

# Optimizing Ionizable Lipid & LNP Properties to Improve Safety

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Acuitas Therapeutics  
December 10, 2024

3<sup>rd</sup> LNP Immunogenicity & Toxicity Summit  
Boston, MA

# LNP Technology Clinically Validated



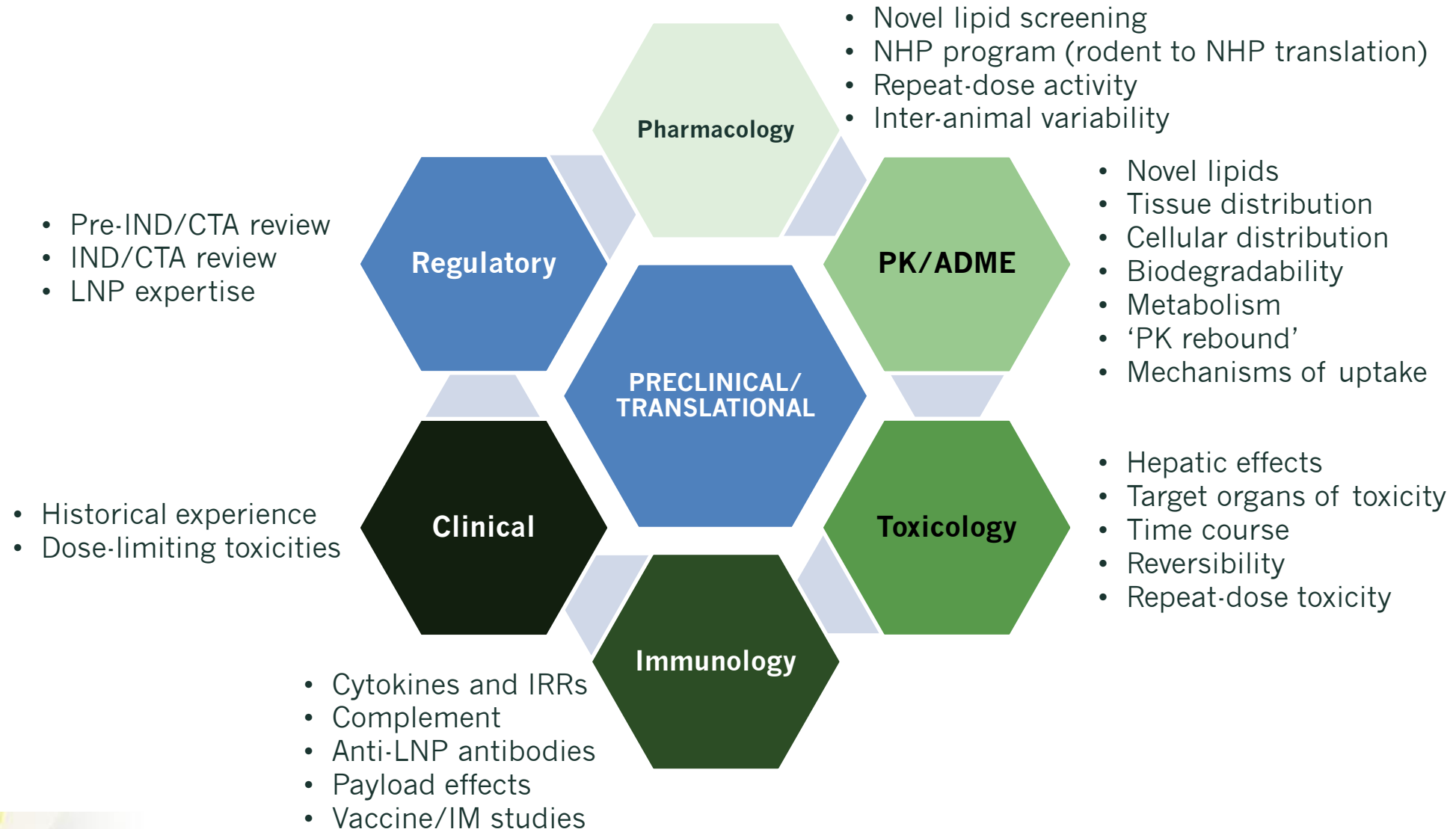
- Acuitas LNP formulation used in ONPATTRO® (Alnylam partnership)
  - First Approved RNAi product (2018)
  - Approved in Canada, US, EU, Japan & elsewhere

- Acuitas LNP formulation used in Comirnaty®  
(BioNTech/Pfizer partnership)

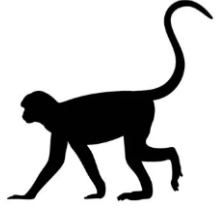
- Emergency authorization in Canada, US, EU, UK and elsewhere (2020)
  - First approved mRNA therapeutic (2021)



# Acuitas LNP Core Research Program

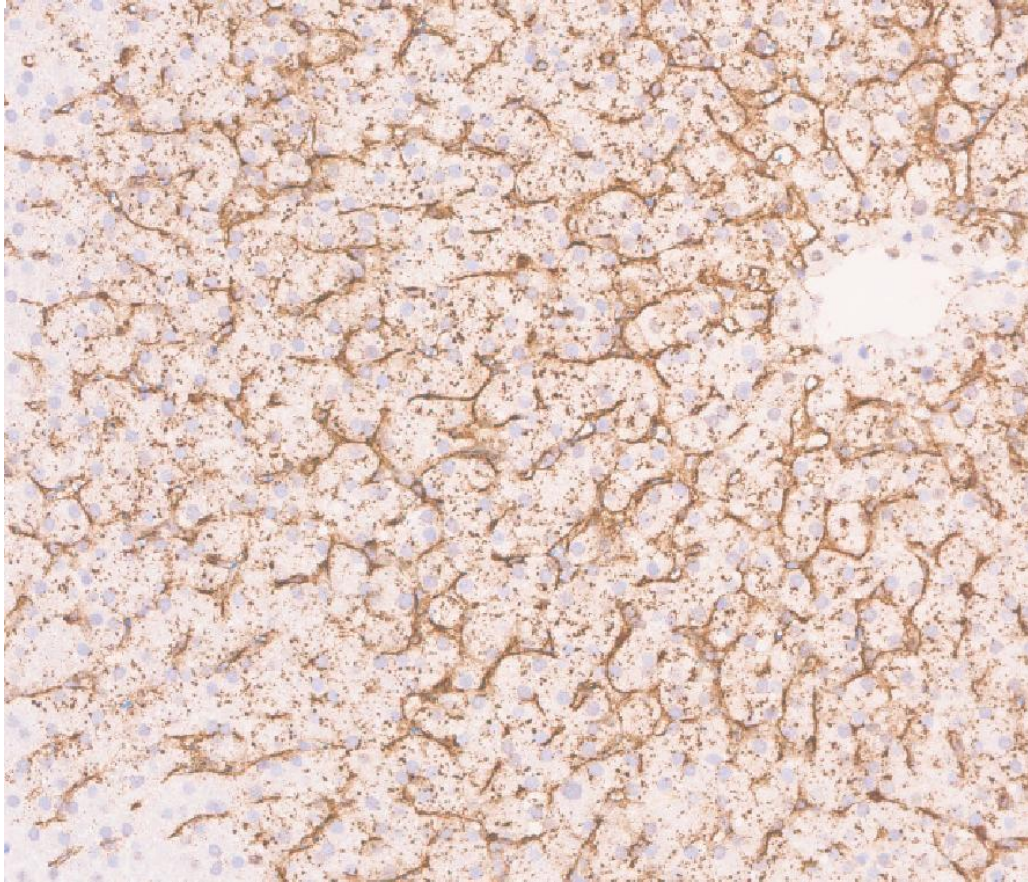


*M. fascicularis*  
2.0 mg/kg  
1 h iv infusion

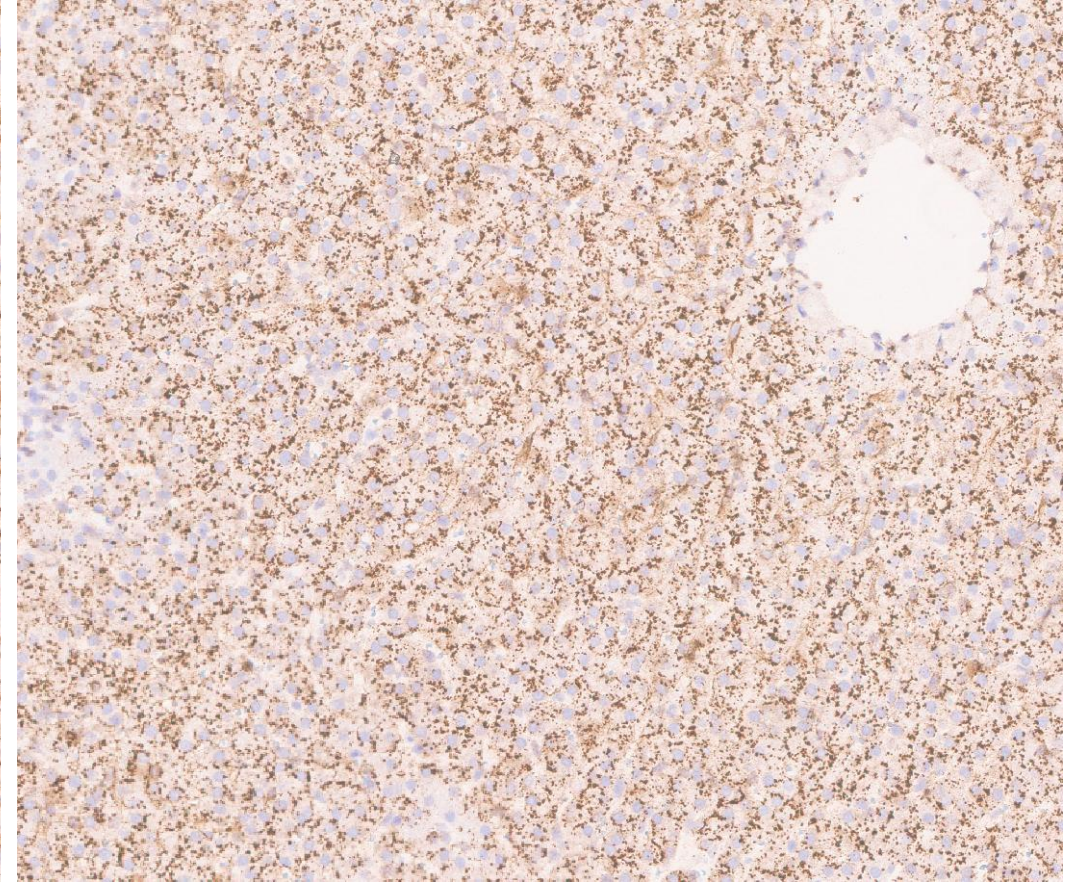


# Optimization

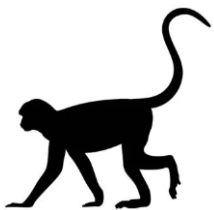
Small Particles Improve Distribution into Hepatocytes and Increase Activity



**LNP 09 (72 nm)**  
**Plasma IgG: 24.6  $\mu\text{g}/\text{mL}$**

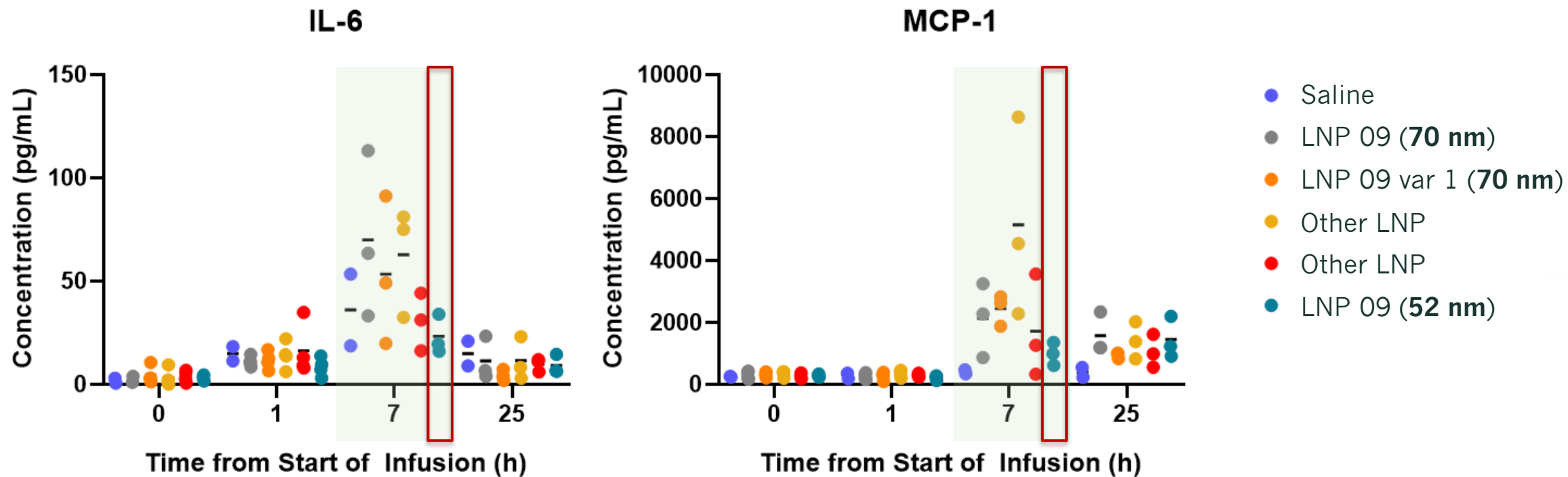


**LNP 09 (54 nm)**  
**Plasma IgG: 91.3  $\mu\text{g}/\text{mL}$**

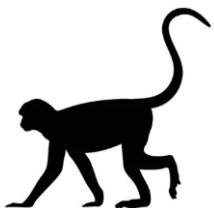


# Optimization

## Small Particles Reduce Cytokine Secretion

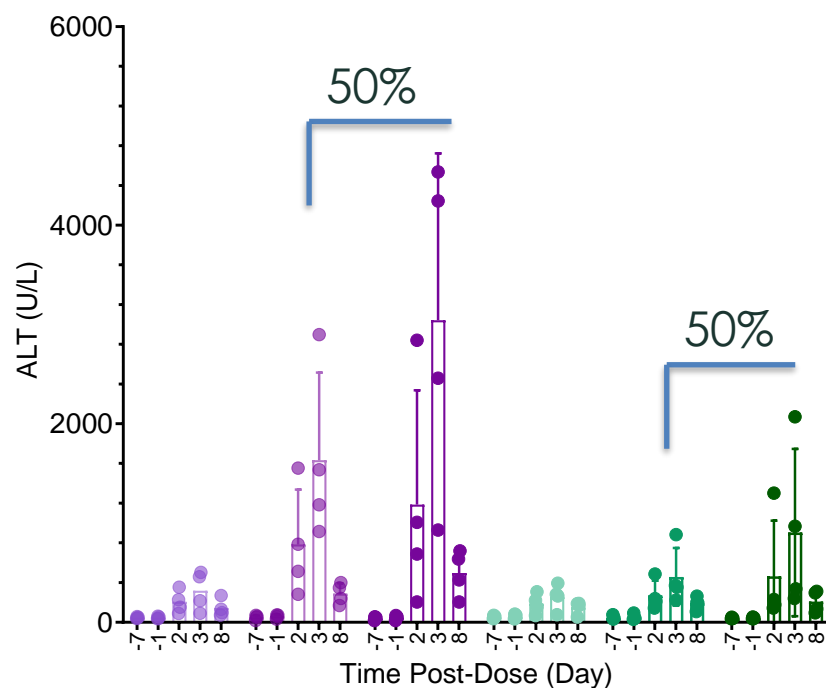


- Transient increases at 6 hours post-infusion; largely returned to baseline by 24 hours post-EOI
- Smaller particles reduce cytokines/chemokines vs larger particles of the same composition



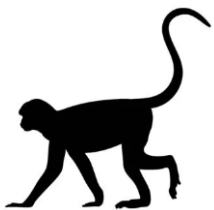
# Optimization

## Reducing Total Lipid Dose Reduces Hepatic Effects



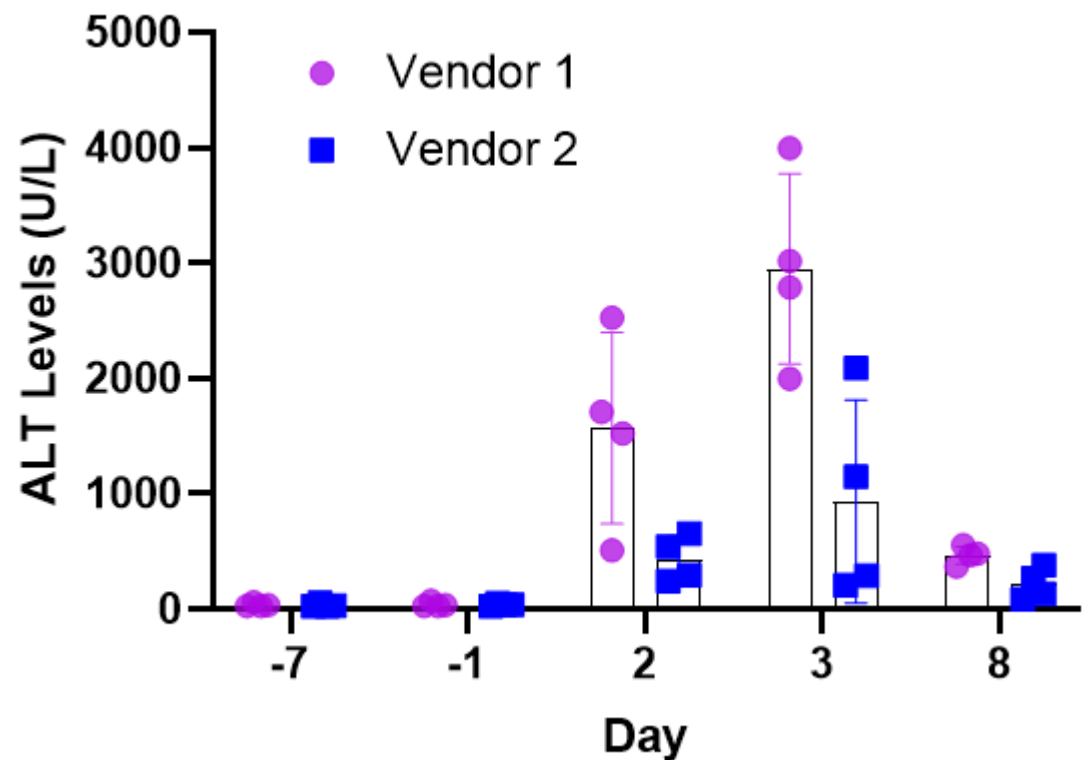
LNP	07	07	07	13	13	13
N:P Ratio	3	4.5	6	3	4.5	6

- N:P ratio - ratio of the amine groups (N) of the ionizable lipid to the phosphate group (P) of the payload
- Lower N:P ratio means less total lipid is dosed for a given RNA dose
- Tolerability benefit must be optimized in parallel with assessing activity of the specific payload

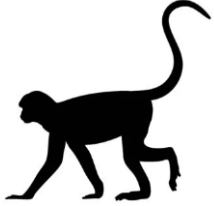


# Optimization

## Payload Quality Affects Toxicity

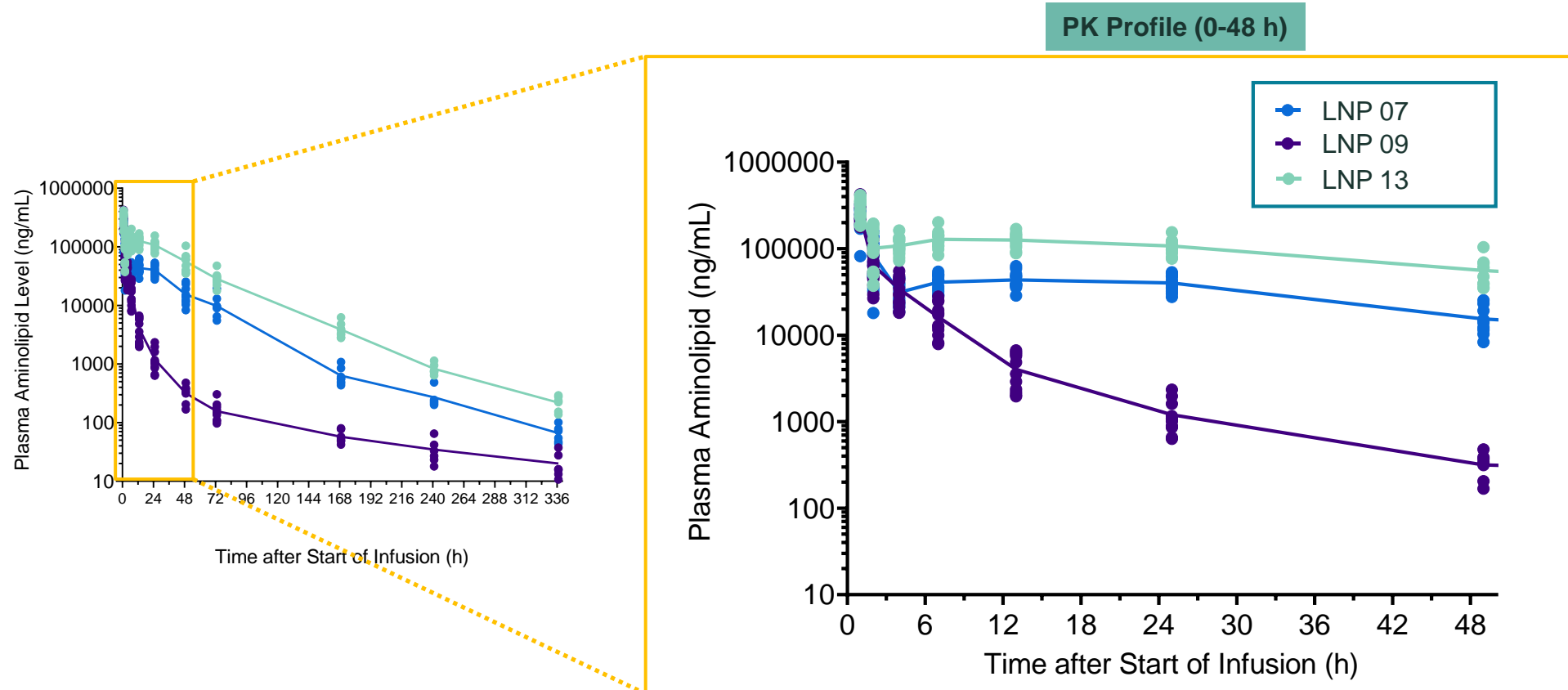


- 1-hour IV infusion
- 1.5 mg/kg
- Male cyno monkeys (same origin and vendor)
- Same LNP composition and particle size
- Same lipid raw materials
- Same IgG mRNA, from different vendors



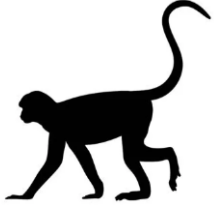
# Pharmacokinetics

## Pharmacokinetic Profiles of Ionizable Lipid in Lead IV LNP in Monkeys



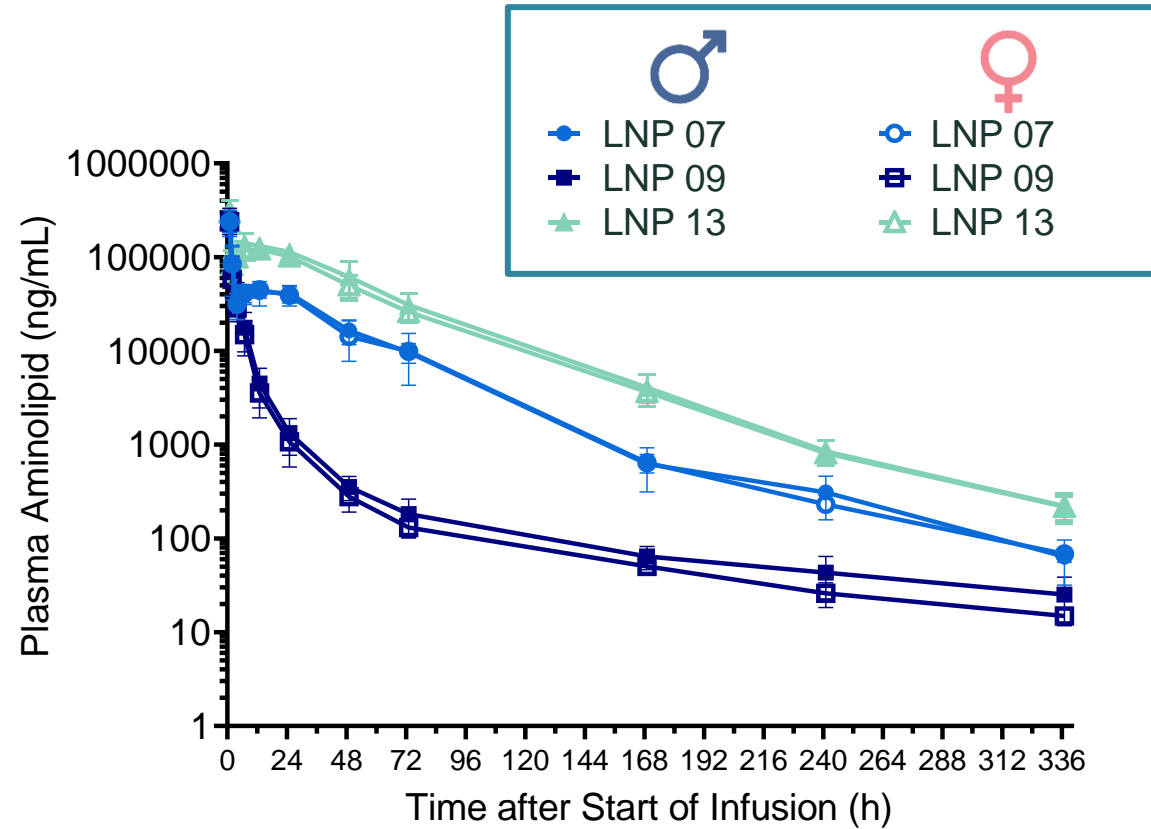
- Broad range of plasma exposures (~13-fold range of plasma AUC)
- 'Rebound' (LNP 07 and LNP 13) and 'traditional' (LNP 09) profiles



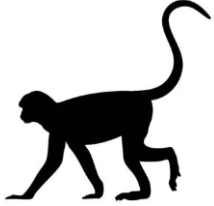


# Pharmacokinetics

## Plasma PK of Ionizable Lipid in Male and Female Monkeys

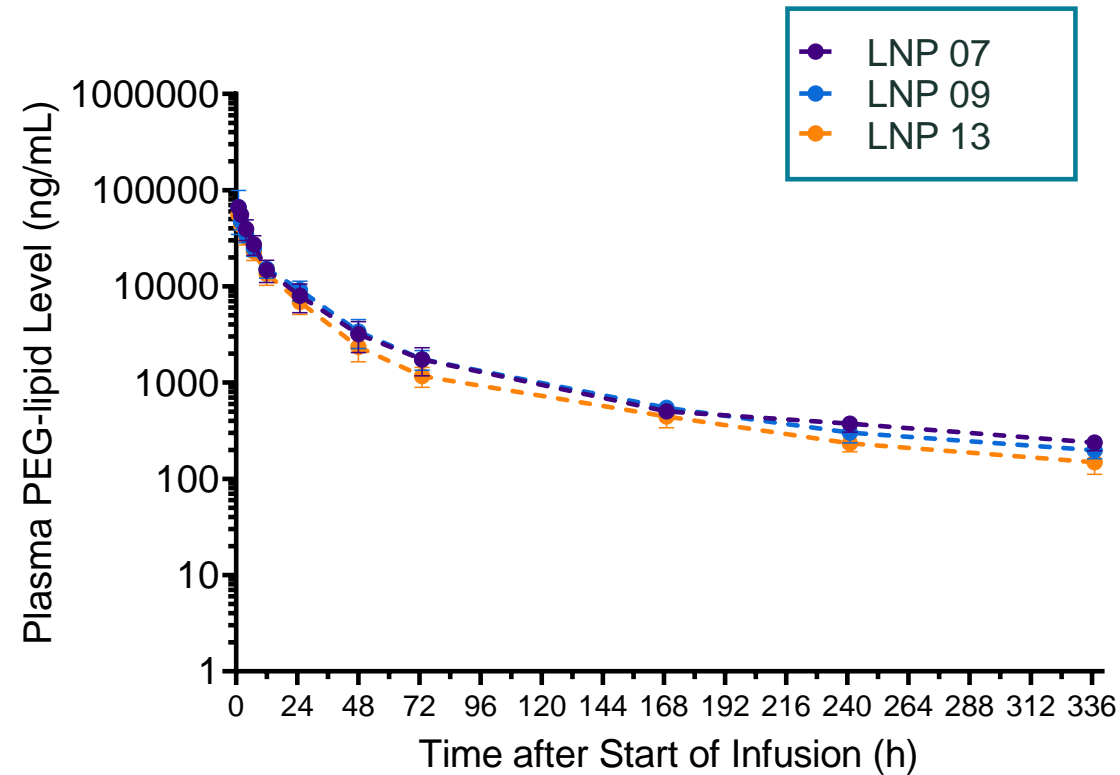


- No difference in plasma PK profile between males and females



# Pharmacokinetics

Plasma PK of PEG-Lipid in Monkeys is Independent of Formulation

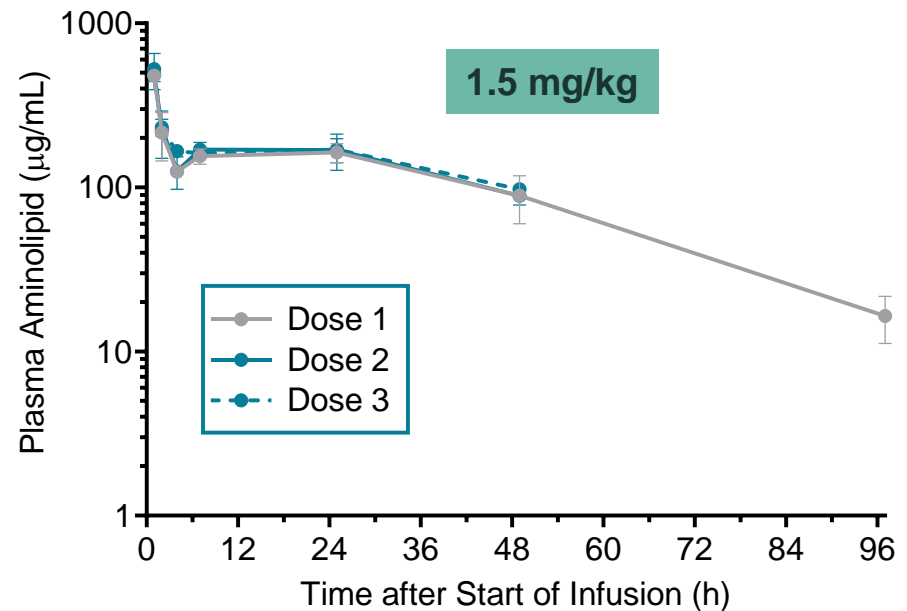
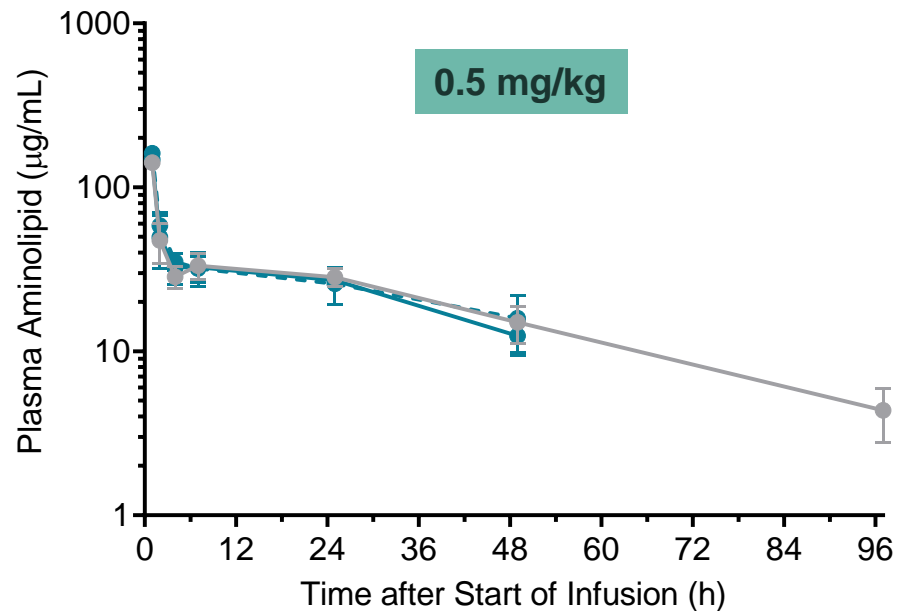
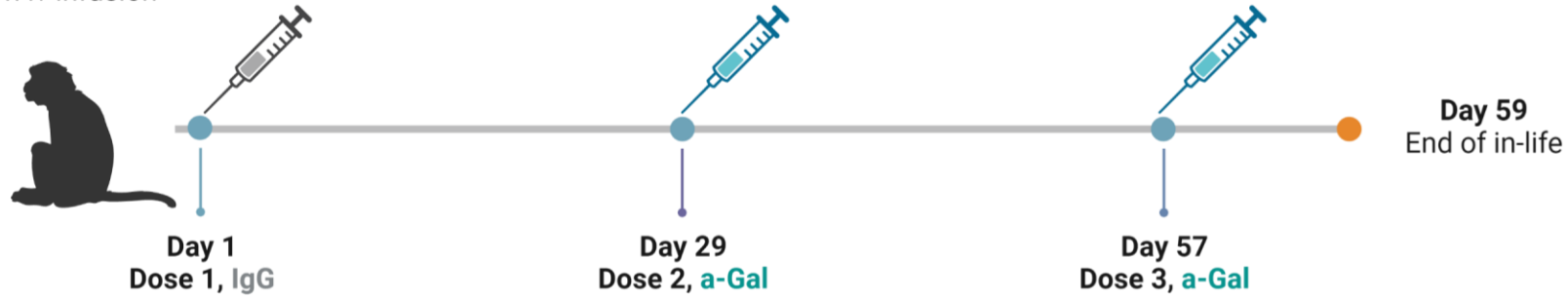


- PEG-lipid exchanges out of LNP in the circulation, resulting in overlapping PEG-lipid profiles for all three LNP formulations

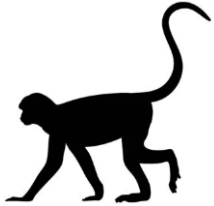
# Pharmacokinetics

## Plasma PK Profile is Payload-Independent and Consistent Upon Repeated Dosing

*M. fascicularis*  
0.5 or 1.5 mg/kg  
1 h iv infusion

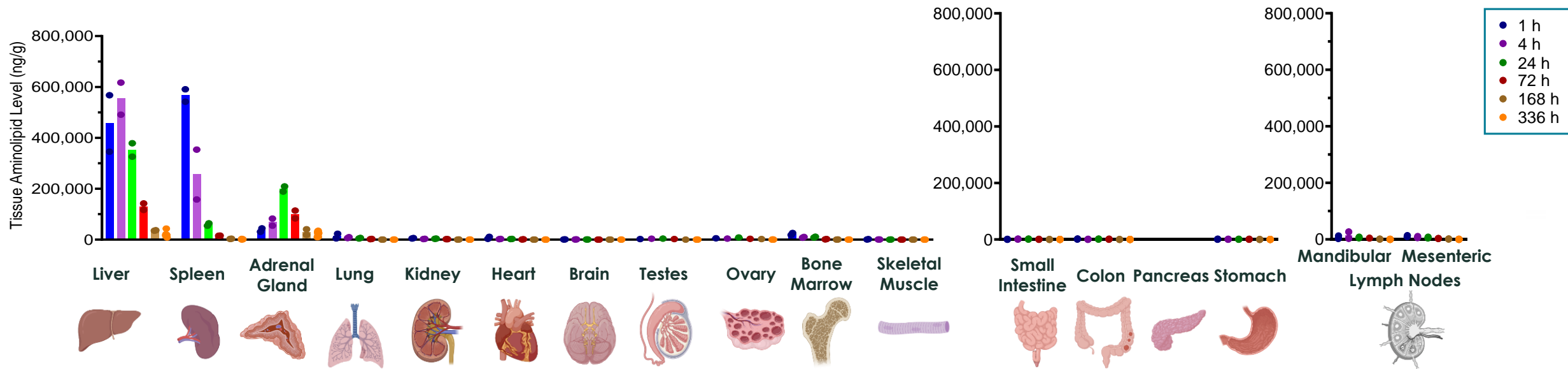


LNP 07

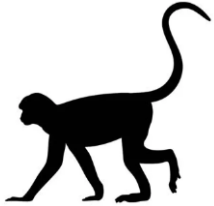


# Distribution

## Ionizable Lipid Tissue Distribution in Monkeys – LNP 07

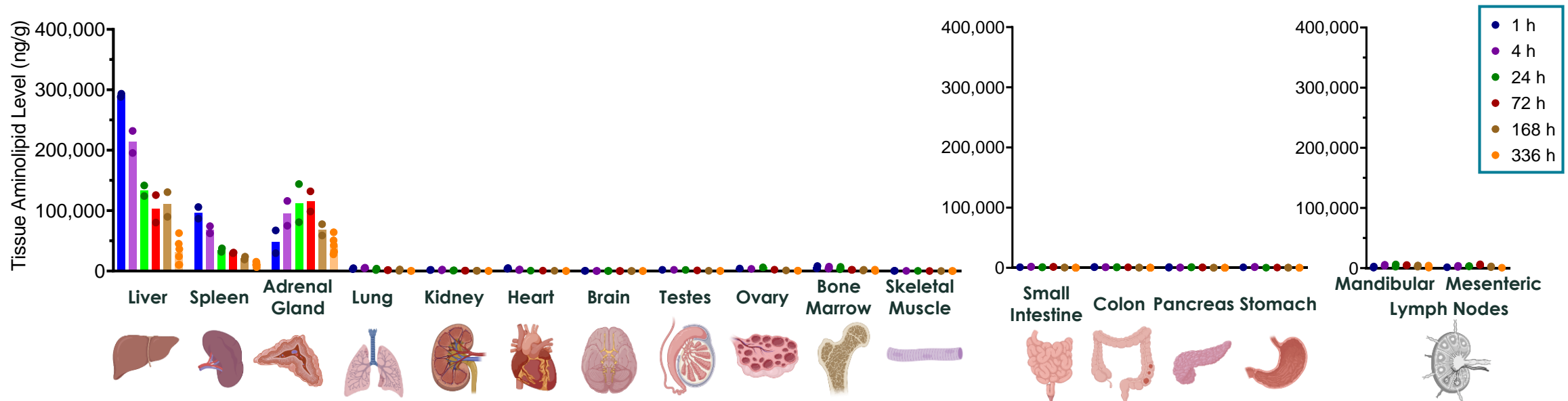


- ⦿ Main tissues: liver  $\approx$  spleen > adrenal gland
- ⦿ No/minimal uptake into other sampled organs

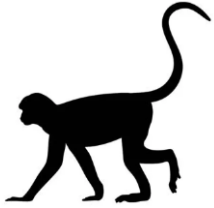


# Distribution

## Ionizable Lipid Tissue Distribution in Monkeys – LNP 09

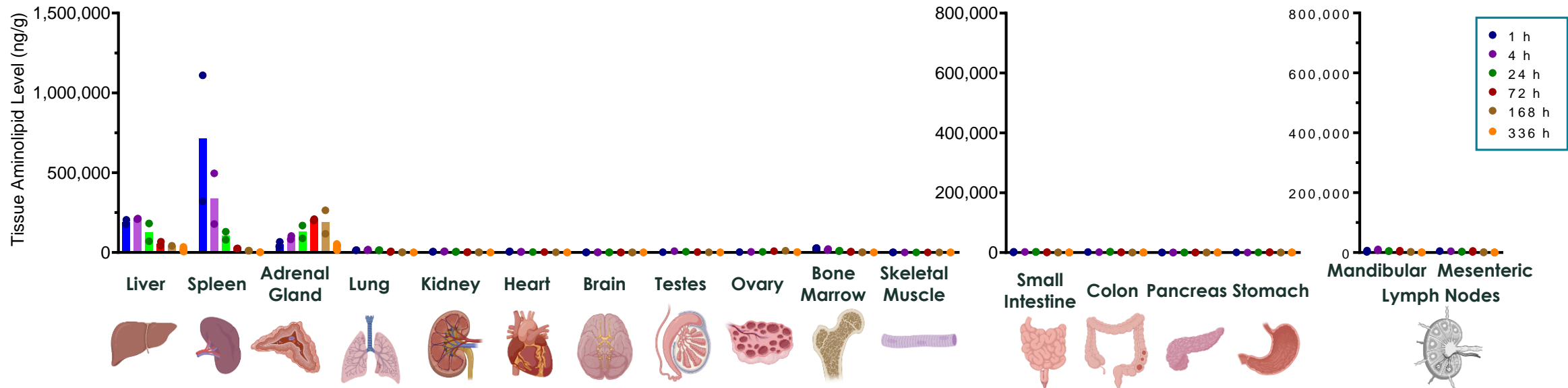


- Main tissues: liver > spleen ≈ adrenal gland
- No/minimal uptake into other sampled organs



# Distribution

## Ionizable Lipid Tissue Distribution in Monkeys – LNP 13



- Main tissues: spleen > liver ≈ adrenal gland
- No/minimal uptake into other sampled organs

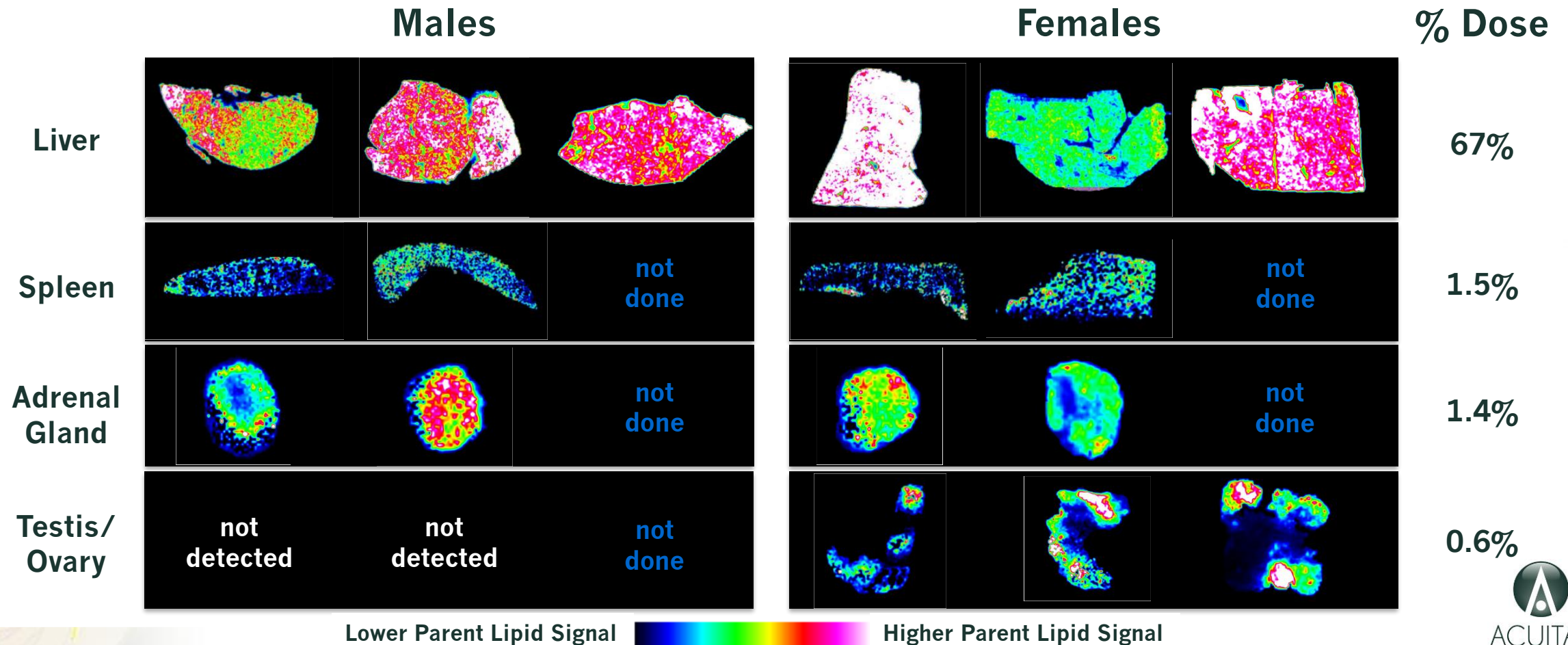
1.0 mg/kg  
IV slow push



# Distribution

Tissue Distribution in Rat is Consistent with Monkey

LNP 09 – Ionizable Lipid (24 hours)



1.0 mg/kg  
IV slow push



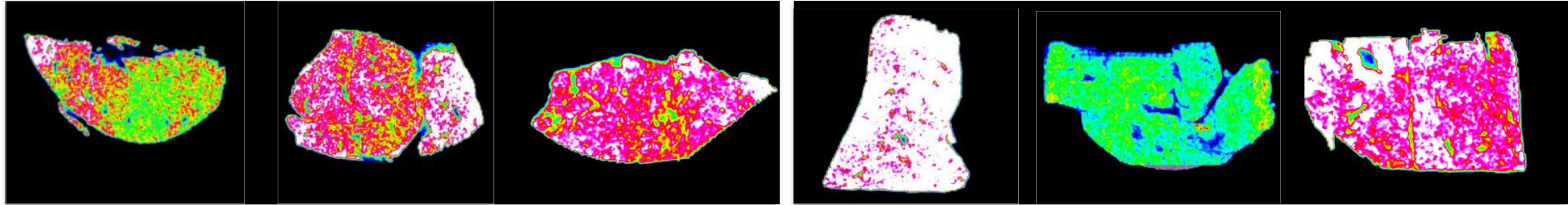
# Distribution

Ionizable Lipid Levels Significantly Decreased by 14 Days Post-Dose

Males

Females

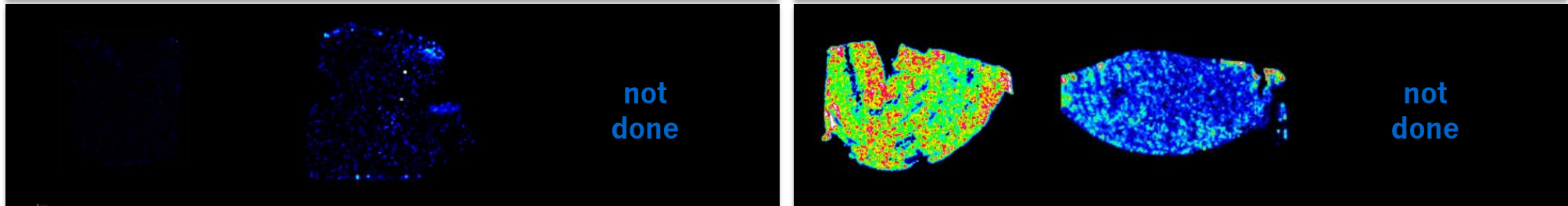
24 h



202  
 $\mu\text{g/g}$

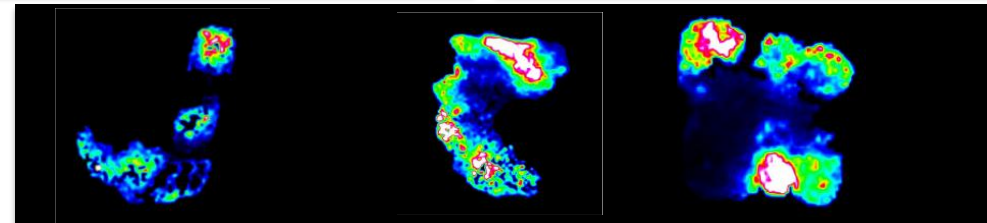
Liver

336 h



81  
 $\mu\text{g/g}$

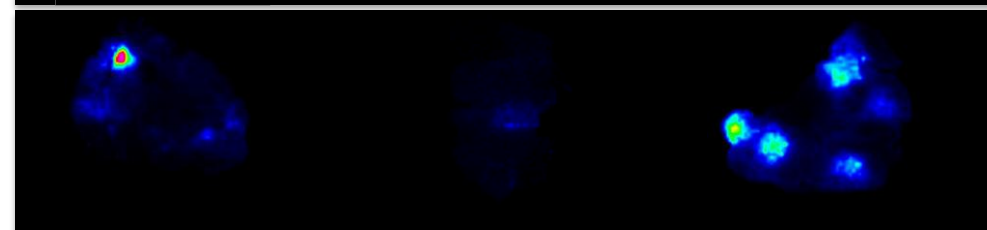
24 h



123  
 $\mu\text{g/g}$

Ovary

336 h

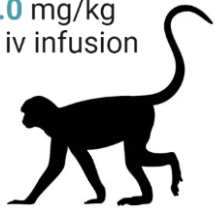


19  
 $\mu\text{g/g}$

0% 20%



*M. fascicularis*  
1.0 mg/kg  
1 h iv infusion



# mRNA Distribution in Monkey Liver (ISH)

Time  
Post-EOI:

1 h

4 h

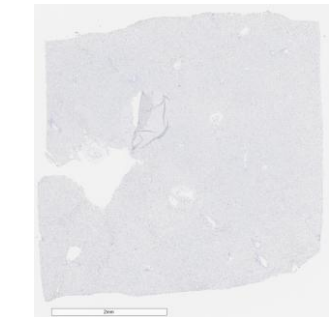
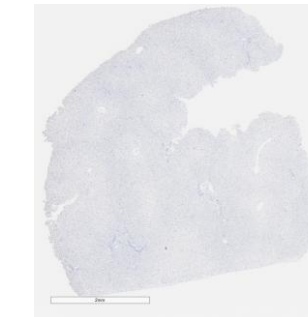
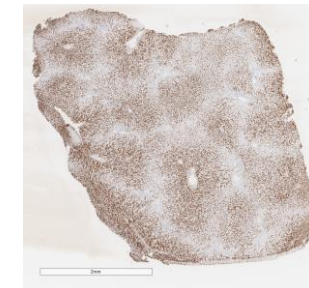
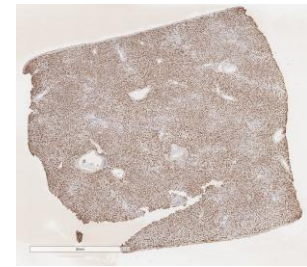
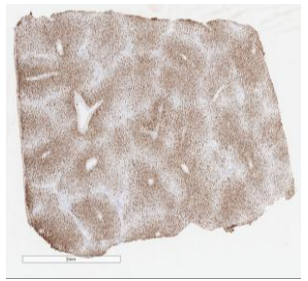
24 h

72 h

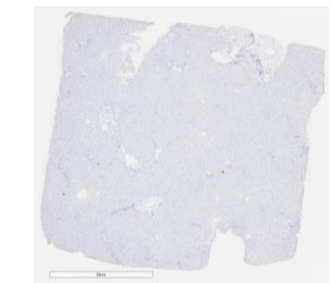
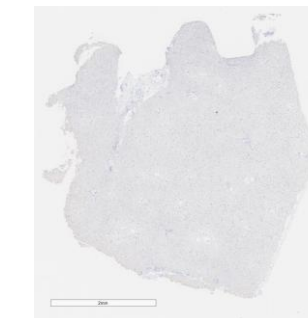
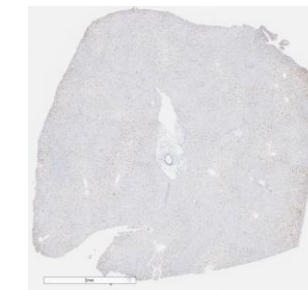
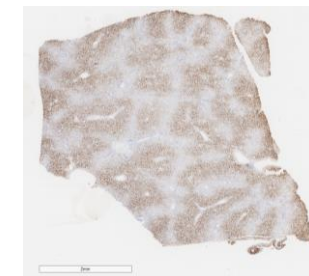
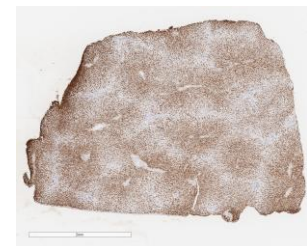
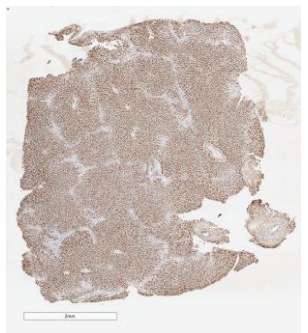
168 h

336 h

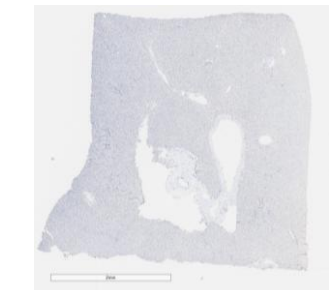
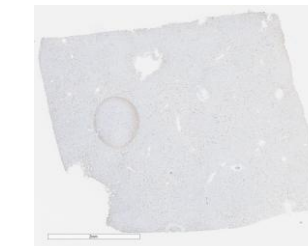
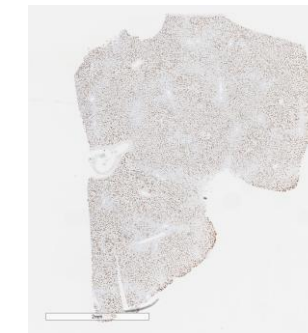
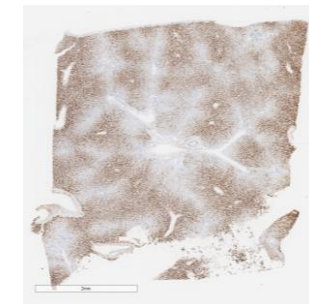
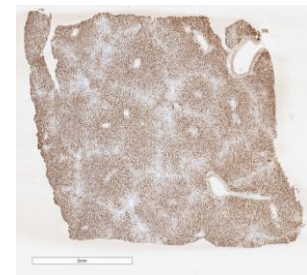
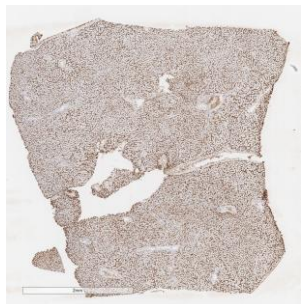
LNP 07



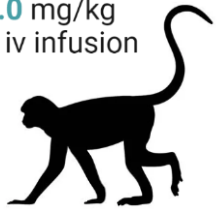
LNP 09



LNP 13



*M. fascicularis*  
1.0 mg/kg  
1 h iv infusion



# mRNA Distribution in Monkey Spleen (ISH)

Time  
Post-EOI:

1 h

4 h

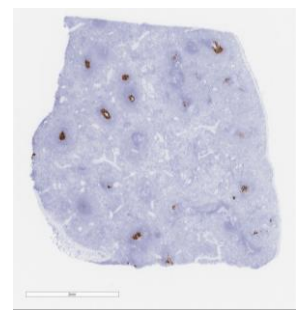
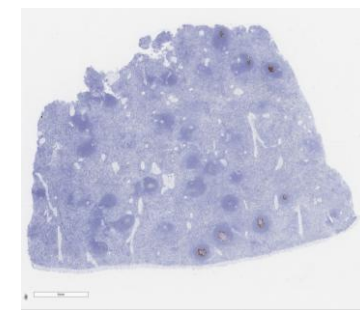
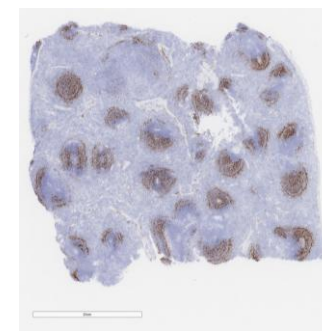
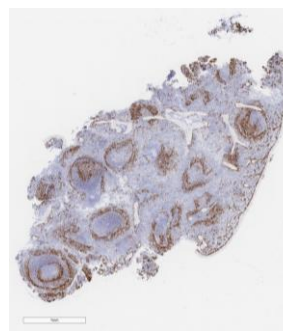
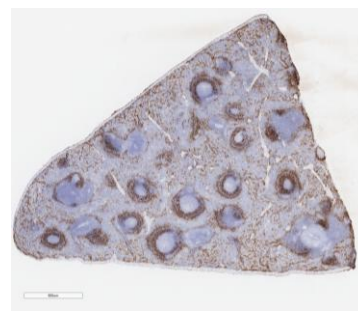
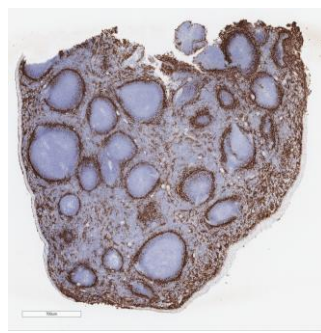
24 h

72 h

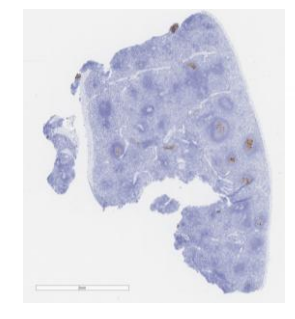
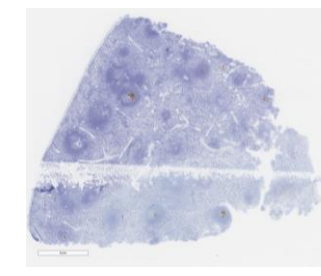
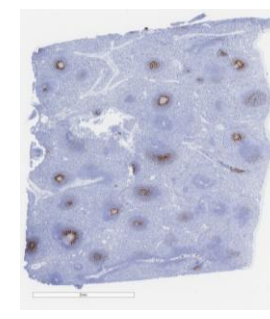
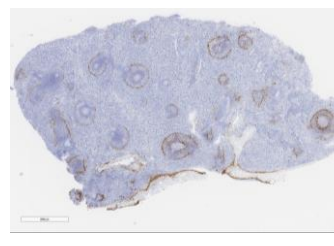
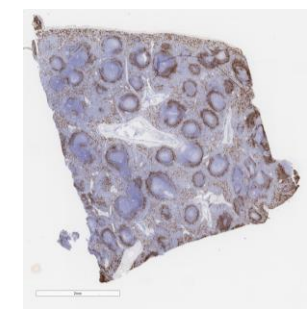
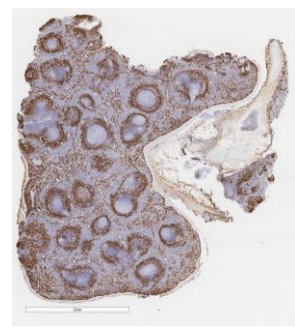
168 h

336 h

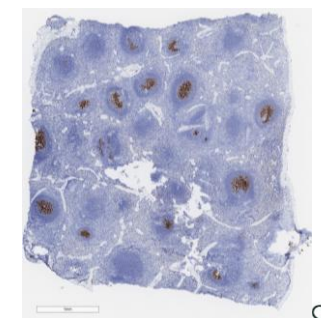
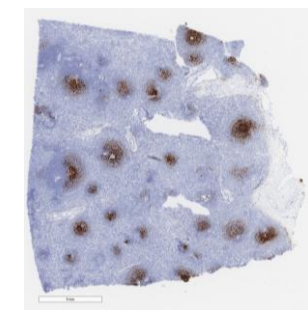
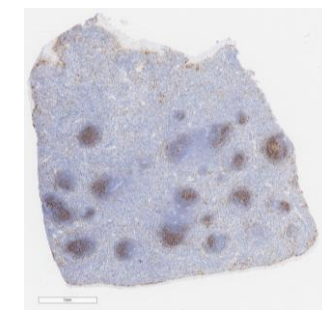
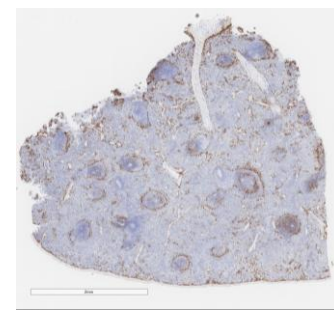
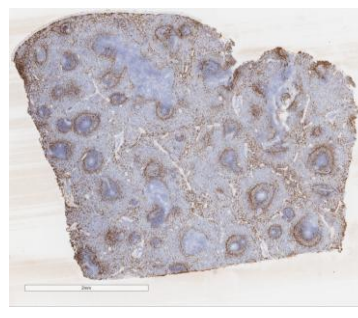
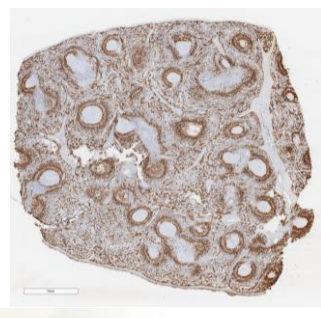
LNP 07

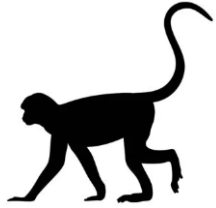


LNP 09



LNP 13





# mRNA Distribution in Monkey Ovaries (ISH)

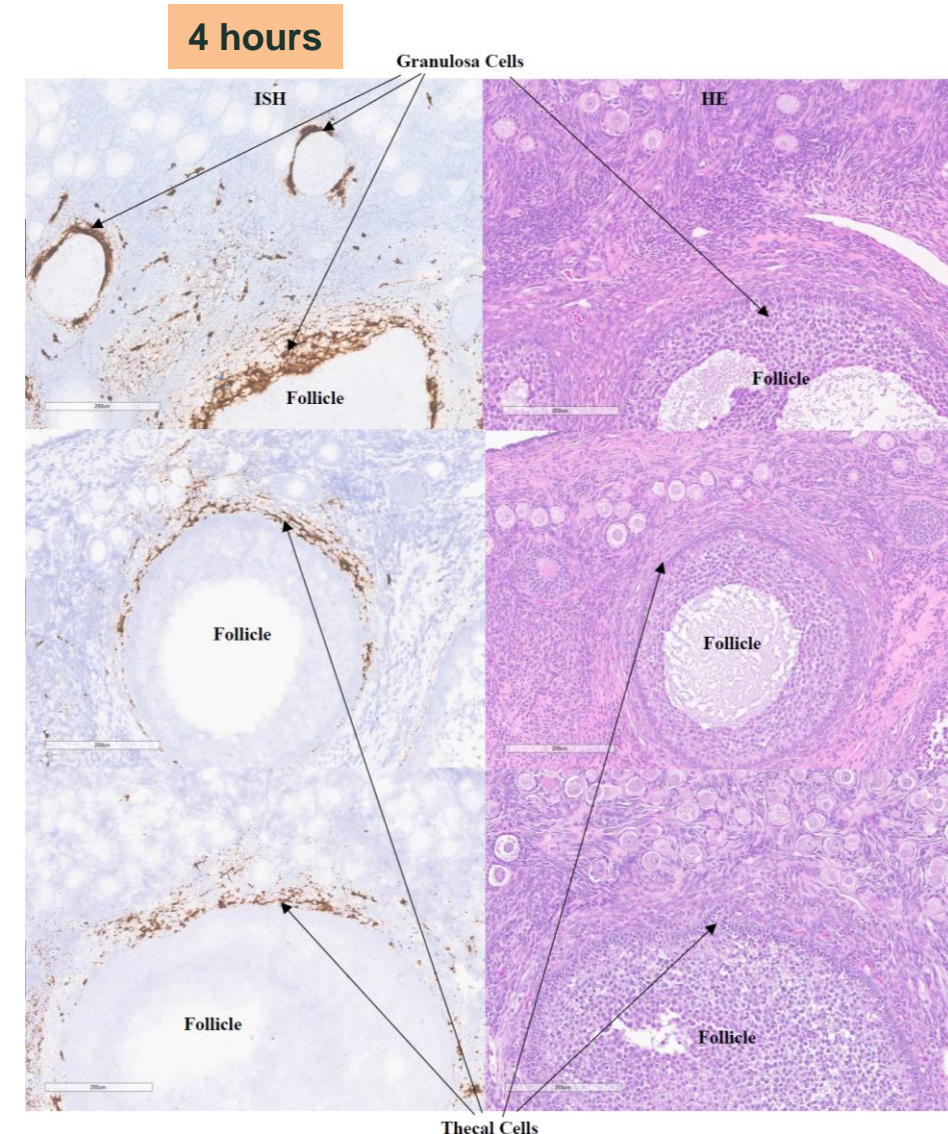
No mRNA Detected in Oocytes

**LNP 07      LNP 09      LNP 13**

	LNP 07	LNP 09	LNP 13
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)
Peri-Ovarian Fat	X (4, 24, 336)	-	X (1, 4)

X = mRNA detected (time in hours ISH label detected)

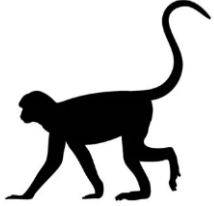
- mRNA was primarily detected in thecal cells, connective tissue and peri-ovarian fat cells
- No mRNA detected in oocytes



**LNP 07**

**LNP 09**

**LNP 13**



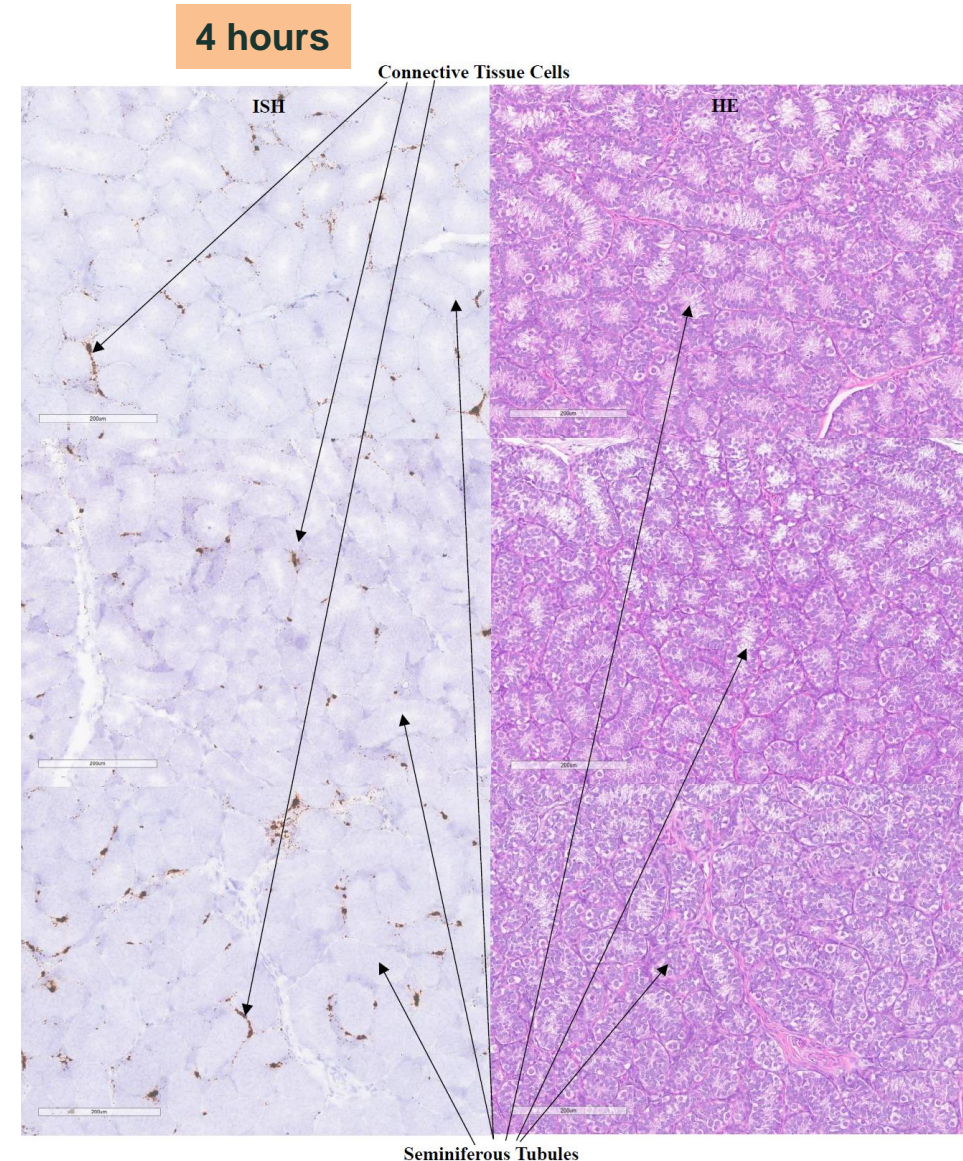
# mRNA Distribution in Monkey Testes (ISH)

## LNP 07    LNP 09    LNP 13

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	-	-	-

X = mRNA detected (time in hours ISH label detected)

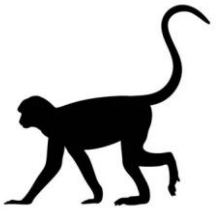
- mRNA detected in connective tissue cells between seminiferous tubules, with lesser amounts in capsule cells and blood vessels
- No mRNA was detected in cells within seminiferous tubules



**LNP 07**

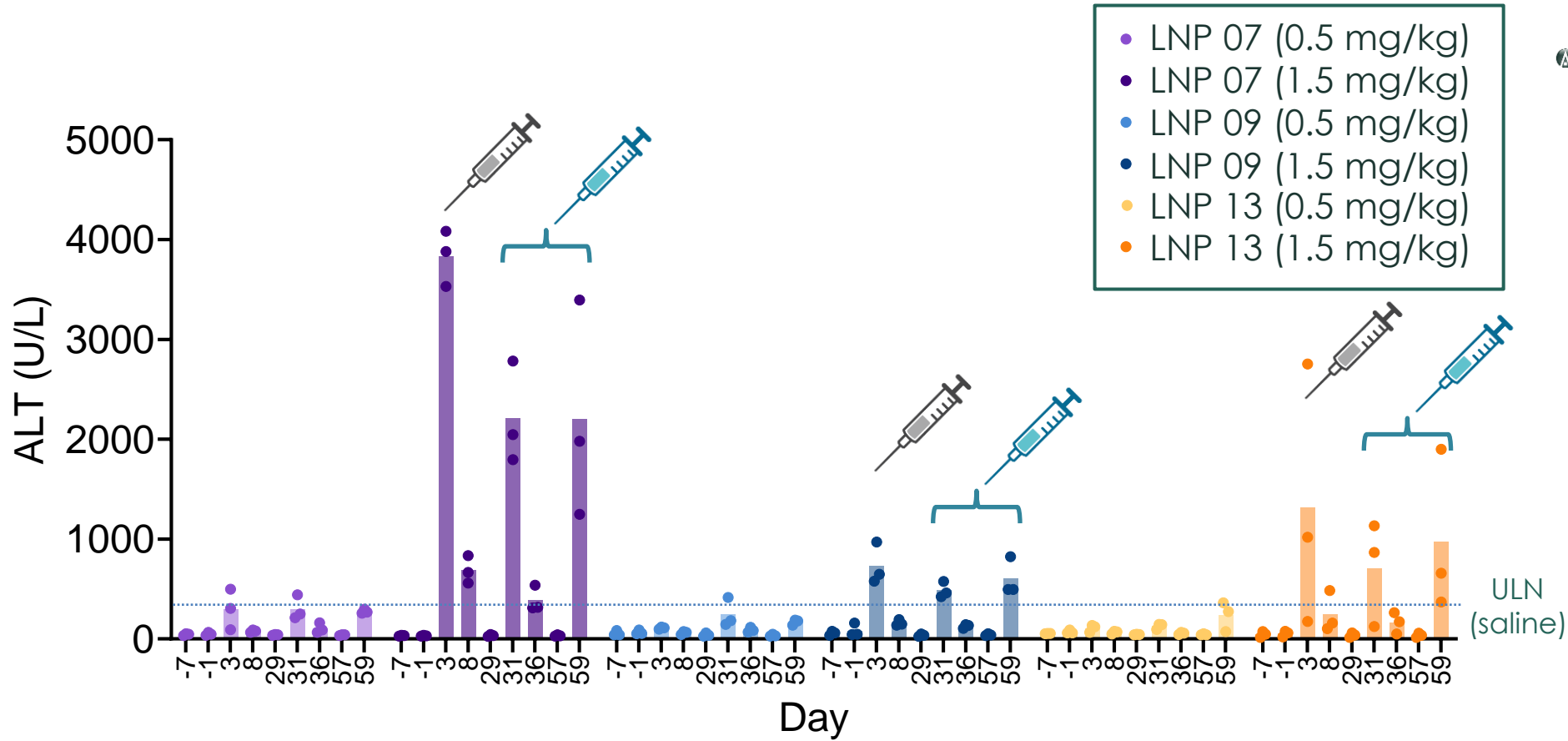
**LNP 09**

**LNP 13**

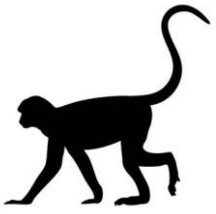


# Safety Assessments

## Dose-Related, Transient Elevations in Liver Transaminases

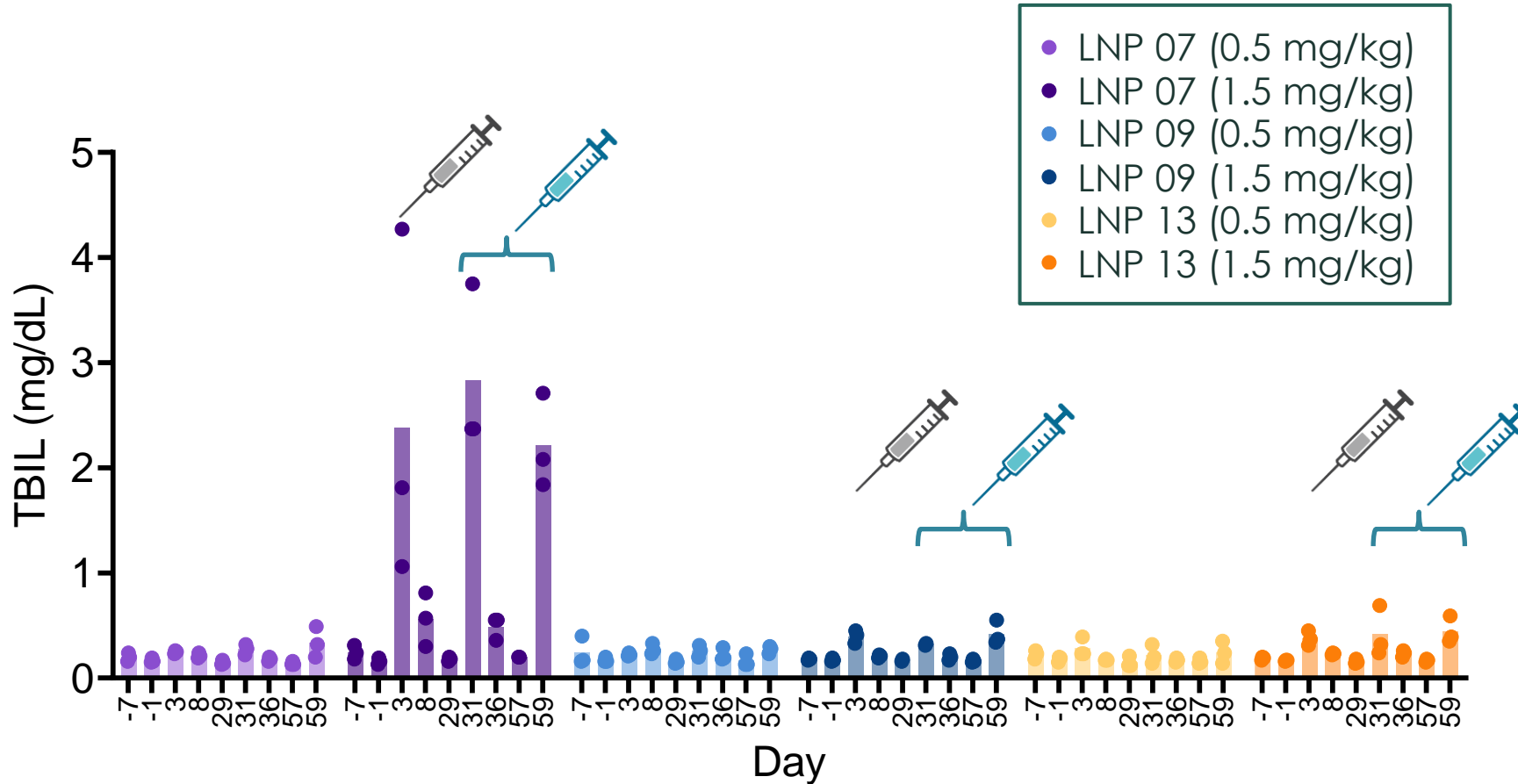


- Transient elevations in ALT (and AST)
- LNP 07 > 13 > 09**
- Lower magnitude with  $\alpha$ Gal payload
- Magnitude of ALT increase consistent between sequential  $\alpha$ Gal doses

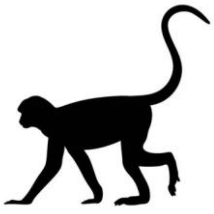


# Safety Assessments

## Dose-Related, Transient Elevations in Total Bilirubin



- TBIL transiently elevated for LNP 07 at 2 days post-dose, but not for LNP 09 and LNP 13



# Safety Assessments: Target Organs

## Histopathology: Day 59 (2 days after last dose; α-Gal mRNA)

Finding	LNP 07		LNP 09		LNP 13	
	0.5	1.5	0.5	1.5	0.5	1.5
<b>LIVER</b>						
Vacuolation, hepatocyte	2/1/0/0 [4]	0/0/3/0 [9]	3/0/0/0 [3]	2/1/0/0 [4]	2/0/0/0 [2]	0/2/1/0 [7]
Inflammation, mixed leukocyte	0/0/0/0 [0]	0/0/0/0 [0]	3/0/0/0 [3]	3/0/0/0 [3]	1/0/0/0 [1]	2/0/1/0 [5]
Single-cell necrosis, hepatocyte	3/0/0/0 [3]	1/0/2/0 [7]	2/0/0/0 [2]	1/2/0/0 [5]	1/0/0/0 [1]	1/0/1/0 [4]
Swollen, hepatocyte	1/0/0/0 [1]	1/2/0/0 [5]	0/0/0/0 [0]	3/0/0/0 [3]	3/0/0/0 [3]	1/0/2/0 [7]

**Grading and Incidence:** Minimal/Mild/Moderate/Marked, e.g., 2/1/0/0 = 2 animals graded minimal and 1 animal graded mild; [sum of severity scores]

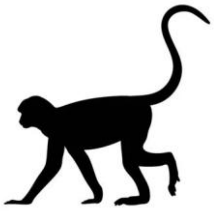
**Description of Finding:**

*Vacuolation, hepatocyte* – small clear vacuoles in cytoplasm of hepatocytes

*Swollen, hepatocyte* - vacuoles appeared to coalesce resulting in swollen cells with pale to clear cytoplasm that occasionally contained blebs of eosinophilic cytoplasm; likely related to exaggerated pharmacology

*Single-cell necrosis, hepatocyte* - shrunken cells with condensed cytoplasm and shrunken darkly basophilic nuclei

*Inflammation, mixed leukocyte* - sinusoidal infiltrates of mixed leukocytes and prominent Kupffer cells



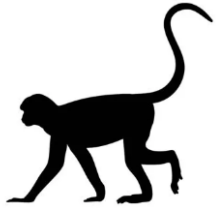
# Safety Assessments: Target Organs

## Histopathology: Day 59 (2 days after last dose; α-Gal mRNA)

Finding	LNP 07		LNP 09		LNP 13	
	0.5	1.5	0.5	1.5	0.5	1.5
<b>SPLEEN</b>						
Vacuolation, cytoplasm, red pulp	3/0/0/0 [3]	1/2/0/0 [5]	3/0/0/0 [3]	3/0/0/0 [3]	0/1/1/0 [5]	0/2/0/0 [4]
Depletion, lymphoid	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/2/1/0 [7]
Necrosis, red pulp	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/2/1 [10]
<b>ADRENAL GLAND</b>						
Depletion, lipid, cortex	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/1 [4]	1/0/0/2 [9]

**Grading and Incidence:** Minimal/Mild/Moderate/Marked, e.g., 2/1/0/0 = 2 animals graded minimal and 1 animal graded mild

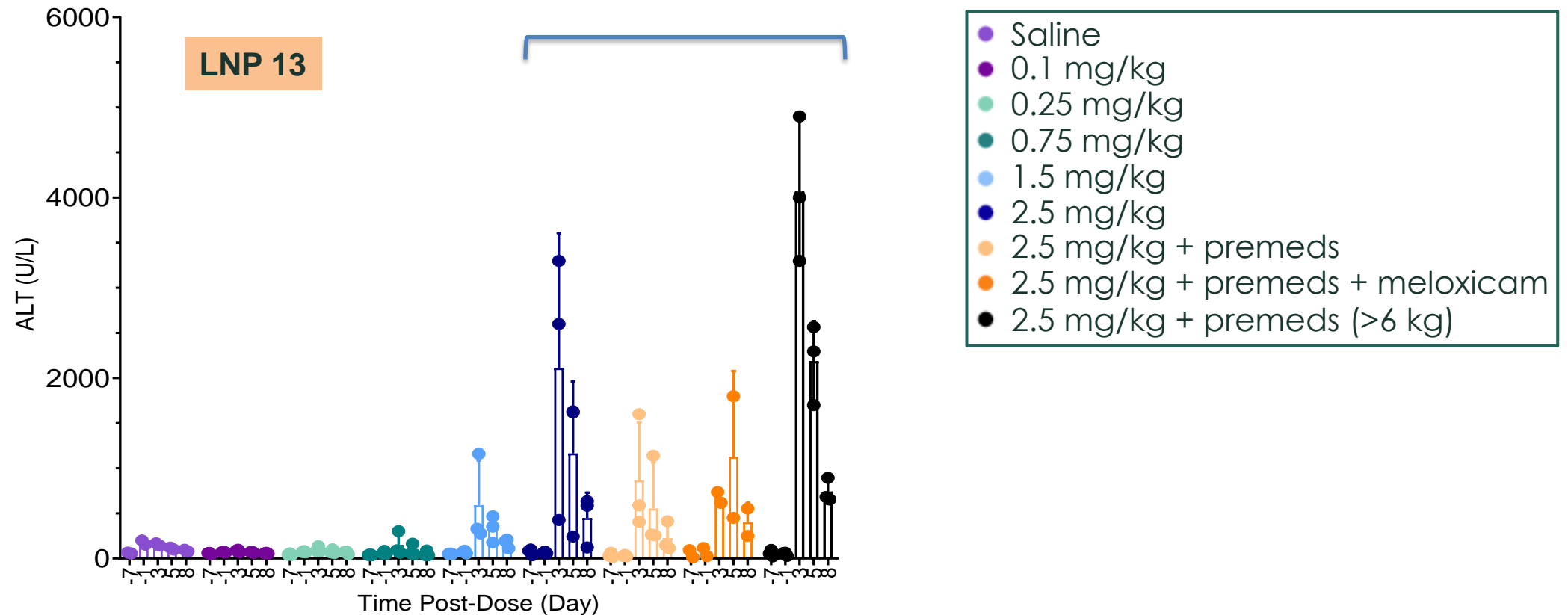




~3 kg or  
6-8 kg

# Tolerability

## Impact of Animal Size and Concomitant Medications on ALT

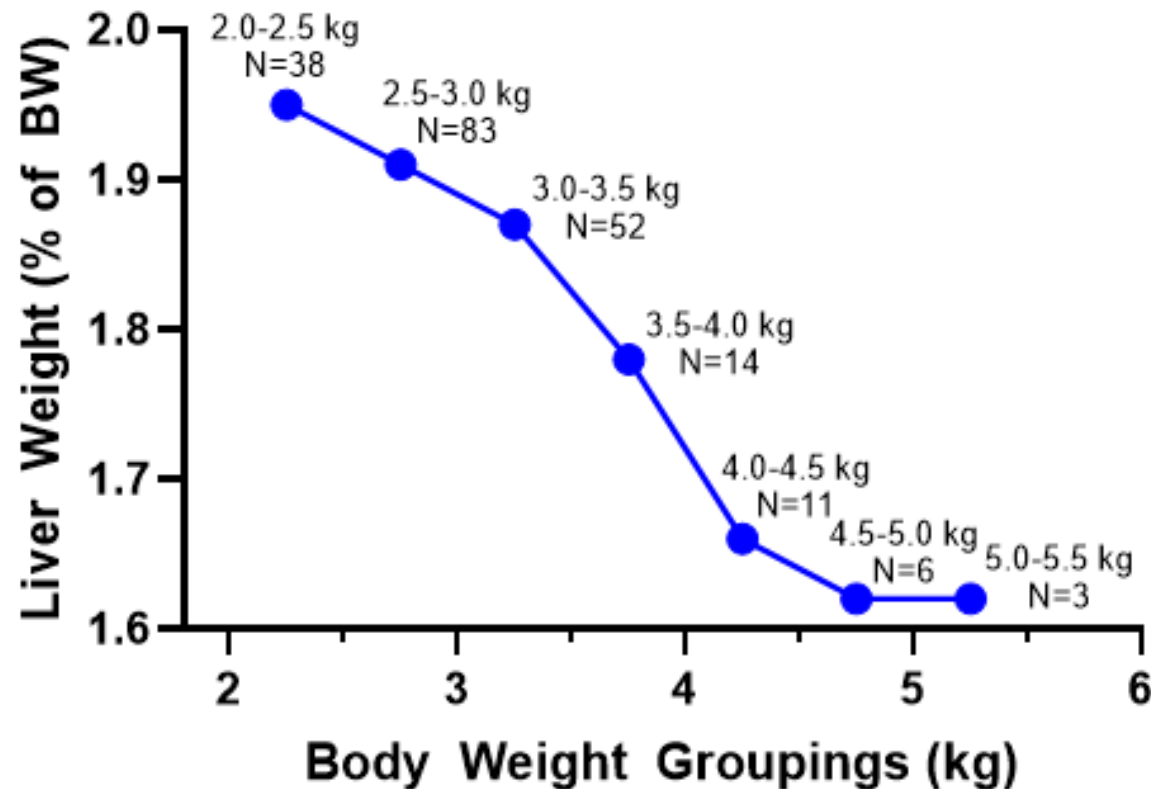


- ALT/AST elevated at  $\geq 1.5$  mg/kg on Day 3, near/nearing baseline on Day 8
- Highest ALT/AST elevations observed in no premed group and large NHPs (>6 kg)

# Tolerability

## Increased Liver Exposure in Larger Monkeys

### Liver Weight as a Percentage of BW

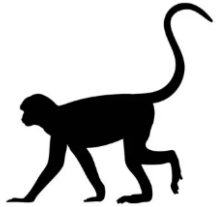


### ● Example:

- Assuming 75% of dose to liver
  - **2.25 kg monkey @ 2 mg/kg**
    - Total dose = 4.5 mg
    - Liver dose = 3.4 mg
    - Liver dose by weight = **72.5 mg/kg liver**
  - **5.25 kg monkey @ 2 mg/kg**
    - Total dose = 10.5 mg
    - Liver dose = 7.9 mg
    - Liver dose by weight = **92.2 mg/kg liver**

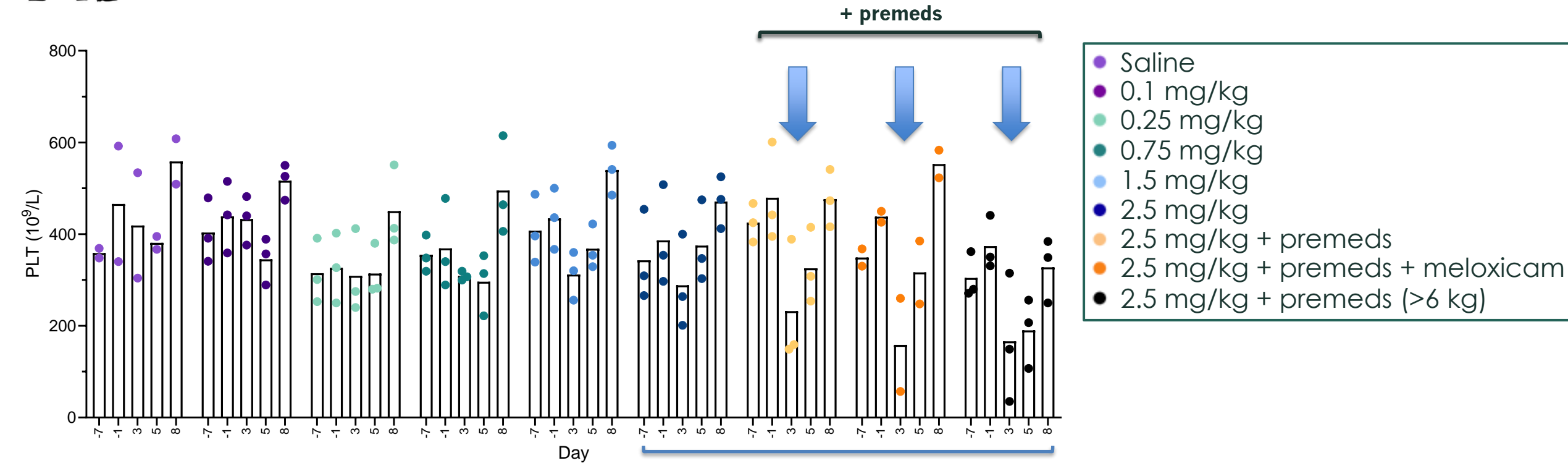
*~27% Liver Exposure Increase in Large vs Small Monkeys*

\* Control data from two CROs (N=207 cynomolgus macaques)



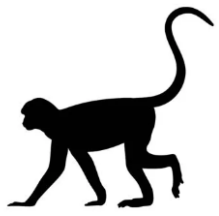
# Tolerability

## Impact of Animal Size and Concomitant Medications on Platelets



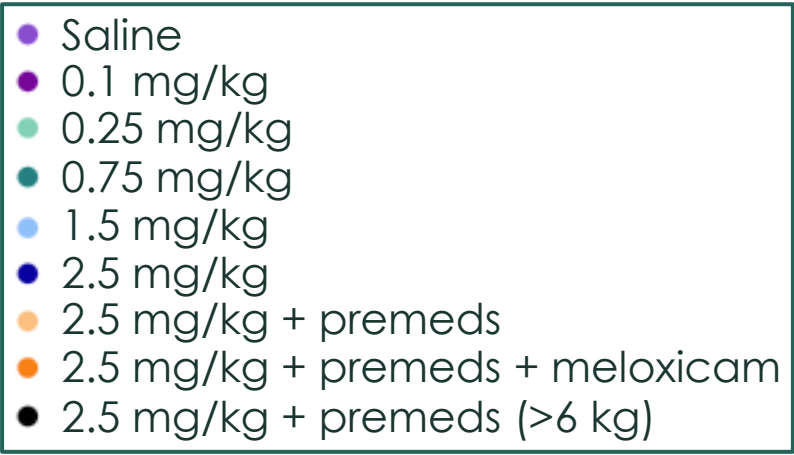
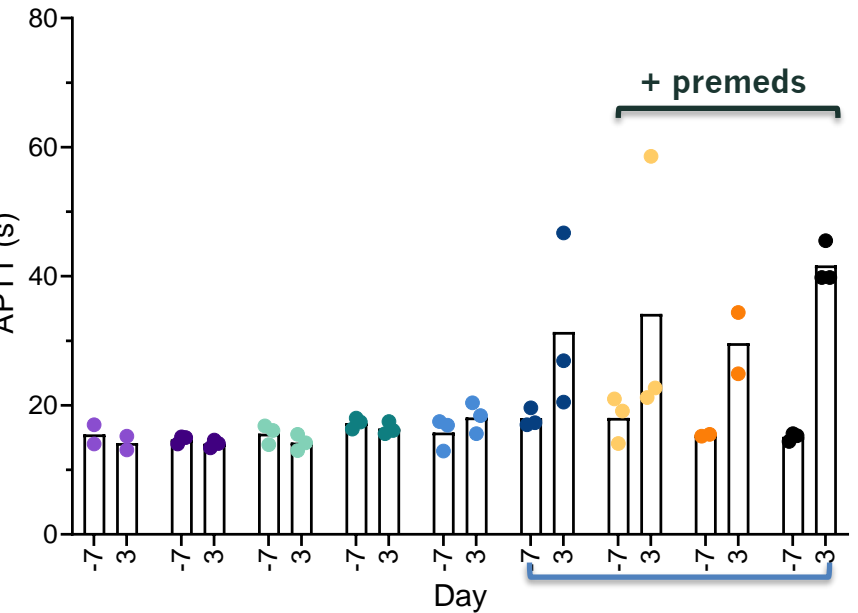
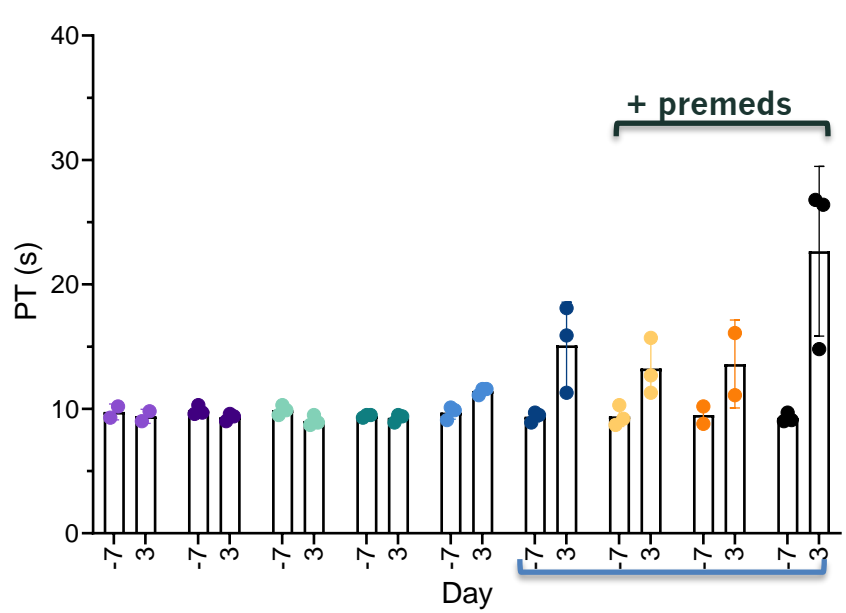
- Minimal to no effect on platelets up to 2.5 mg/kg of LNP13
- Decreases on Day 3 in 2.5 mg/kg groups given premeds; red, patchy skin in most animals
- Largest decrease in 2.5 mg/kg animals given meloxicam and in animals >6 kg

1 h iv infusion



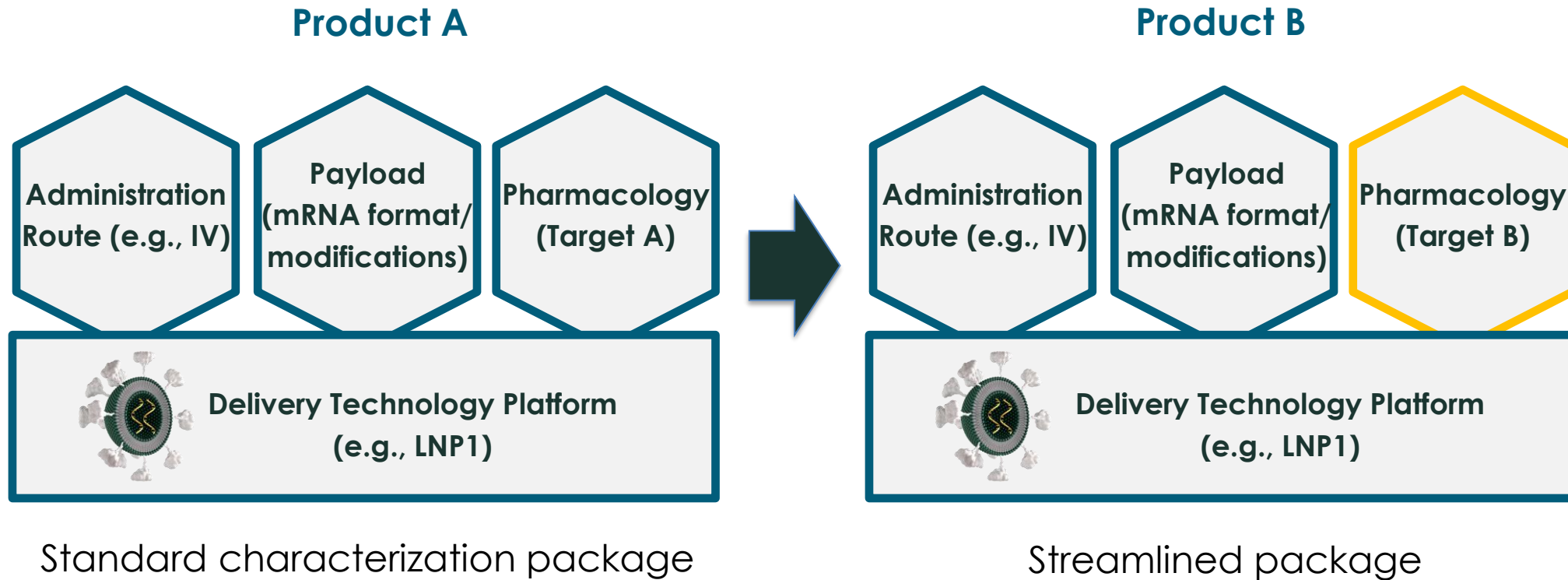
# Tolerability

## Impact of Animal Size and Concomitant Medications on Coagulation



- PT and APPT increases in all groups given 2.5 mg/kg
- Greatest increases in 2.5 mg/kg animals given meloxicam and in animals >6 kg

# Leveraging an LNP Delivery Platform



- Same LNP composition can be used across multiple therapeutic programs, providing opportunities to leverage CMC and nonclinical information (“prior knowledge”).

# Summary

- **LNP toxicity is predictable, dose-related, monitorable and most effects resolve within 7-14 days**
  - Target organs of toxicity in monkeys are most commonly liver, spleen and adrenal glands
  - Peak hepatic effects typically occur 2 days post-dose and include ALT/AST elevations and microscopic changes of vacuolation, mixed cell infiltration and single-cell necrosis
  - Repeated doses of LNP elicit effects of very similar magnitude and timing for each dose
  - Small particles improve activity and reduce cytokines/chemokines in monkeys
  - Payload quality and total lipid dose impact the degree of toxicity (and activity)
- **Pharmacokinetics and distribution are dictated by the LNP, not the payload**
  - Different payloads encapsulated in the same LNP (and particle size) will have the same PK properties
  - LNP can be administered repeatedly without changes in the PK profile
  - Different ionizable lipids may have a significant impact on plasma AUC, but still distribute to the liver, spleen and adrenal glands
- **No evidence of distribution into germline cells observed for three different LNP formulations with ionizable lipids from different chemical classes and a broad range of plasma exposures**
- **LNP are highly adaptable to a platform technology approach to product development**