mRNA-lipid nanoparticle delivery systems for effective genetic and epigenetic editing medicines.

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Abstract

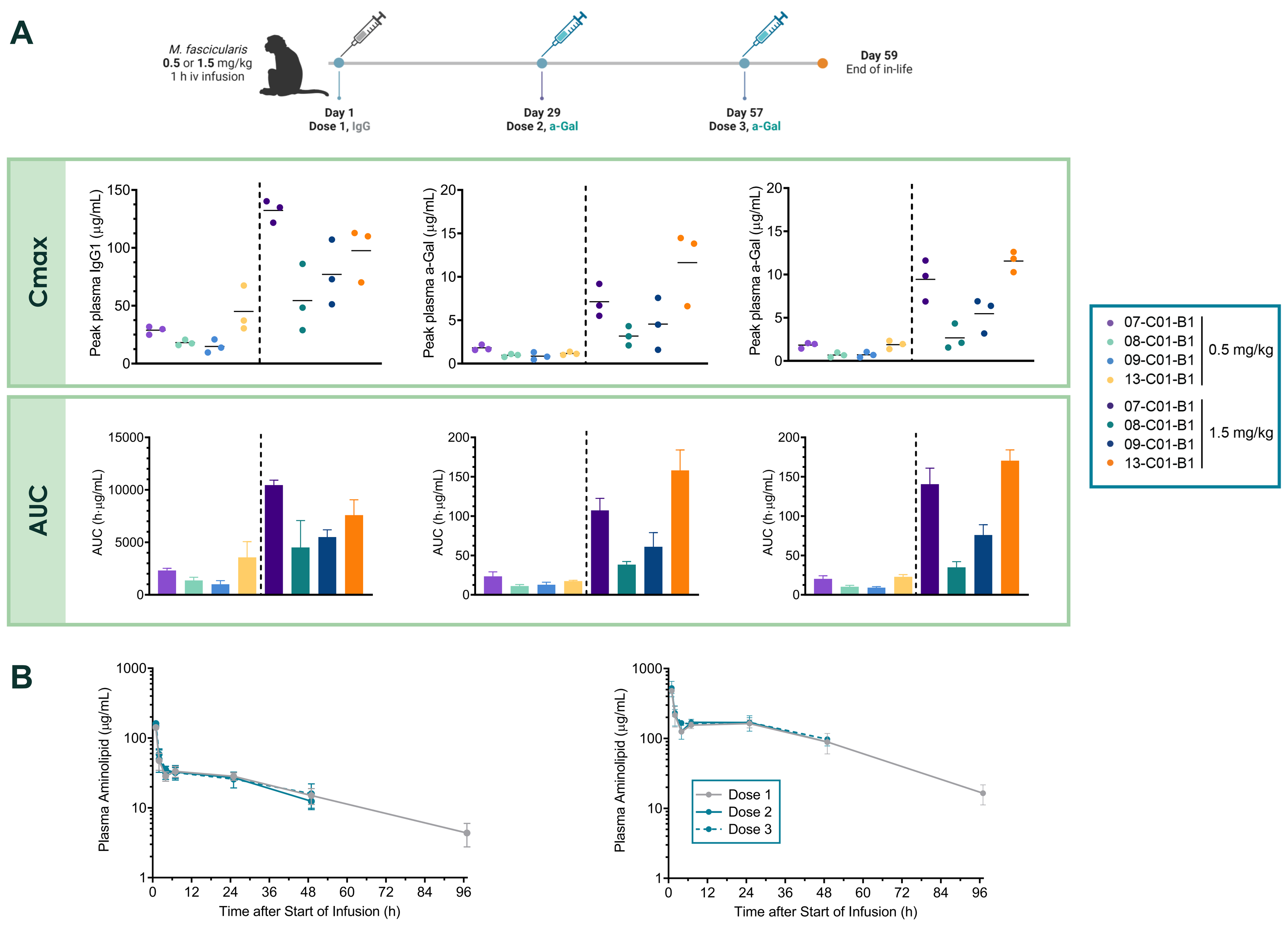
Lipid nanoparticles (LNP) are a highly effective and versatile delivery platform for nucleic acid medicines, currently enabling the validation of numerous examples of genetic and epigenetic editing medicines in the clinic. LNP systems are highly flexible and have been validated preclinically and clinically as an effective delivery system for numerous editing modalities, including zinc-finger nucleases, TALENS, and CRISPR/Cas9-based systems among others. Their capacity to encapsulate a wide range of payload sizes and nucleic acid structures makes LNP ideal vehicles for delivering mRNAs encoding more advanced editing systems such as prime editors, which are more complex and therefore require larger messenger (mRNA) and guide RNA (gRNA) payloads. Furthermore, LNP are capable of co-encapsulating and co-delivering multiple payloads (e.g., mRNA and gRNA required to enable CRISPR-based systems) to the same target cells and enable multiplexed editing therapeutics (e.g., mRNA-encoded CRISPR delivered with multiple gRNAs) to mediate editing at multiple loci with a single therapeutic. The low immunogenicity of LNP compared to viral systems enhances the safety profile and allows repeat dosing without compromising potency or safety, which is not possible with viral delivery vectors. This enables "dose-to-effect" strategies by which the medicine can be administered multiple times until the desired pharmacological effect is achieved, also allowing the use of lower and better tolerated doses. In addition to LNP-associated factors, another important safety consideration for mRNA medicines is the fact that mRNA is not associated with the potential for insertional mutagenesis inherent with DNA delivery. In addition, the transient expression of mRNA payloads delivered by LNP greatly reduces the potential for off-target editing associated with persistent expression from virally delivered editing systems.

The effective delivery of gene editing systems to hepatocytes has been extremely well validated preclinically and clinically, facilitated by the natural tendency of systemically administered mRNA-LNP to accumulate in the liver (i.e., approx. 60-80% of an injected dose). Multiple rodent and non-human primate models have demonstrated efficient and saturating editing in hepatocytes at well tolerated doses, using a broad range of editing systems. Notably, therapeutically relevant pharmacological effects have been observed for mRNA-LNP gene editing medicines in clinical studies for the treatment of monogenic inherited, cardiovascular and chronic infectious diseases. This includes a personalized base-editing medicine – developed within 6 months – to treat an infant born with a severe urea cycle disorder, resulting in a significant reduction in disease symptoms, the subsequent weaning from standard-of-care medicines, and allowing the infant to begin to thrive and leave the hospital for the first time since birth.

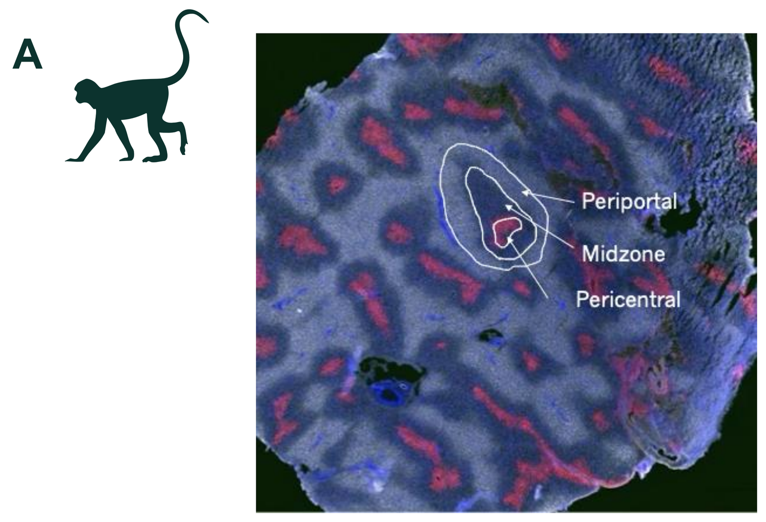
While LNP delivery to the liver is well established, efforts are being made to expand effective LNP delivery to reach extrahepatic targets. Delivery to non-liver target cells can dramatically increase the range of diseases that could be treated with gene editing medicines. Although challenging, good progress has been made using various strategies to access extrahepatic cell targets. One approach is "active" targeting, whereby ligands are conjugated to LNP to facilitate direct recognition and uptake by target cells accessible within the vascular compartment. As an example, we have used designed ankyrin repeat proteins (DARPins) as high-affinity ligands to actively target LNP delivery to specific target cells. These small proteins, covalently coupled to LNP with extended pharmacokinetic characteristics, allow effective delivery and expression of the LNP-encapsulated mRNA to circulating and splenic T-lymphocytes, as well as cells resident in the bone marrow compartment. In an alternative, more direct approach to extrahepatic tissue targeting, we have used aerosolized mRNA-LNP to reach, transfect and edit pulmonary epithelial cells. Specific technology has been identified which allows effective aerosolization of the LNP with minimal or no impact on biophysical properties or potency. Optimization of lipid composition has allowed effective migration through mucous associated with cystic fibrosis, a barrier that has previously posed a significant challenge for LNP therapies in this patient population. Additionally, the appropriate selection of ionizable lipids enables highly effective transfection of lung cells based on 3D airway cultures.

This work described highlights the role of LNP as a leading and highly versatile delivery platform to enable mRNA-based genetic and epigenetic editing therapeutics. The promise of these medicines in the clinic is already being realized for the treatment of a range of liver disorders, including chronic diseases such as hypercholesterolemia, monogenic inherited diseases, such as urea cycle disorders and transthyretin amyloidosis, and infectious diseases such as chronic hepatitis B infection. In the future, continued advances in LNP delivery technology to achieve effective mRNA delivery to extrahepatic tissues will allow these therapies to be applied to a progressively wider range of therapies such as *in vivo* generation of CAR-T cells to treat autoimmune and malignant disease, editing of hematopoietic stem cells to treat sickle-cell disease and editing of airway epithelial cells as a treatment for cystic fibrosis.

Repeat Dosing of a mRNA-LNP Adenine Base Editor

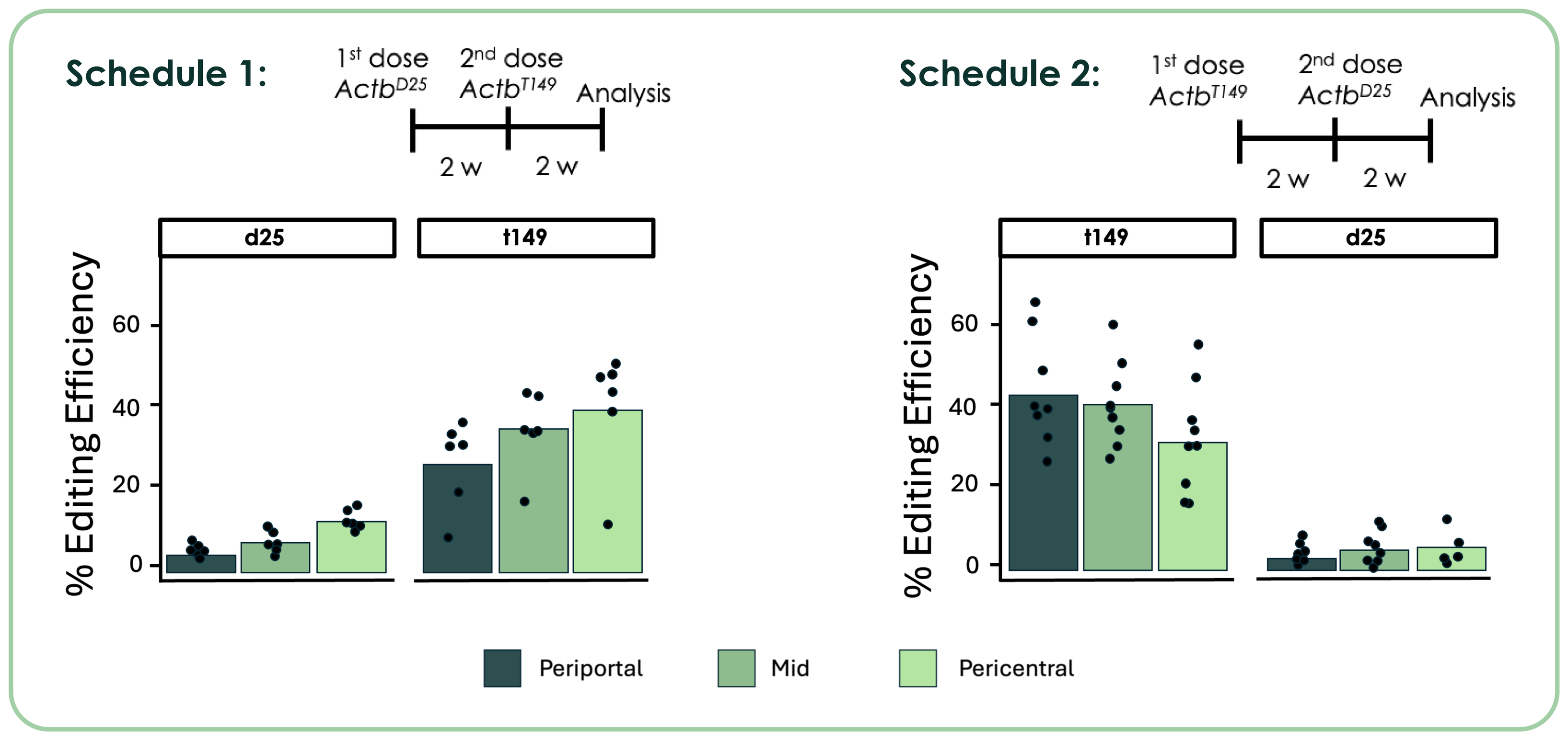


Pharmacodynamics and pharmacokinetics. A: Pharmacodynamic activity following repeat IV administration of 4 different LNP encapsulating mRNA encoding human IgG (Dose 1) and human α-galactosidase (α-gal; Doses 2 and 3) at 0.5 and 1.5 mg/kg doses. **B:** Plasma PK of the ionizable lipid following repeat IV administration a mRNA-LNP at doses of 0.5 and 1.5 mg/kg.

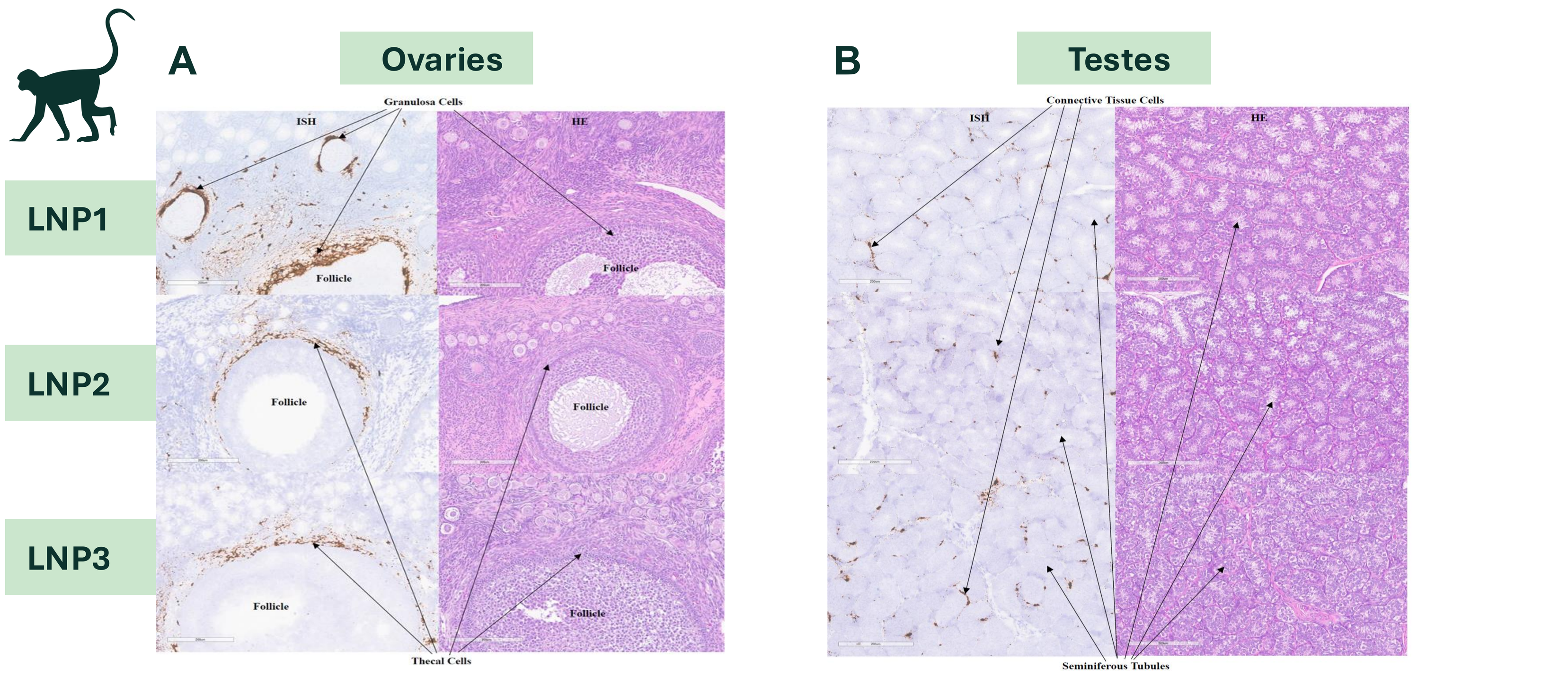


Detection of base editing by *In Situ* Sequencing (ISS) in the liver of LNP-RNA treated Cynomolgus macaques. A: Liver zonation where liver tissue sections were stained for ASS1 to mark the periportal zone and GS to mark the pericentral zone. **B:** Cynomolgus macaques were treated with either mRNA-LNP encoding ABEs targeting $ACTB^{D25}$ and two weeks later $ACTB^{T149}$ (Schedule 1) or $ACTB^{T149}$ and two weeks later $ACTB^{D25}$ (Schedule 2). Editing rates in liver biopsies was determined by NGS and quantification of editing rates in the three zones within single cells was assessed by ISS using barcoded padlock probes for the $ACTB^{D25}$ and $ACTB^{T149}$ target loci.

NOTE: this work was performed in collaboration with the Schwank Lab at the University of Zurich.



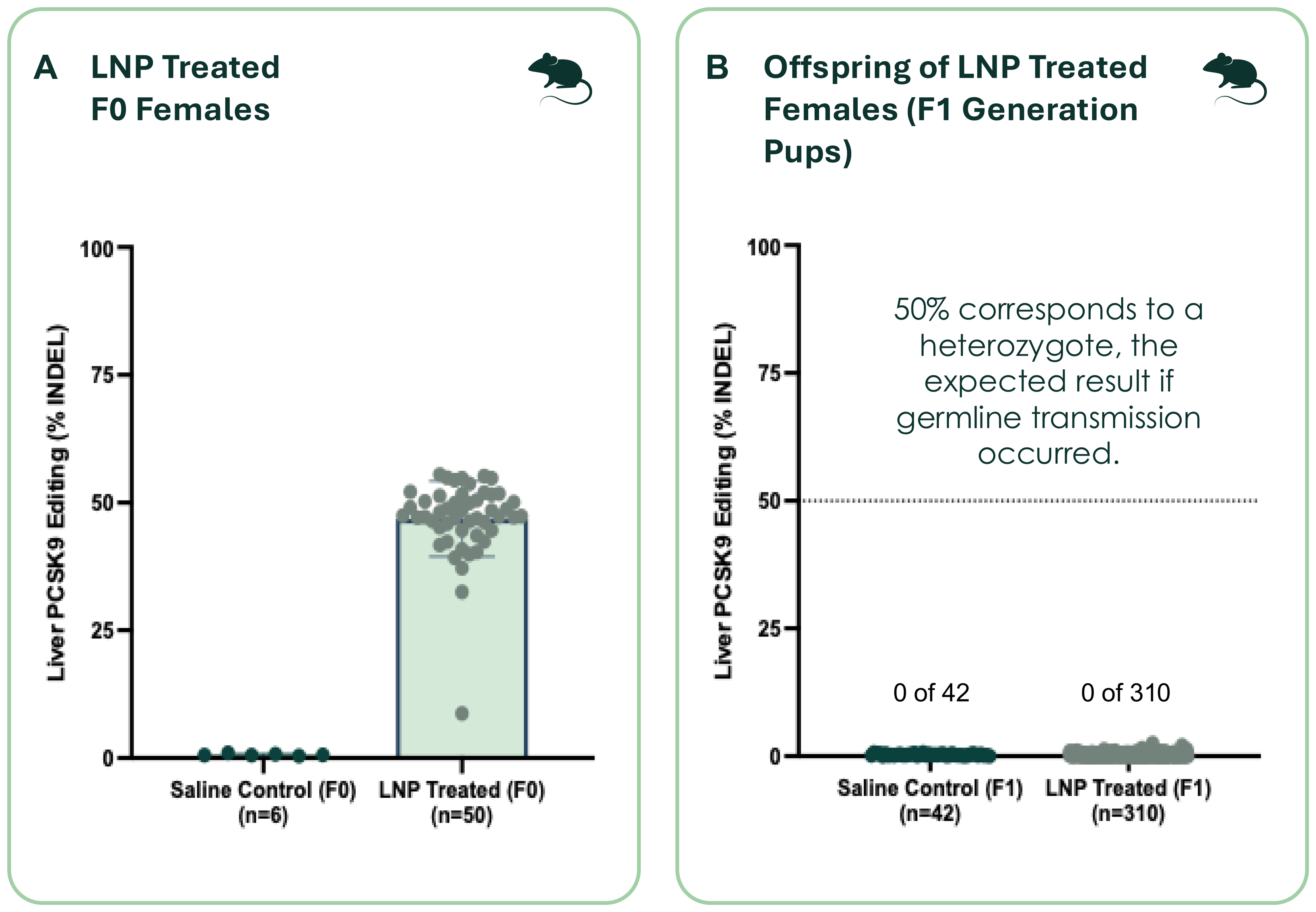
Distribution of mRNA-LNP Gene Editor to Ovaries/Testes



Localization/Cell Type	LNP1	LNP2	LNP3
Follicle			
• Oocyte	--	--	--
• Flat Follicular Epithelial Cell	--	--	--
• Cuboidal Follicular Epithelial Cell	X (4)	--	--
• Antrum	X (1)	--	--
• Granulosa Cell	X (1,4)	--	--
• Theca Cell	X (1,4)	X (1,4,24,72)	X (1,4,24,72)
Corpora Lutea	--	--	X (336)
Corpora Albicans	--	X (1,4,72)	X (4)
Connective Tissue (Spindle) Cells	X (1,4,24)	X (1,4,72)	X (1,4,24,72)
Capsule Cells	X (1,4)	X (1,4)	X (1)
Blood Vessel			
• Intima	X (4)	X (1,4,24)	X (1,4)
• Media	--	--	--
• Adventitia	X (4)	X (1,4)	X (4,24,336)

Localization/Cell Type	LNP1	LNP2	LNP3
Leydig Cells	--	--	--
Spermatogonia*	--	--	--
Sertoli Cells	--	--	--
Connective Tissue/Spindle Cell	X (1,4,24,72,336)	X (1,4,24,72)	X (1,4,24,72,168)
Capsule Cells	X (1)	X (1)	X (4)
Blood Vessel		--	--
• Intima	--	X (1)	X (1,4)
• Media	--	--	--
• Adventitia	--	--	--

Detection of mRNA in Ovaries (A) and Testes (B) of Cynomolgus Monkeys. mRNA detection in tissue sections was evaluated for 14 days by *in situ* hybridization, following a 1 mg/kg intravenous infusion of mRNA-LNP in cynomolgus monkeys. Brown staining represents mRNA delivered by LNP. Tissue localization and cell types containing mRNA signal were evaluated by a board-certified pathologist (tables indicate presence of signal and time points, hour). Oocytes were evaluated both manually and using a computer algorithm.

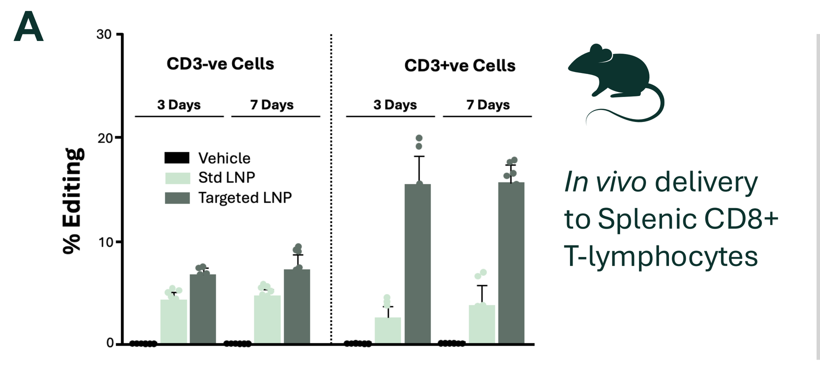


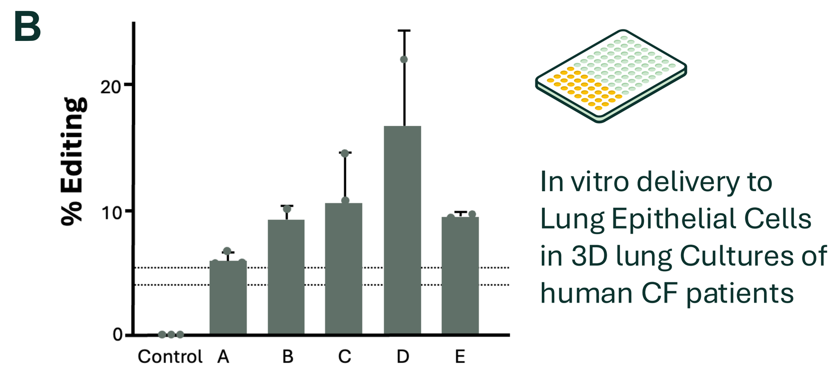
F1 progeny study. PCSK9 editing rates in livers of female (F0) and offspring (F1) mice following IV treatment of F0 females with either saline or 1 mg/kg LNP carrying Cas9 mRNA and PSCK9 gRNA (n=50); 14 days later, all female animals were bred. Livers from F0 female dams, 42 pups from saline treated dams and 310 pups from LNP treated dams (the F1 generation) were collected for PCSK9 editing analysis. DNA was extracted and PCR was used to amplify the PCSK9 locus containing the editing site followed by library preparation and next-generation sequencing.

Extrahepatic mRNA-LNP Delivery of Gene Editors

mRNA-LNP Mediated Editing in Extrahepatic Target Cells. A: Editing in splenocytes isolated from wild type mice 3 or 7 days following IV treatment with either saline or 1 mg/kg of a LNP targeted with DARPins directed against murine CD8. LNP encapsulated a mRNA encoded gene editor. CD3 negative and positive populations were immunomagnetically isolated and assessed for editing. **B:** Editing in 3D-lung cultures derived from cells from cystic fibrosis patients. Five different mRNA-LNP encapsulating a mRNA encoded gene editor was applied to the 3D cultures and editing in airway epithelial cells was assessed. Editing rates were 2-3x higher than benchmarks previously reported in similar model systems.

NOTE: the work in (B) was performed in collaboration with the Hedtrich Lab at the University of British Columbia





Summary

1. mRNA-LNP can be repeat dosed with no impact on potency or pharmacokinetic behavior.
2. Repeat administration of a mRNA encoded base editor does not impact level or distribution of editing, enabling dosing to effect.
3. No mRNA is detected in oocytes or spermatogonia following IV administration of mRNA-LNP formulations.
4. No indication of germline transmission is observed following IV administration of LNP encapsulating a mRNA-encoded gene editor.
5. Effective extrahepatic editing following delivery of LNP encapsulating a mRNA-encoded gene editor in T-lymphocytes and airway epithelial cells.

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