Ionizable Lipid Nanoparticles for *In Vivo* mRNA Delivery to the Placenta during Pregnancy

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Introduction: Ionizable lipid nanoparticles (LNPs) are the most clinically advanced non-viral platform for mRNA delivery. While they have been explored for applications including vaccines and gene editing technologies, to our knowledge, LNPs have not been investigated for placental disorders during pregnancy. Pre-eclampsia is a placental disorder caused by insufficient blood flow in the placenta that increases maternal blood pressure and restricts fetal growth. Therefore, therapies that improve vasodilation in the placenta have the potential to benefit both maternal and fetal health. Here, we engineer ionizable LNPs for selective mRNA delivery to the placenta with applications in mediating placental vasodilation using vascular endothelial growth factor (VEGF-A) mRNA **(Fig. 1A)**.

Materials and Methods: A library of novel ionizable lipids was synthesized using S_N2 chemistry and used to formulate LNPs via microfluidic mixing with mRNA. *In vitro* LNP-mediated luciferase mRNA delivery was evaluated in JEG-3 cells — an immortalized human placental trophoblast cell line — to identify top performing LNPs for placental delivery. *In vivo* luciferase mRNA delivery was assessed in non-pregnant and pregnant mice. Finally, VEGF-A mRNA LNPs were administered to pregnant mice to evaluate local VEGFR1 expression and vasodilation in the placenta via immunohistochemistry.

Results and Discussion: A library of 15 ionizable lipids was synthesized using three different epoxide tails and five unique polyamine cores. In addition to these lipid materials, two industry-standard ionizable lipids — C12-200 and DLin-MC3-DMA — were used to formulate LNPs with luciferase mRNA for *in vitro* screening in placental trophoblasts. One lead formulation LNP X4 mediated potent and reproducible mRNA delivery with little toxicity, while cells treated with the industry standard C12-200 LNP had poor luciferase expression **(Fig. 1B)**. For *in vivo* luciferase mRNA delivery, the lead LNP X4 showed potent extrahepatic delivery to the spleen while C12-200 showed potent delivery to the liver in both pregnant and non-pregnant mice **(Fig. 1D)**. Additionally, the lead LNP demonstrated potent luciferase expression to the placentas in pregnant mice, with no luciferase expression observed in the fetuses **(Fig. 1C)**. To explore a clinically relevant mRNA cargo, pregnant mice were treated with VEGF-A mRNA LNPs to evaluate local placental VEGF receptor 1 (VEGFR1) expression which is upregulated in response to increased VEGF-A expression. In the labyrinth region of the placenta, increased VEGFR1 was observed, particularly surrounding the maternal and fetal blood vessel spaces **(Fig. 1E)**. Our lead LNP formulation X4 demonstrated significantly (**$p < 0.0021$) increased VEGFR1 expression compared to untreated mice **(Fig. 1F)**.

Conclusion: This work involved the design and optimization of an LNP platform for potent mRNA delivery to the placenta. Future work will exploit this local VEGF-A mRNA expression to mediate vasodilation and treat placental disorders such as pre-eclampsia.

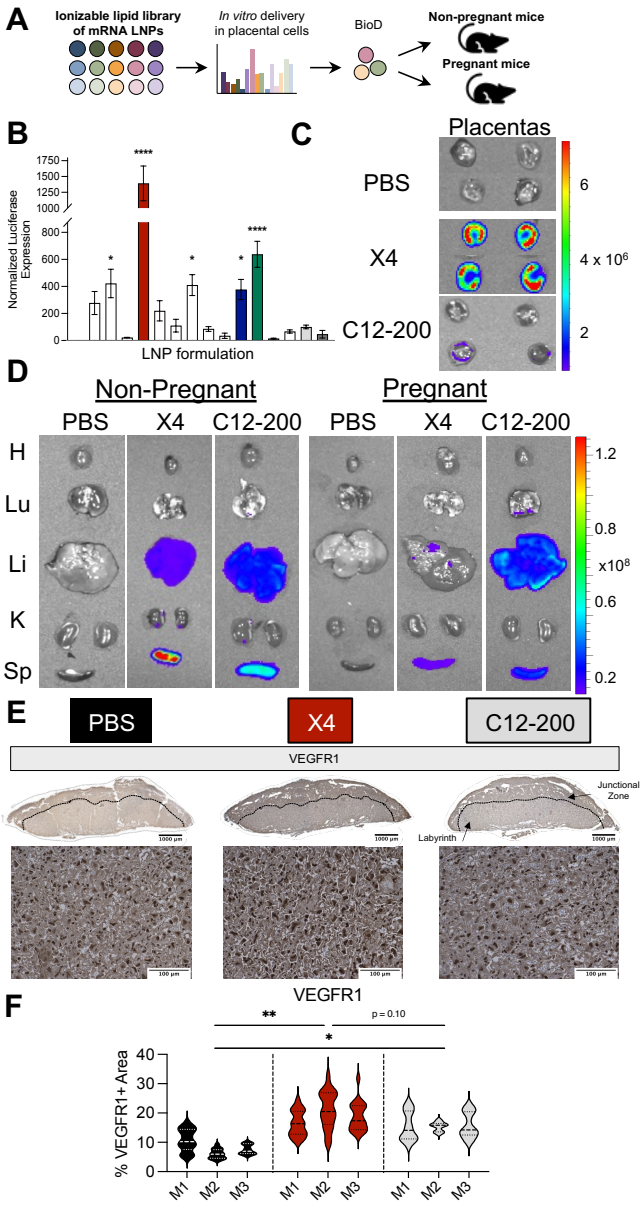

Figure 1. (A) Engineering mRNA LNPs for delivery to the placenta. **(B)** *In vitro* screening of LNP library for mRNA delivery to placental trophoblasts. **(C)** *In vivo* LNP-mediated luciferase mRNA delivery to the placentas in pregnant mice for the lead formulation LNP X4 and C12-200 LNP. **(D)** Luciferase expression in the liver (Li) and spleen (Sp) in non-pregnant and pregnant mice. **(E)** Placentas stained with VEGFR1 from pregnant mice 48 h after treatment with VEGF-A mRNA LNPs. **(F)** Quantification of percent VEGFR1 positive area (*: $p < 0.05$, **: $p < 0.0021$, ****: $p < 0.0001$)