Pharmacokinetics and Distribution of Lipid Nanoparticle Formulations of mRNA in Gonadal Tissues in Rat and Monkey

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Background:

With the increasing use of lipid nanoparticles (LNP) for gene-editing and other RNA-based therapeutics, a detailed understanding of LNP distribution is essential to support the safety assessment of these therapeutics and to identify cell types for further investigation of on- and off-target editing. The distribution and persistence of LNP components in gonadal tissue is of particular interest, given the potential for chemical and/or biological effects of LNP components on reproductive status. To address these questions, we have evaluated the pharmacokinetics and distribution of different mRNA-LNP formulations in cynomolgus monkeys and Wistar Han rats, with emphasis on distribution into testes and ovaries.

Methods:

Naïve cynomolgus monkeys (~3-4 years old) received a 1-hour intravenous infusion of one of three distinct mRNA-LNP formulations (identified as LNP1, LNP2 and LNP3). One male and one female were terminated at 1, 4-, 24-, 72- and 168-hours post-dose and three males and three females were terminated at 336-hours post-dose for tissue collection. Plasma samples and urine and feces were collected at various time points prior to termination.

Wistar Han, male and female rats (6-7 weeks; 112-212 g) were given a single 1 mg/kg intravenous dose of mRNA-LNP (LNP2). Animals were terminated at specific time points and processed for whole body imaging, or isolated organs were collected and prepared for LC-MS/MS or imaging.

mRNA localization and persistence within tissues and cells was evaluated by *in situ* hybridization. Ionizable lipid and PEG-lipid concentrations in plasma and tissues were quantified by LC-MS/MS and/or MALDI-MS.

Results:

Plasma PK/Distribution in NHP: Distinct plasma pharmacokinetic (PK) profiles and large differences in plasma exposure of the ionizable lipids were observed in monkeys for the three different mRNA-LNP compositions evaluated; however, the PK profiles of the PEG-lipid component of each mRNA-LNP formulation were nearly identical, consistent with the intended exchange of the PEG-lipid out of the LNP *in vivo*.

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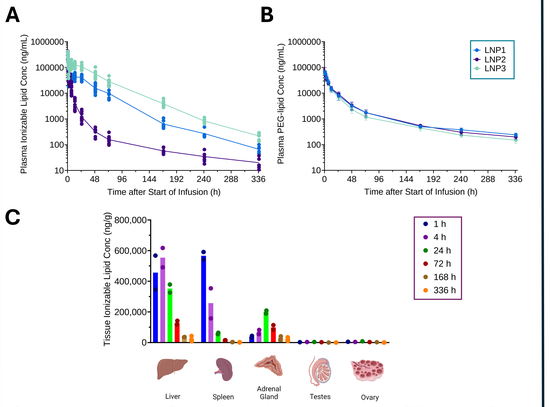
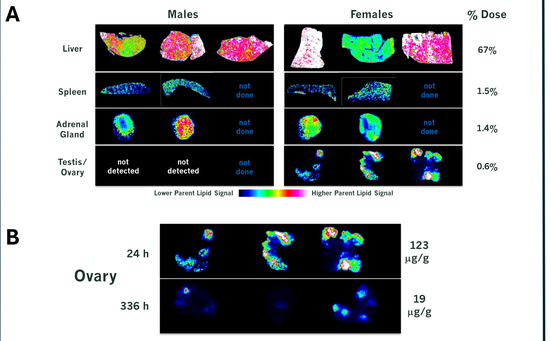
NHP - Plasma PK/Distribution



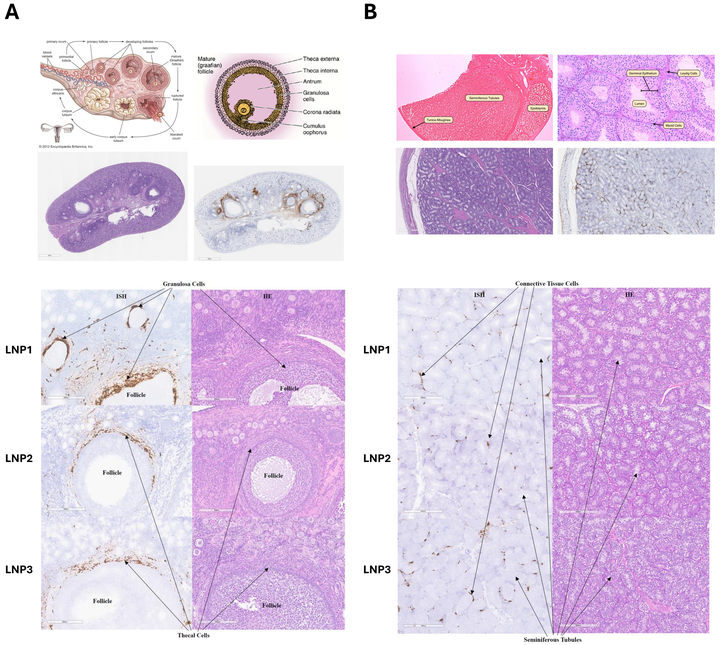
Pharmacokinetics and Distribution of Ionizable Lipid and PEG-lipid in Three Distinct mRNA-LNP in Cynomolgus Monkeys. A: Plasma PK of ionizable lipid for LNP1, LNP2 and LNP3. **B:** Plasma PK of PEG-lipid for LNP1, LNP2 and LNP3. **C:** Ionizable lipid distribution (LNP1) in key tissues.

Rat - Distribution



Distribution of Ionizable Lipid in Rat Tissues by MALDI-MS. A: Detection of parent ionizable lipid (in LNP2) in liver, spleen, adrenal glands and gonadal tissues in rat (24 h post-dose). **B:** Decreased detection of parent ionizable lipid in rat ovaries after 14 days.

NHP – Distribution 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	--	--	--

* Includes all cells originating from germ cells

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.

Results (continued):

Ionizable lipids, PEG-lipid and mRNA were detected mainly in liver, spleen and adrenal glands for all three mRNA-LNP, and much lower levels of ionizable lipids were detected in ovaries and testes, which further decreased to near the limit of detection by 336 hours.

LNP (mRNA) Distribution in Gonadal Tissue: Low levels of mRNA payload were also detected in ovaries and testes, with most of the signal present for only 24 hours post-dose and declining rapidly thereafter, consistent with mRNA degradation. In ovaries, mRNA was observed mainly in theca cells surrounding the follicles and in connective tissue (spindle cells), while in testes, mRNA was detected mainly in connective tissue and capsule cells. Importantly, no mRNA was detected within oocytes or spermatogonia at any timepoints, for any of the formulations.

Distribution of Ionizable Lipid in Rat: Ionizable lipid (parent molecule) in LNP2 was detected in rat liver, spleen, adrenal gland and ovaries at 24 hours post-dose and declined substantially by 336 hours. No signal was detected in testes. Overlays of serial MALDI-MS images and H&E sections of ovary samples indicated lipid signal was associated with corpora lutea.

Summary:

1. Ionizable lipids, PEG-lipid and mRNA were detected mainly in liver, spleen and adrenal glands for all three LNP, and much lower levels of ionizable lipids were detected in ovaries and testes.

2. In ovaries, mRNA was observed mainly in theca cells and in connective tissue, while in testes, mRNA was detected mainly in connective tissue and capsule cells, and no mRNA was detected in seminiferous tubules.

3. No mRNA was detected in oocytes or spermatogonia at any timepoints, for any mRNA-LNP formulations.

4. Tissue distribution of mRNA-LNP in rat was consistent with monkey.

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