14, indicating that most formulations were ionizable (pKa < 7). Following *in utero* injections in mice, IVIS revealed that three LNPs yielded strong luciferase mRNA delivery in fetuses compared to the other LNPs as well as DLIn-MC3-DMA and JetPEI, two commercially available *in vivo* delivery platforms (Fig 1B, 1C). Further analysis of the extracted organs revealed that livers had the strongest delivery with two LNP formulations also delivering mRNA to the fetal lungs and intestines, and no LNP formulations resulting in maternal mRNA delivery. Two of the top-performing LNPs were also used to deliver GFP or erythropoietin mRNA to demonstrate the ability of these platforms to deliver multiple types of mRNA cargo. Lastly, LNPs were evaluated for fetal toxicity by survival and immunogenicity, and both resulted in >90% survival at birth with no significant changes in cytokine levels compared to non-treated controls.

Conclusions: We developed and screened LNPs for mRNA delivery *in utero* with measurable delivery in fetal livers, lungs, and intestines. These platforms yielded higher transfection with advantageous safety compared to commercially available reagents and improved delivery compared to naked mRNA, demonstrating their high potency for fetal delivery. In future work, we will utilize these ionizable lipids and LNPs to deliver therapeutic nucleic acids in animal models of fetal disease to treat congenital disorders.

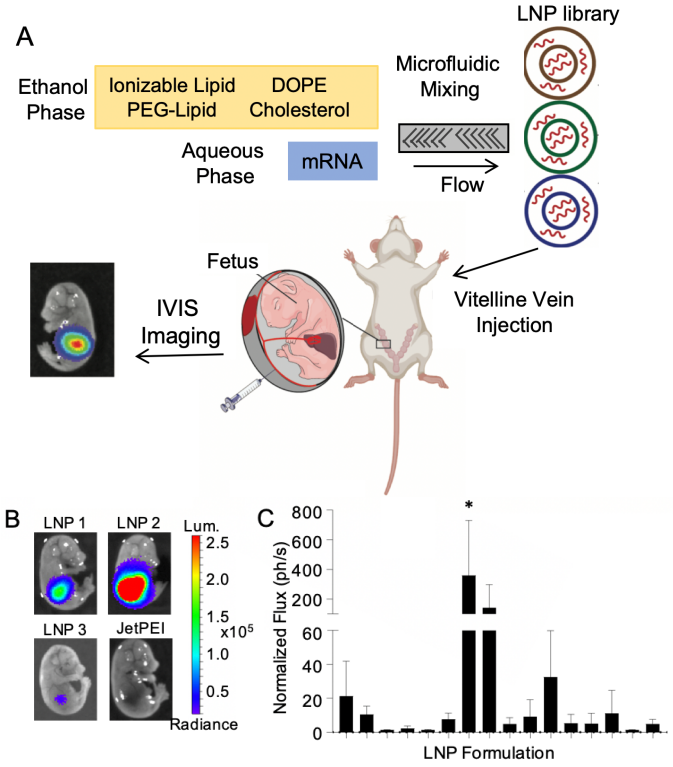

Fig 1: (A) Schematic overview of LNP formulation and fetal injections. LNPs were injected into individual fetuses via the vitelline vein, and fetuses were extracted for IVIS imaging. (B) Representative images of fetuses injected with three of the LNPs or JetPEI. (C) Quantification of luminescence from fetuses injected with each of the 16 LNPs. Data collected from at least 3 fetuses per treatment, $p<0.001$.

References: (1) Ferreira C.R., Trans. Sci. Rare Dis., 2017, 2, 1-71. (2) Almeida-Porada G., Mol. Ther. Clin. Dev., 2016, 5. (3) Ricciardi A.S., *Nat Commun,* 2018. (4) Oberli M.A., Nano. Lett., 2017, 17, 1326-1335.