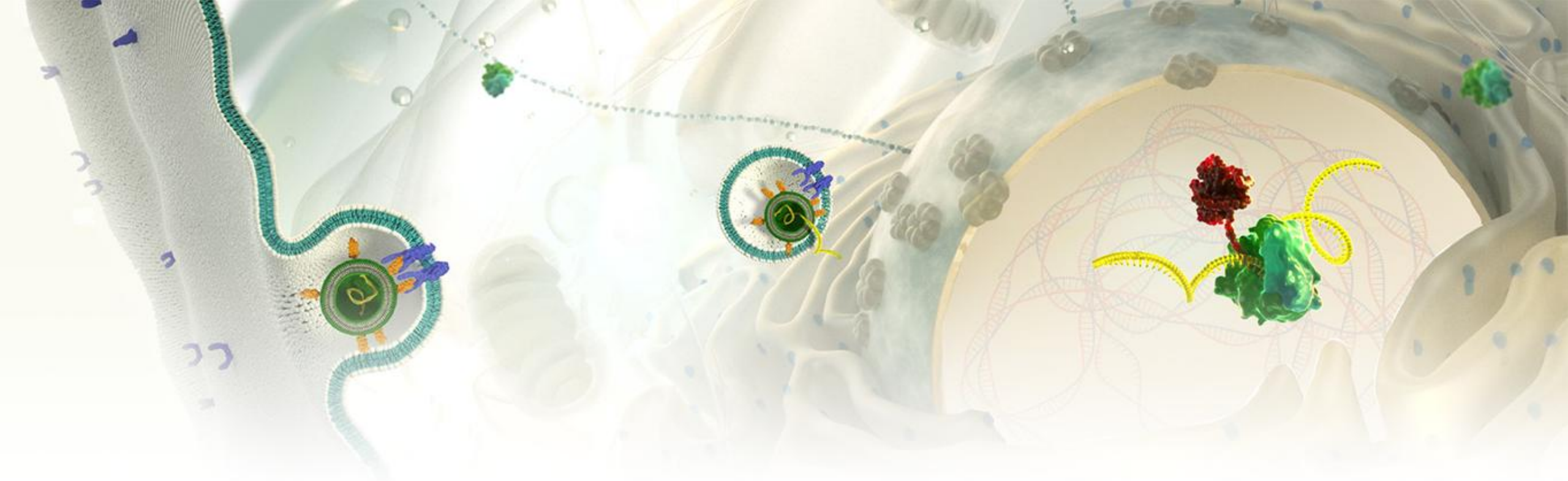


Optimizing Ionizable Lipid & LNP Properties to Improve Safety

Sean Semple
Acuitas Therapeutics
December 10, 2024

3rd LNP Immunogenicity & Toxicity Summit
Boston, MA

ACUITAS
THERAPEUTICS

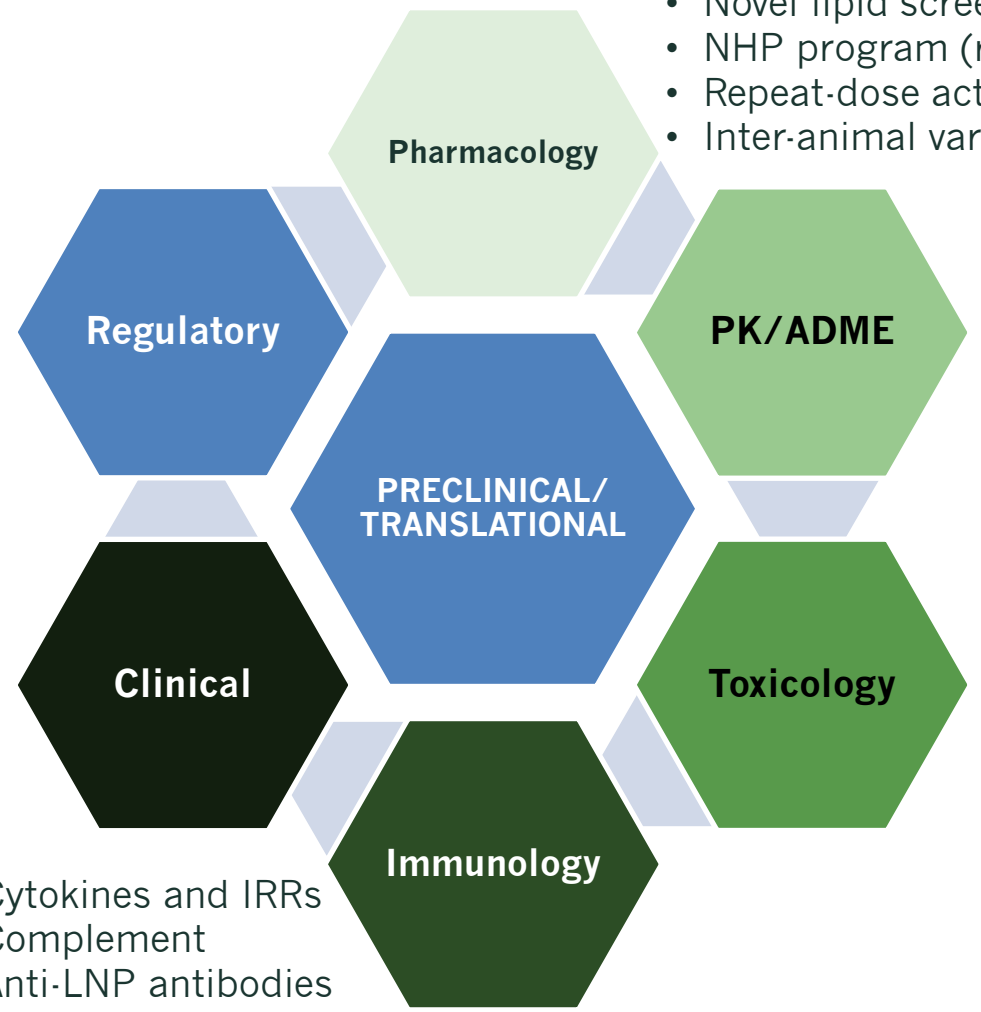
LNP Technology Clinically Validated

- 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 THERAPEUTICS

Acuitas LNP Core Research Program



- Novel lipid screening
- NHP program (rodent to NHP translation)
- Repeat-dose activity
- Inter-animal variability

- Pre-IND/CTA review
- IND/CTA review
- LNP expertise

- Novel lipids
- Tissue distribution
- Cellular distribution
- Biodegradability
- Metabolism
- 'PK rebound'
- Mechanisms of uptake

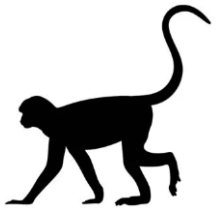
- Historical experience
- Dose-limiting toxicities

- Hepatic effects
- Target organs of toxicity
- Time course
- Reversibility
- Repeat-dose toxicity

- Cytokines and IRRs
- Complement
- Anti-LNP antibodies
- Payload effects
- Vaccine/IM studies

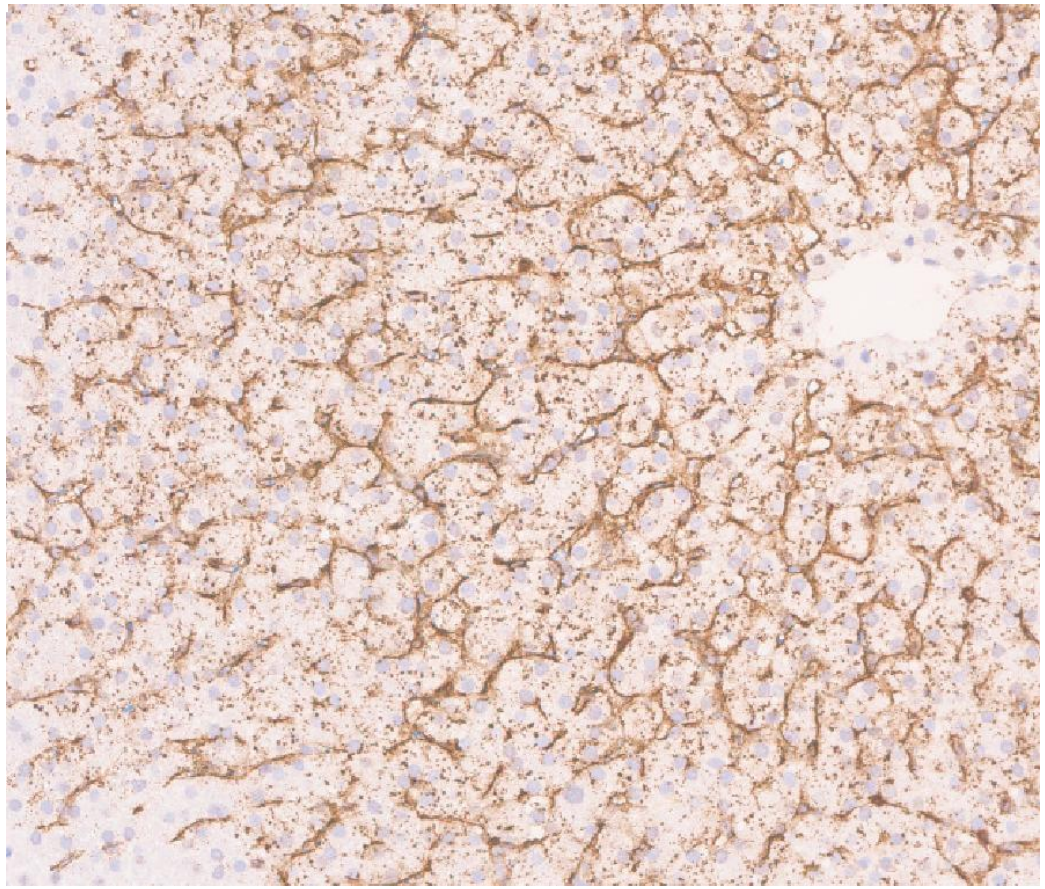
ACUITAS THERAPEUTICS

M. fascicularis
2.0 mg/kg
1 h iv infusion

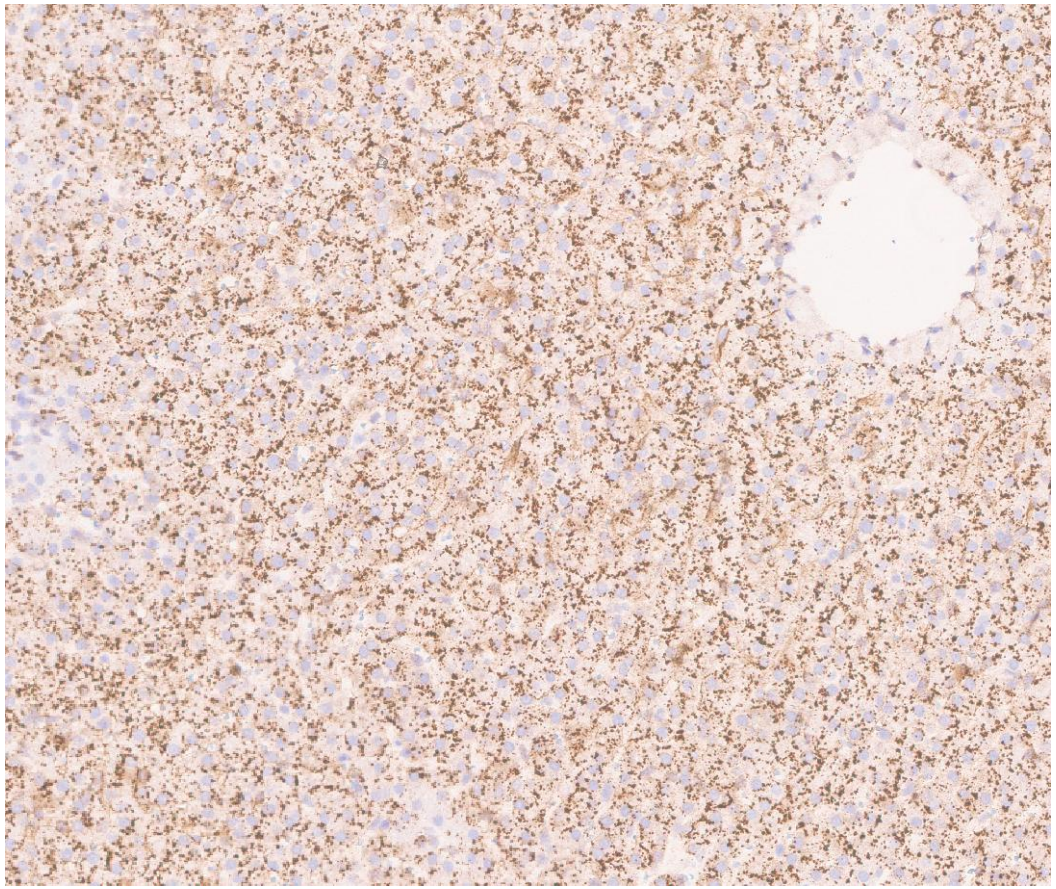


Optimization

Small Particles Improve Distribution into Hepatocytes and Increase Activity



LNP 09 (72 nm)
Plasma IgG: 24.6 μg/mL

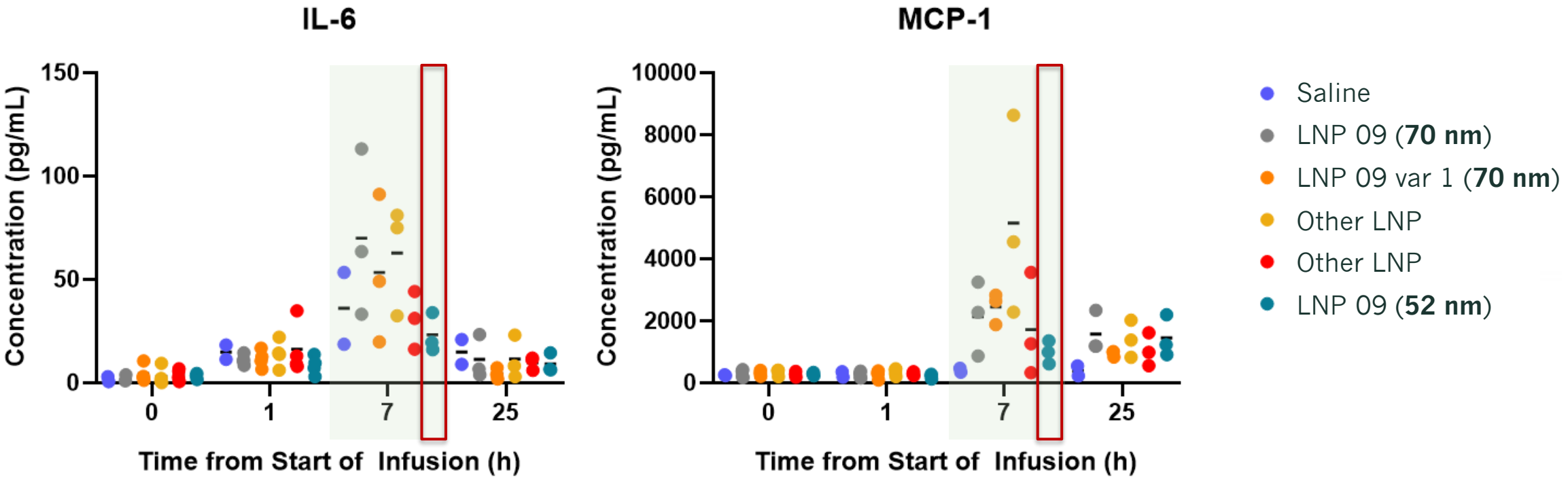


LNP 09 (54 nm)
Plasma IgG: 91.3 μg/mL

ACUITAS THERAPEUTICS

Optimization

Small Particles Reduce Cytokine Secretion

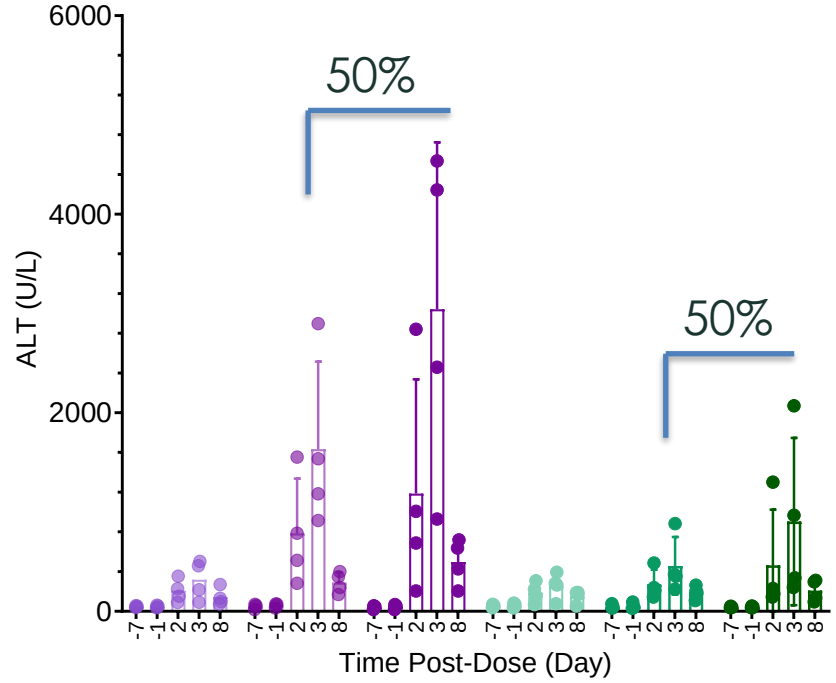


- Transient increases at 6 hours post-infusion; largely returned to baseline by 24 hours post-EOI
- Smaller particles reduce cytokines/chemokines vs larger particles of the same composition

ACUITAS THERAPEUTICS

Optimization

Reducing Total Lipid Dose Reduces Hepatic Effects

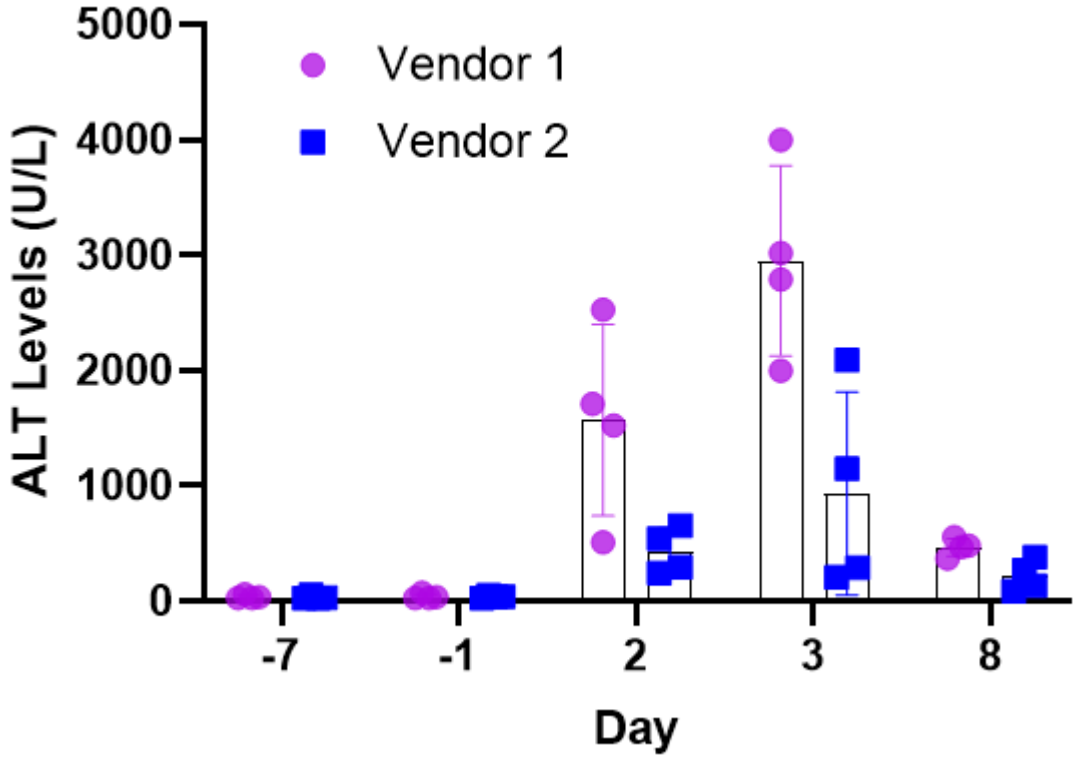


LNP	07	07	07	13	13	13
N:P Ratio	3	4.5	6	3	4.5	6

- 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

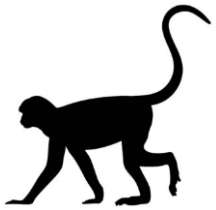
Payload Quality Affects Toxicity



- 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

ACUITAS THERAPEUTICS

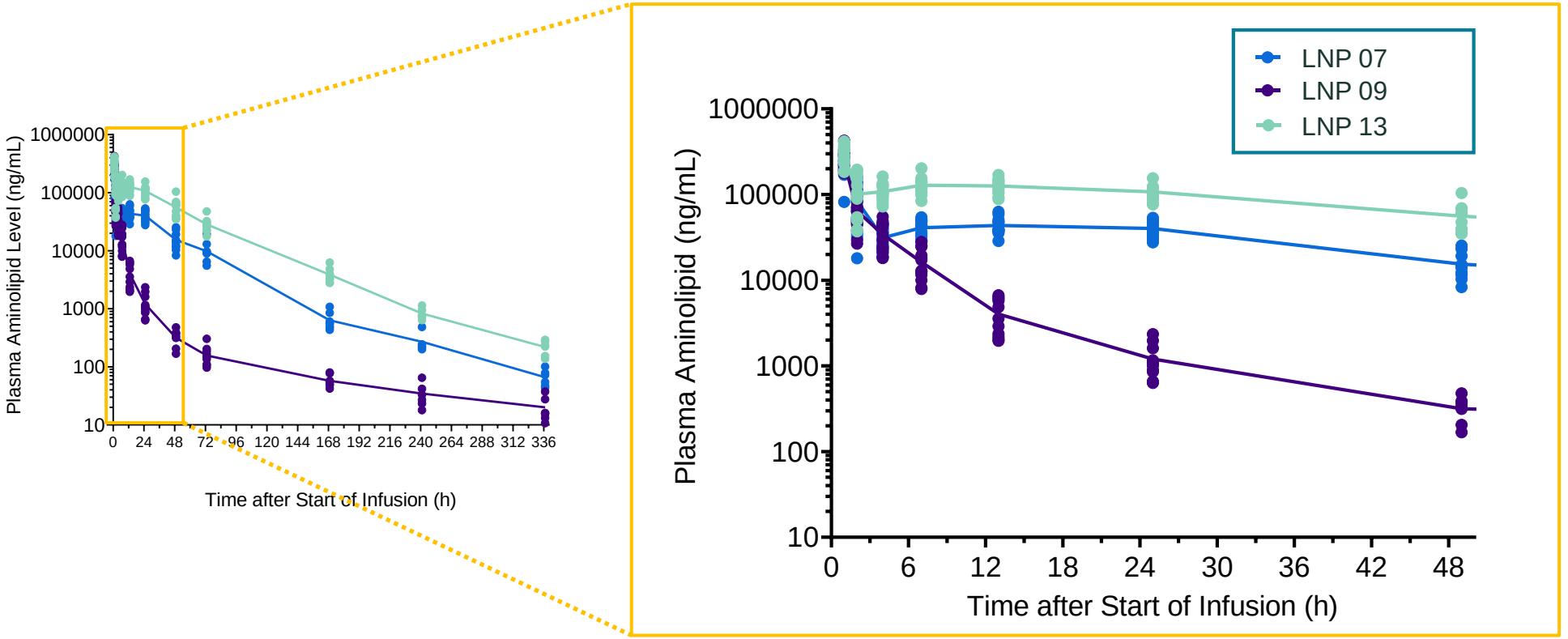
M. fascicularis
1.0 mg/kg
1 h iv infusion



Pharmacokinetics

Pharmacokinetic Profiles of Ionizable Lipid in Lead IV LNP in Monkeys

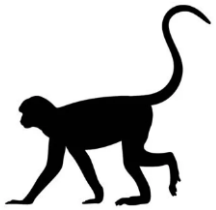
PK Profile (0-48 h)



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

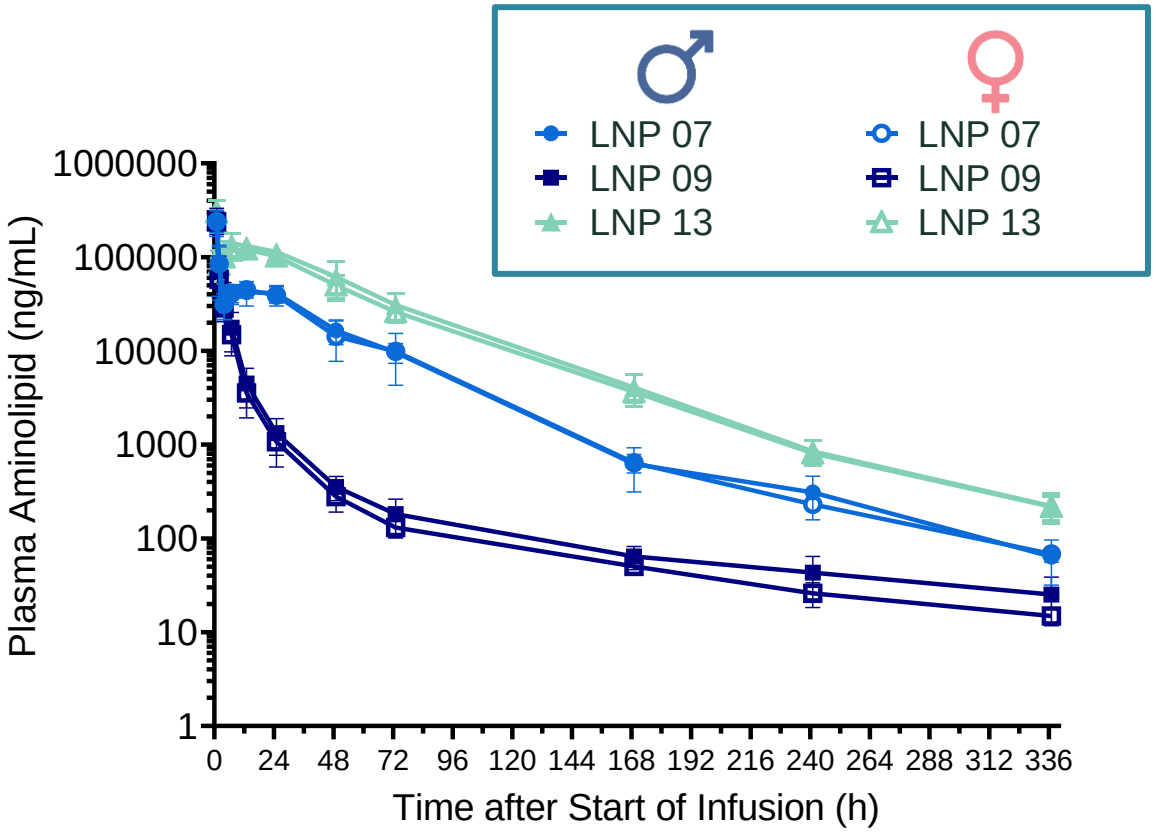
ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion



Pharmacokinetics

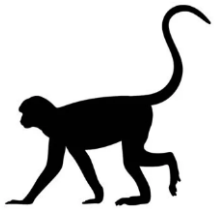
Plasma PK of Ionizable Lipid in Male and Female Monkeys



- No difference in plasma PK profile between males and females

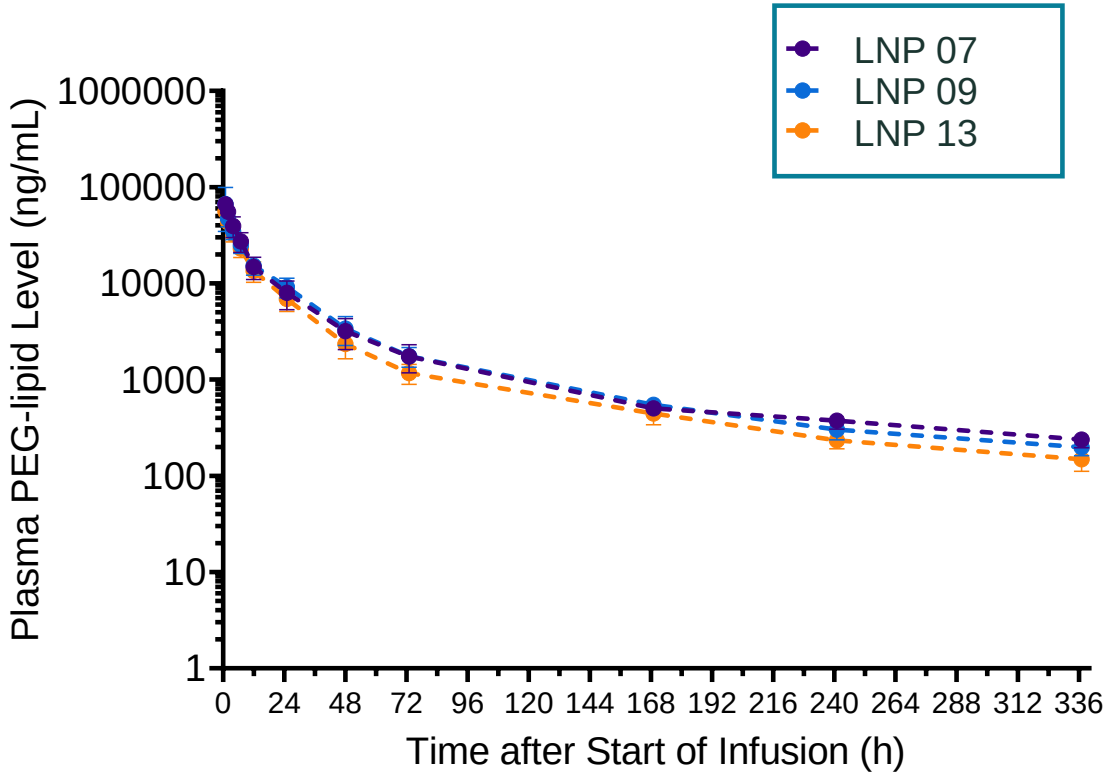
ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion



Pharmacokinetics

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

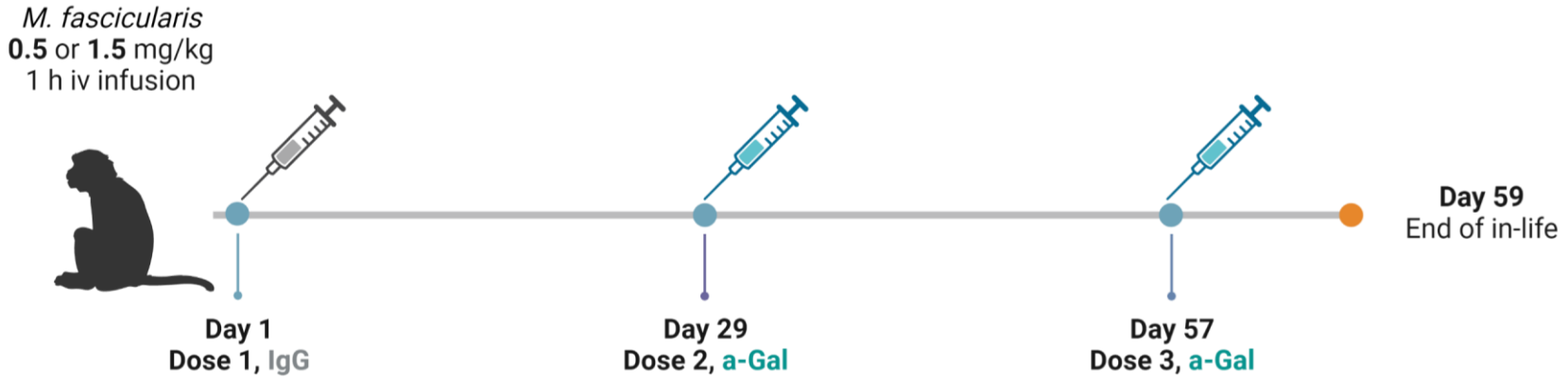


- PEG-lipid exchanges out of LNP in the circulation, resulting in overlapping PEG-lipid profiles for all three LNP formulations

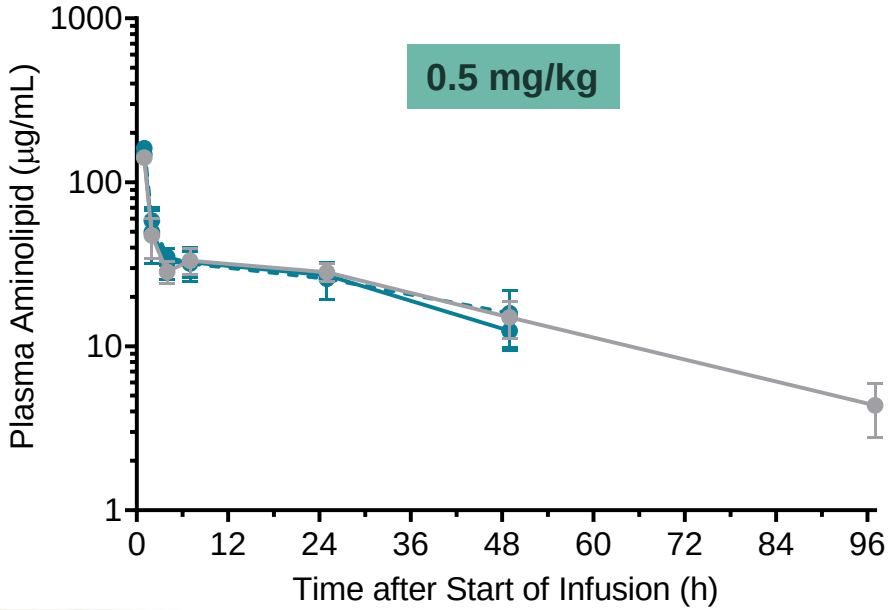
ACUITAS THERAPEUTICS

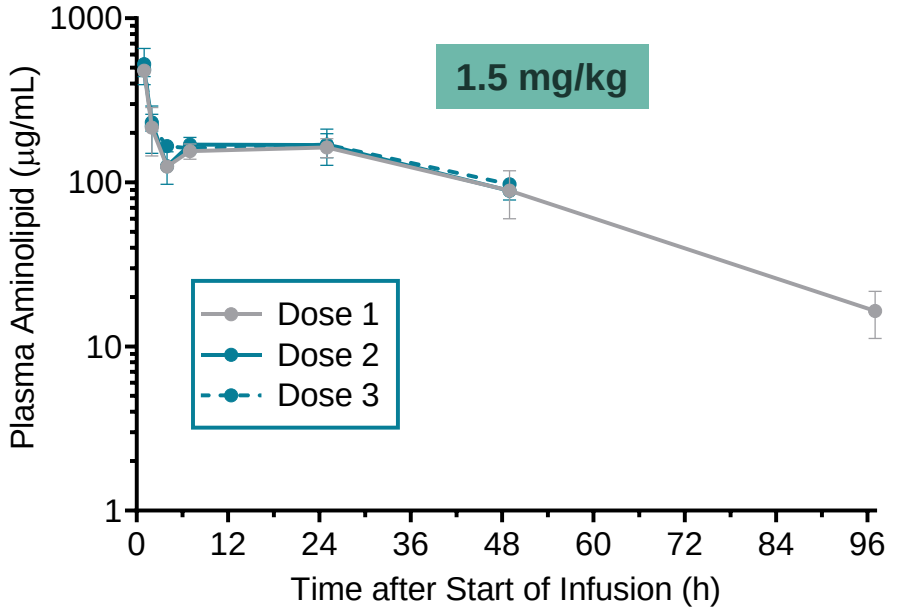
Pharmacokinetics

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



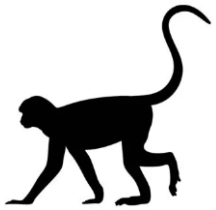
LNP 07





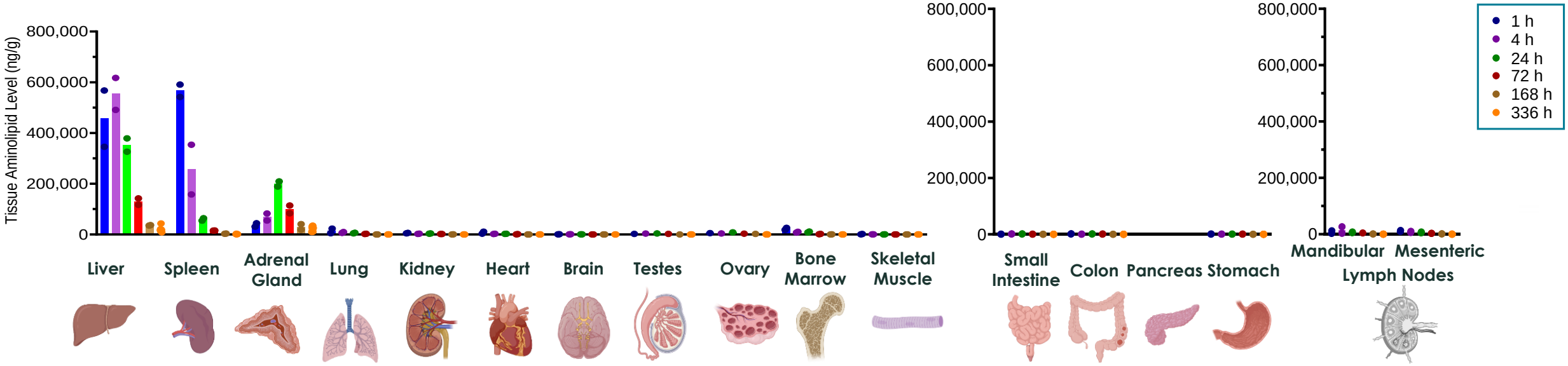
ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion



Distribution

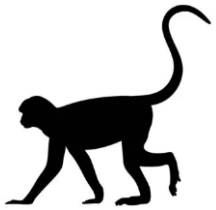
Ionizable Lipid Tissue Distribution in Monkeys – LNP 07



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

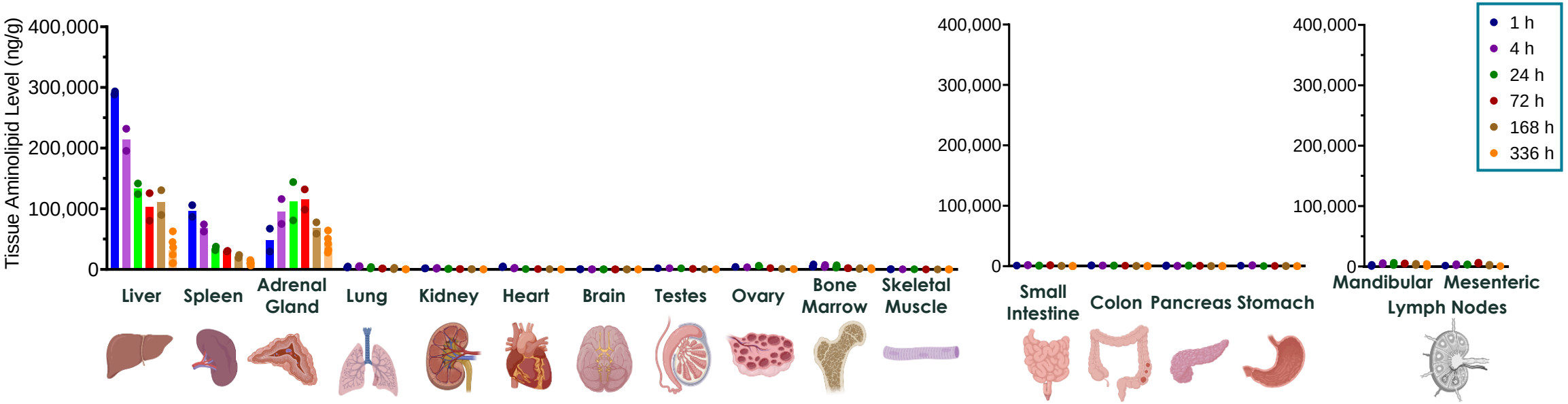
ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion



Distribution

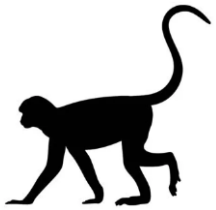
Ionizable Lipid Tissue Distribution in Monkeys – LNP 09



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

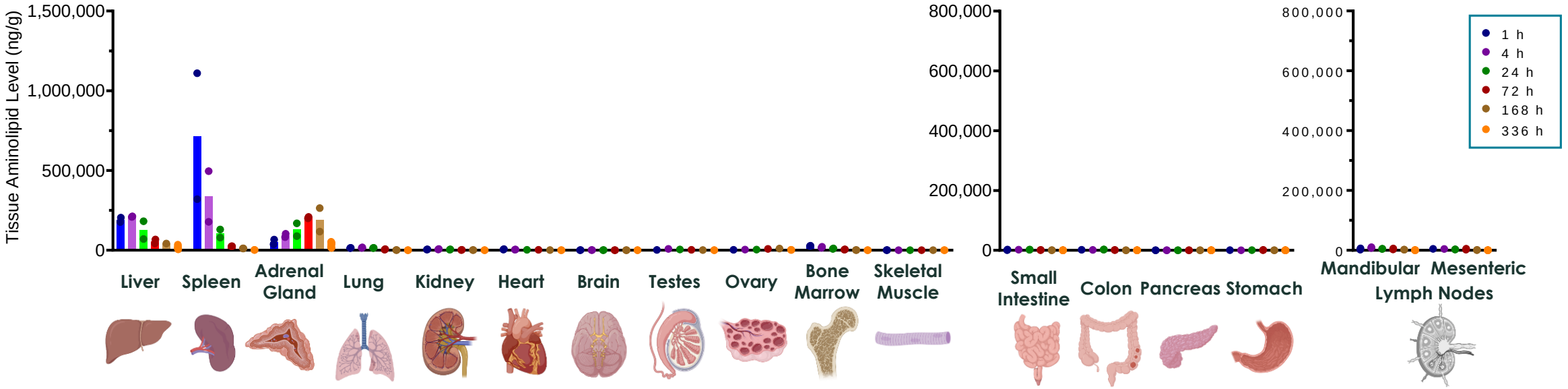
ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion



Distribution

Ionizable Lipid Tissue Distribution in Monkeys – LNP 13



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

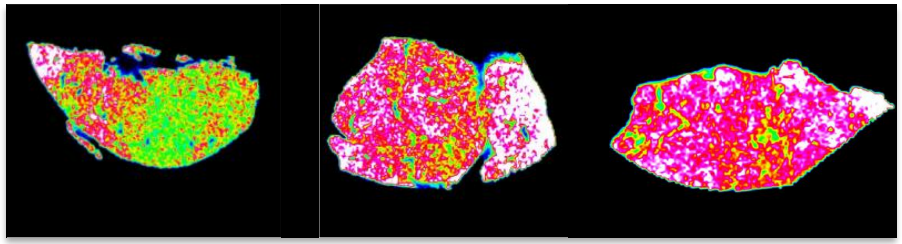
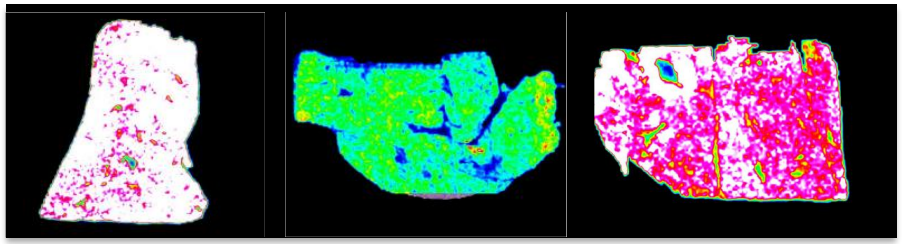
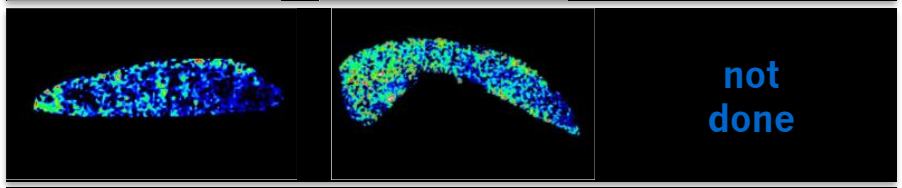
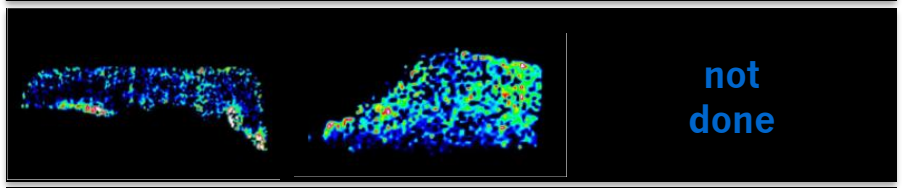
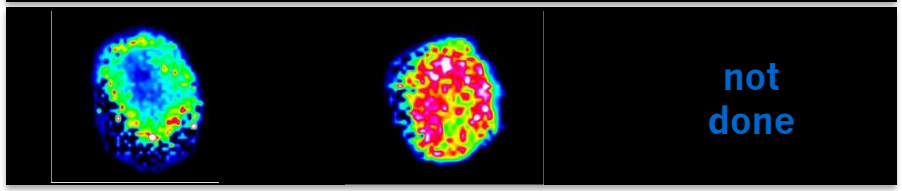
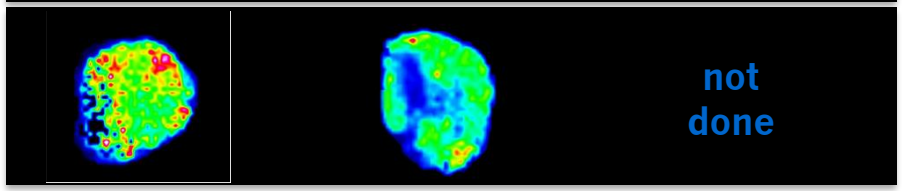
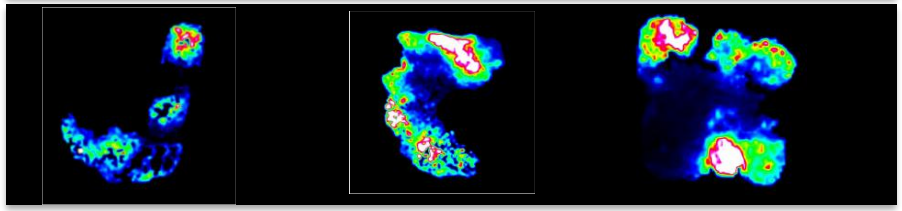
ACUITAS THERAPEUTICS

1.0 mg/kg
IV slow push

Distribution

Tissue Distribution in Rat is Consistent with Monkey

LNP 09 – Ionizable Lipid (24 hours)

	Males			Females			% Dose
Liver							67%
Spleen			not done			not done	1.5%
Adrenal Gland			not done			not done	1.4%
Testis/ Ovary	not detected	not detected	not done				0.6%

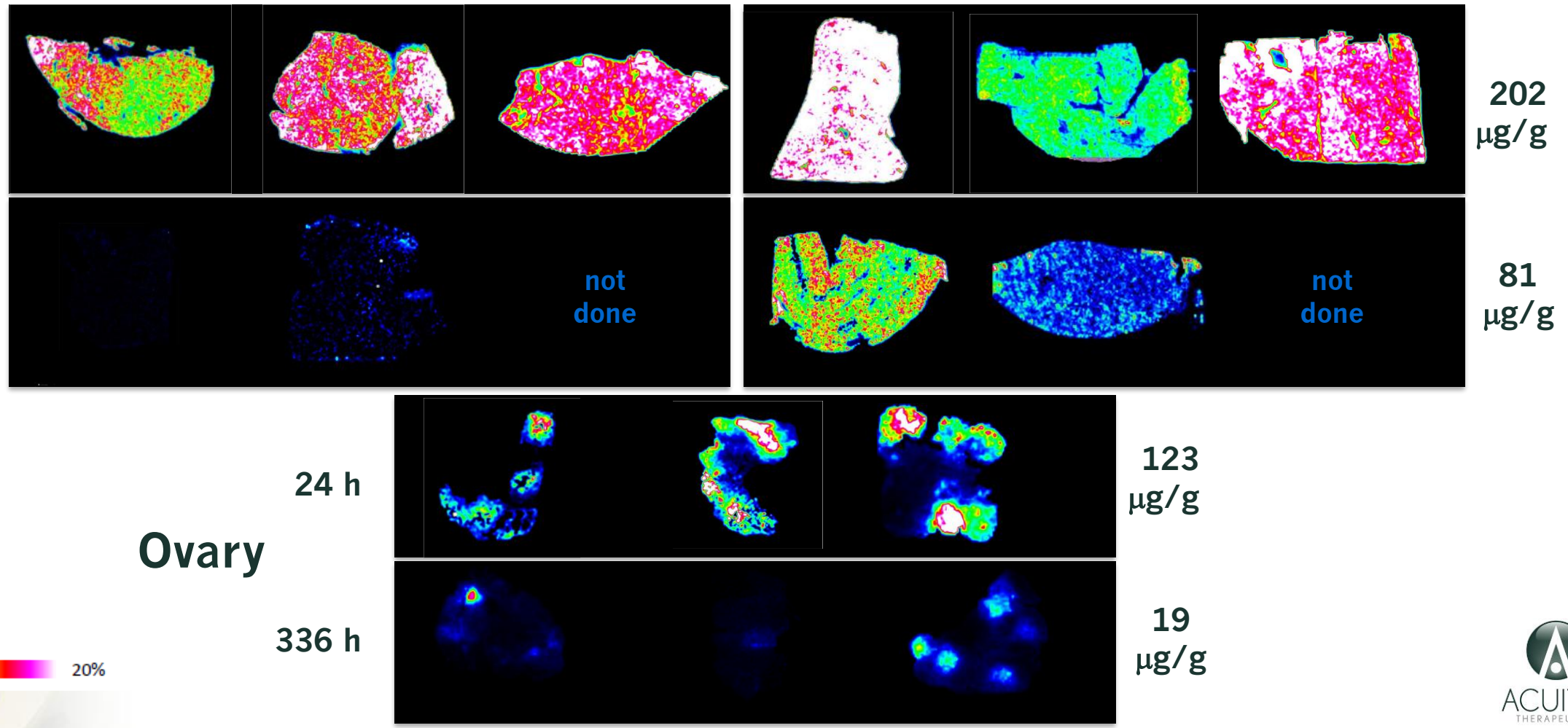
Lower Parent Lipid Signal  Higher Parent Lipid Signal

ACUITAS THERAPEUTICS

1.0 mg/kg
IV slow push

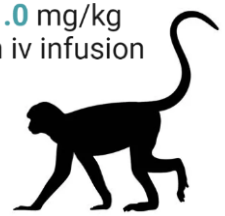
Distribution

Ionizable Lipid Levels Significantly Decreased by 14 Days Post-Dose

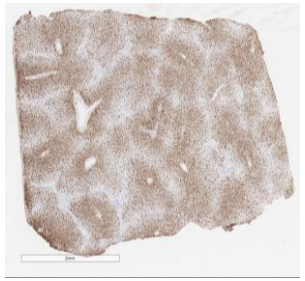
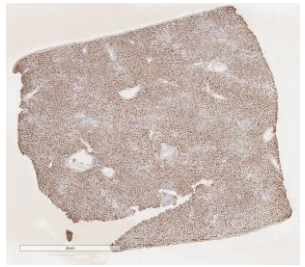
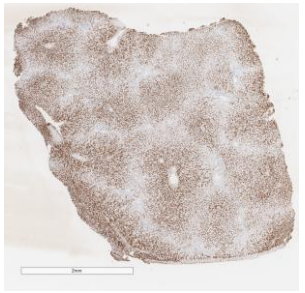
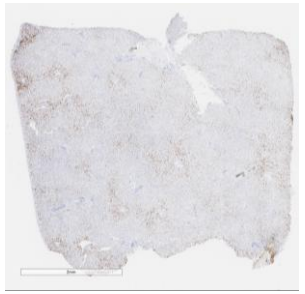
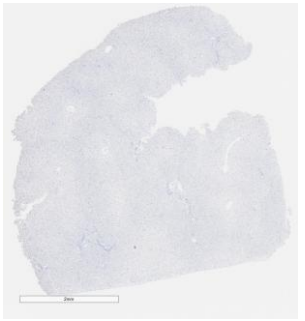
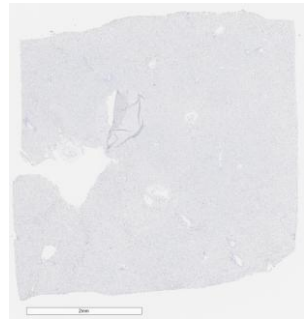
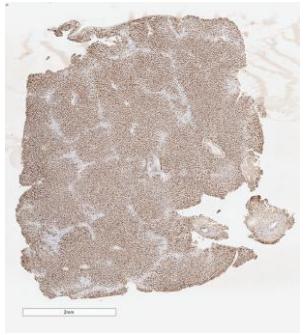
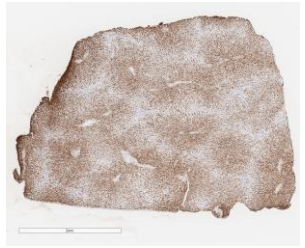
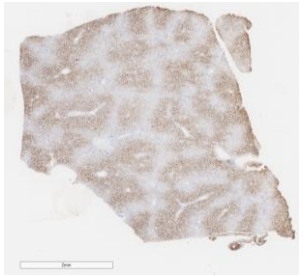
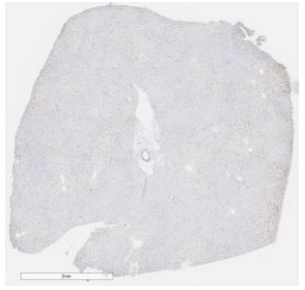
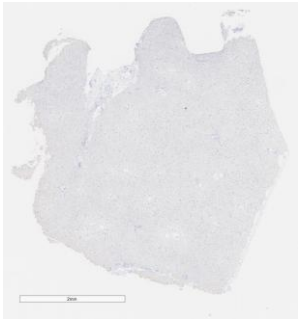
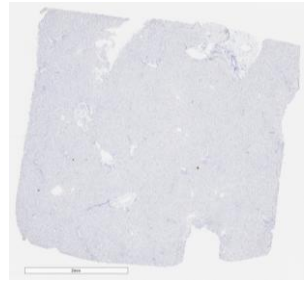
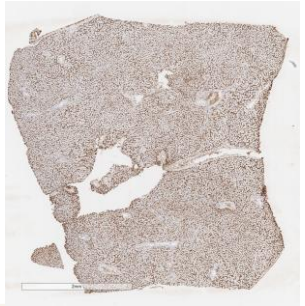
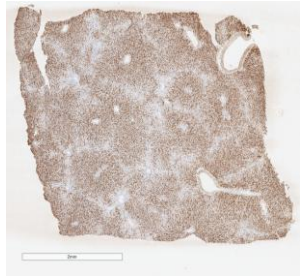
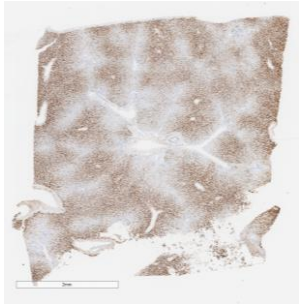
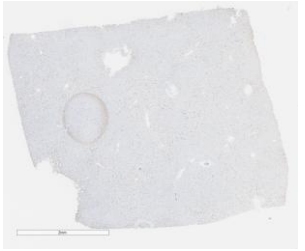
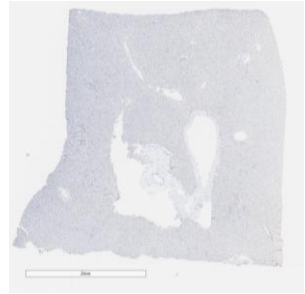


ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion

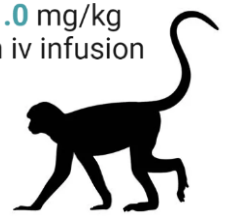


mRNA Distribution in Monkey Liver (ISH)

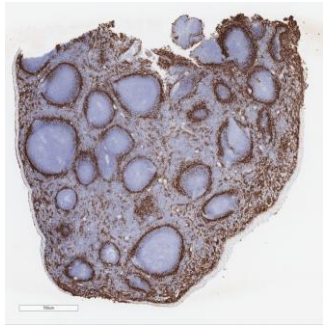
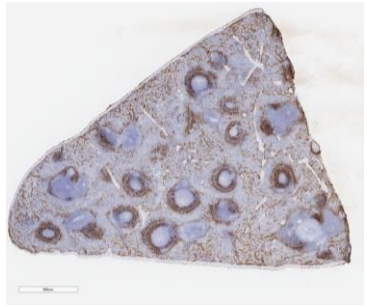
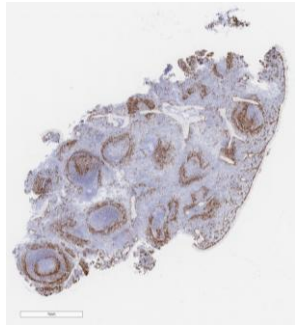
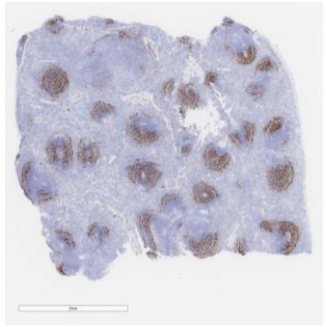
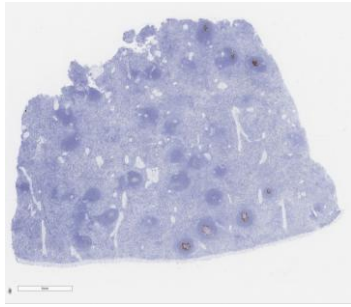
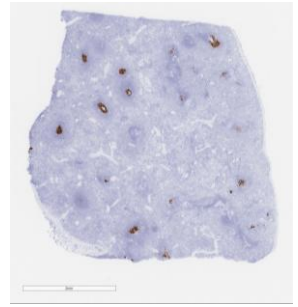
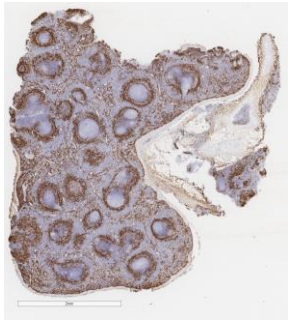
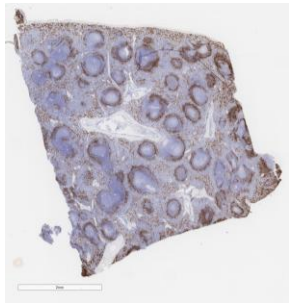
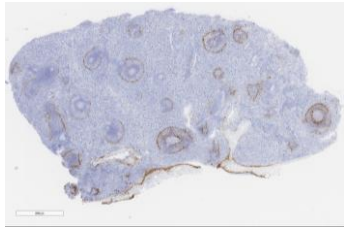
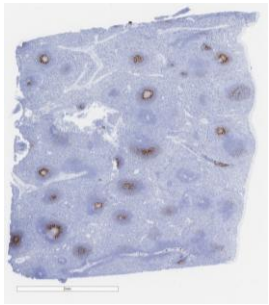
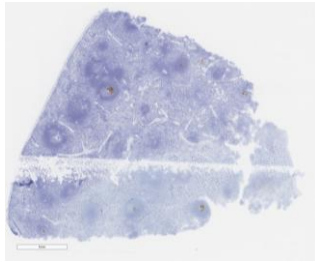
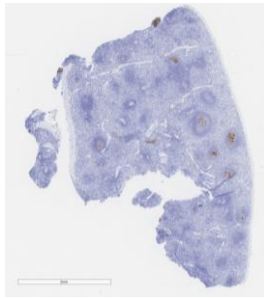
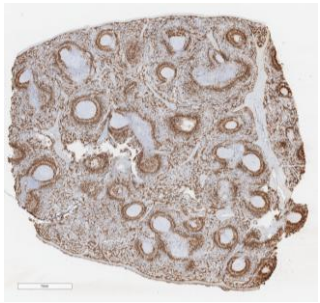
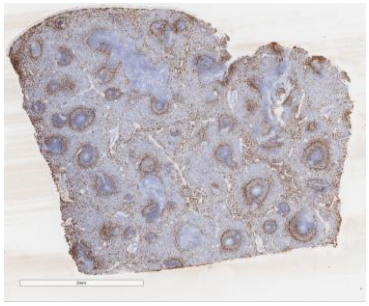
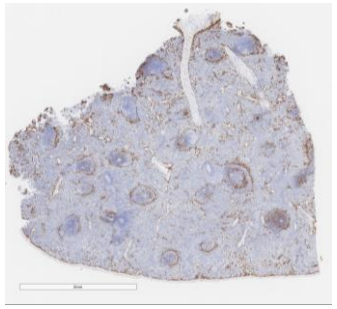
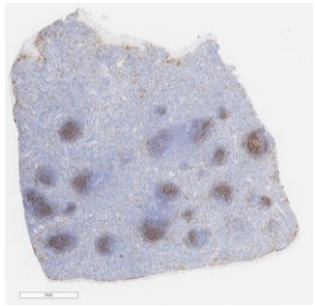
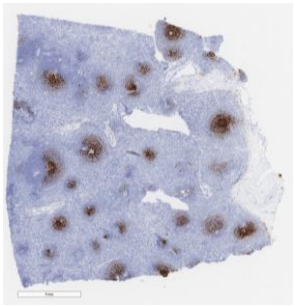
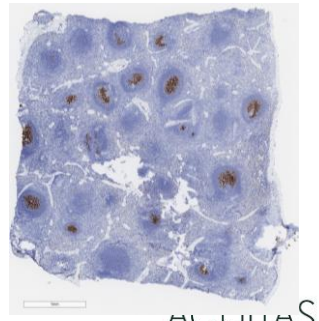
Time Post-EOI:	1 h	4 h	24 h	72 h	168 h	336 h
LNP 07						
LNP 09						
LNP 13						

ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion

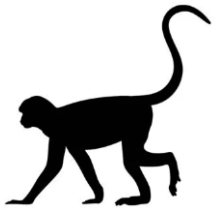


mRNA Distribution in Monkey Spleen (ISH)

Time Post-EOI:	1 h	4 h	24 h	72 h	168 h	336 h
LNP 07						
LNP 09						
LNP 13						

ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion



mRNA Distribution in Monkey Ovaries (ISH)

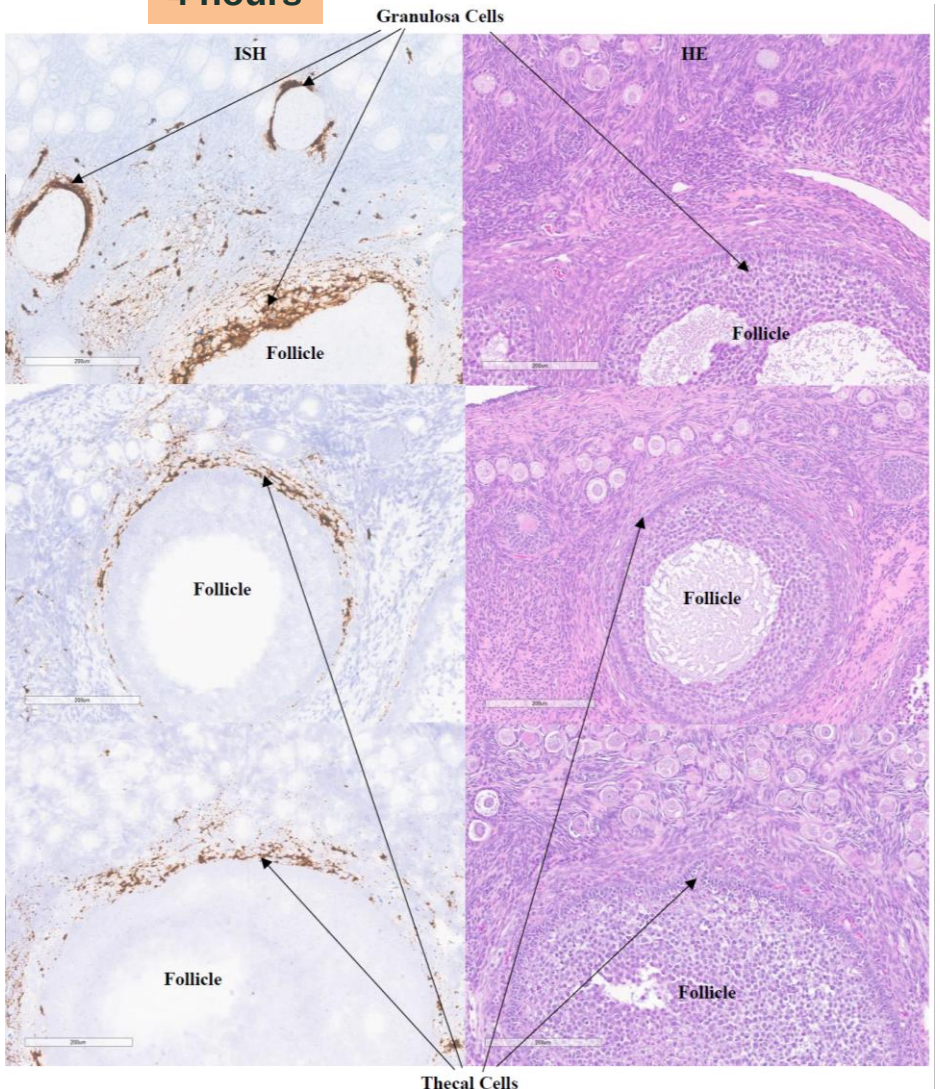
No mRNA Detected in Oocytes

	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

4 hours



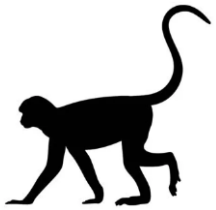
LNP 07

LNP 09

LNP 13

ACUITAS THERAPEUTICS

M. fascicularis
1.0 mg/kg
1 h iv infusion



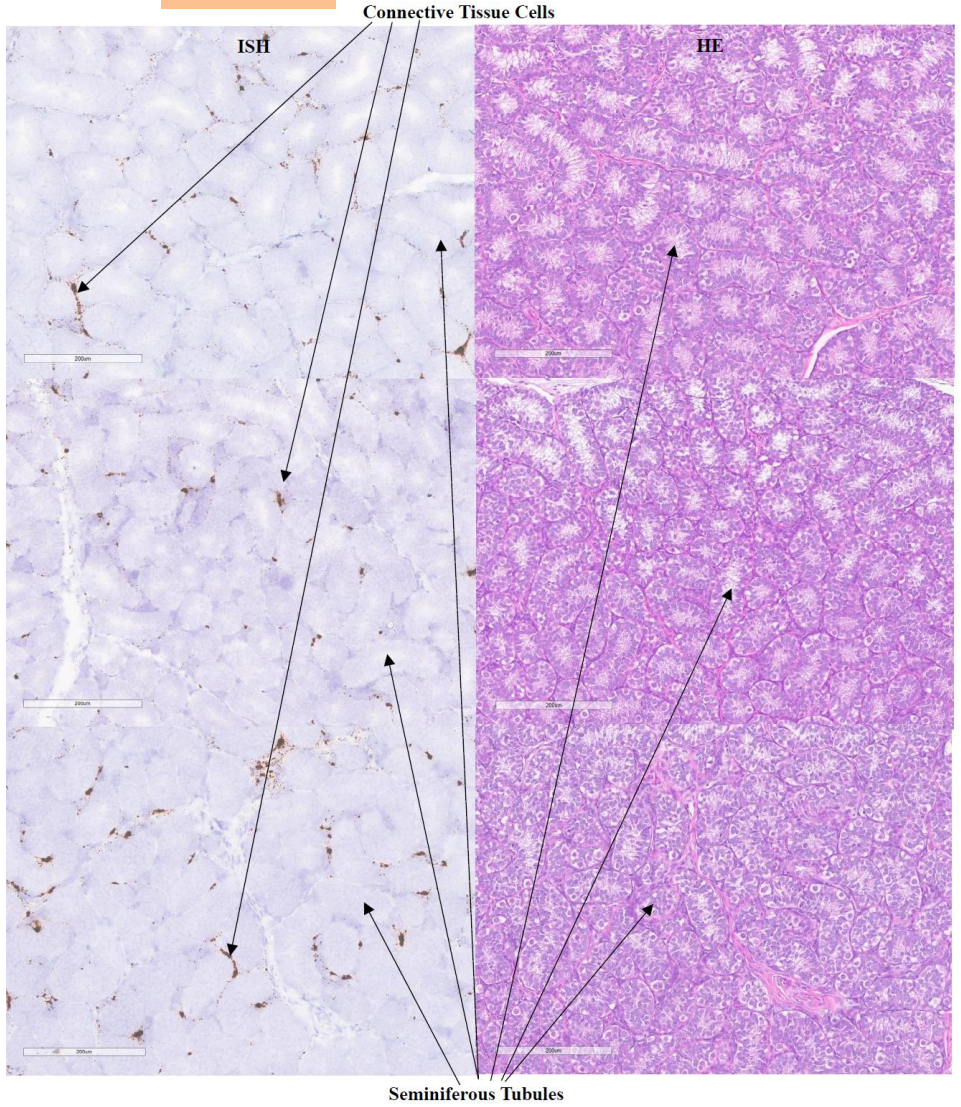
mRNA Distribution in Monkey Testes (ISH)

	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

4 hours



LNP 07

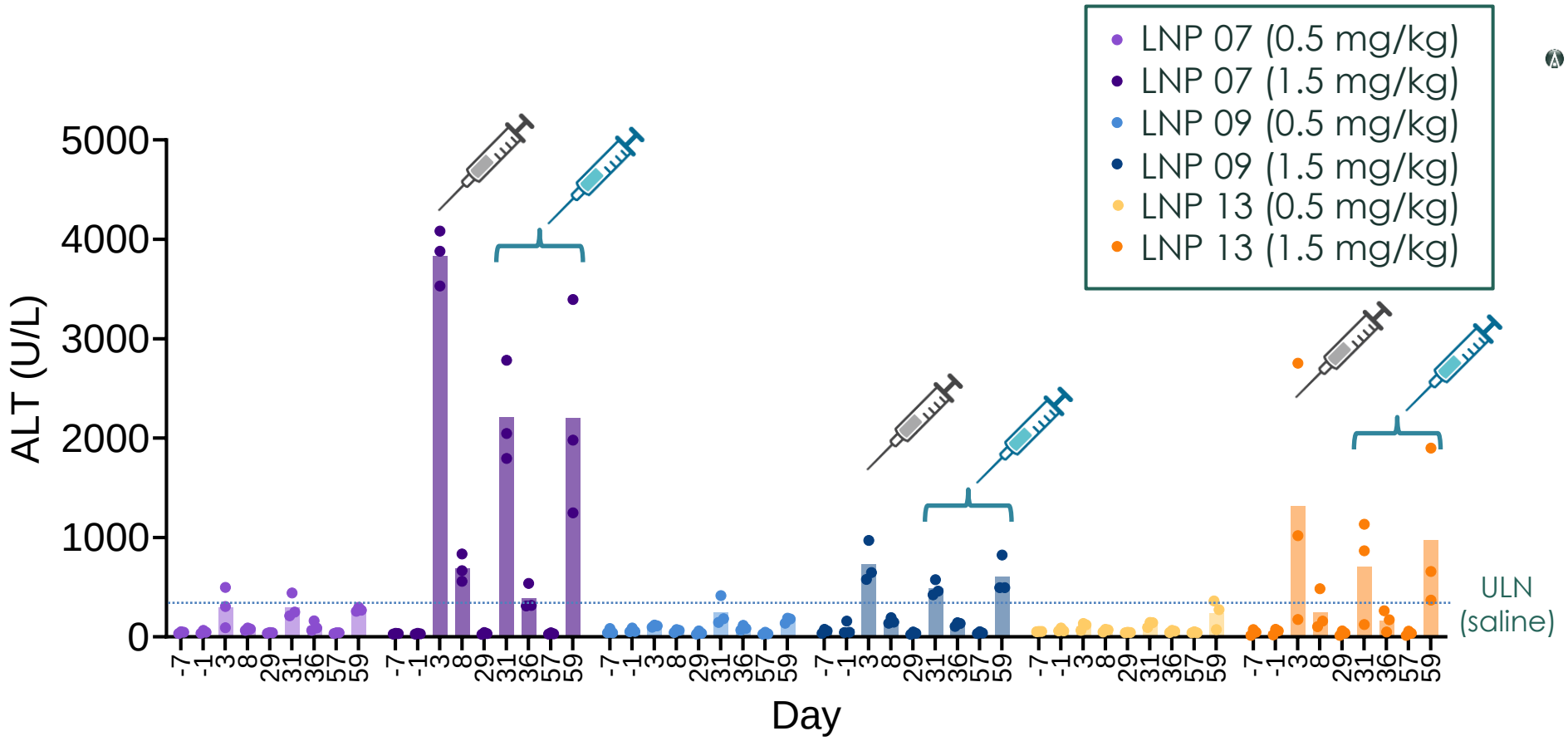
LNP 09

LNP 13

ACUITAS THERAPEUTICS

Safety Assessments

Dose-Related, Transient Elevations in Liver Transaminases

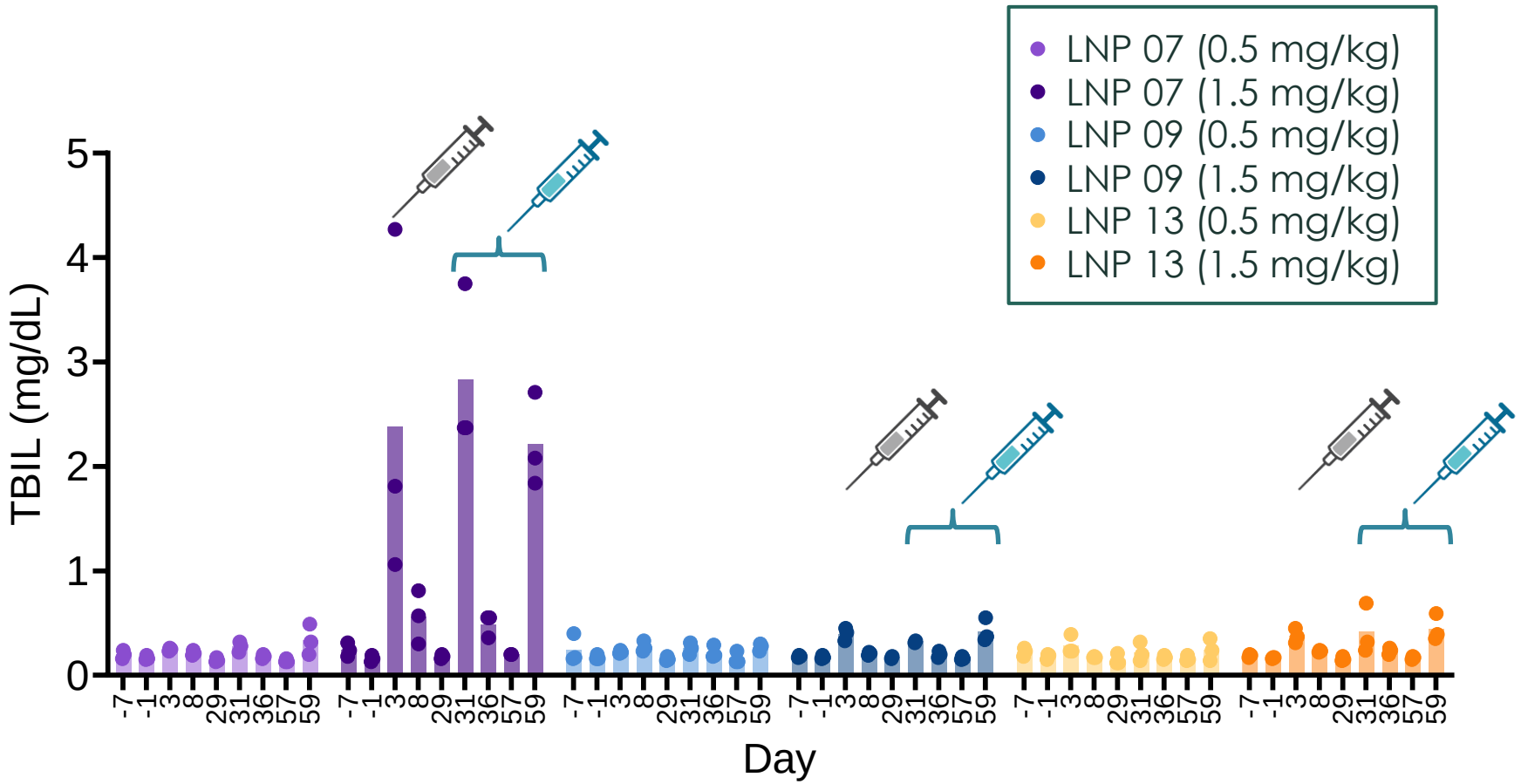


- Transient elevations in ALT (and AST)
  - **LNP 07** > **13** > **09**
  - Lower magnitude with αGal payload
  - Magnitude of ALT increase consistent between sequential αGal doses

ACUITAS THERAPEUTICS

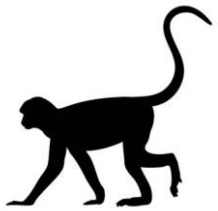
Safety Assessments

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

ACUITAS THERAPEUTICS



Safety Assessments: Target Organs

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

Finding	LNP 07		LNP 09		LNP 13	
	0.5	1.5	0.5	1.5	0.5	1.5
LIVER						
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:

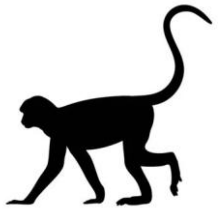
Vacuolation, hepatocyte – small clear vacuoles in cytoplasm of hepatocytes

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

ACUITAS THERAPEUTICS



Safety Assessments: Target Organs

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

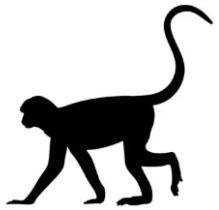
Finding	LNP 07		LNP 09		LNP 13	
	0.5	1.5	0.5	1.5	0.5	1.5
SPLEEN						
Vacuolation, cytoplasm, red pulp	3/0/0/0 [3]	1/2/0/0 [5]	3/0/0/0 [3]	3/0/0/0 [3]	0/1/1/0 [5]	0/2/0/0 [4]
Depletion, lymphoid	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/0/0/0 [0]	0/2/1/0 [7]
Necrosis, red pulp	0/0/0/0 [0]	0/0/0/0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

ACUITAS THERAPEUTICS

M. fascicularis

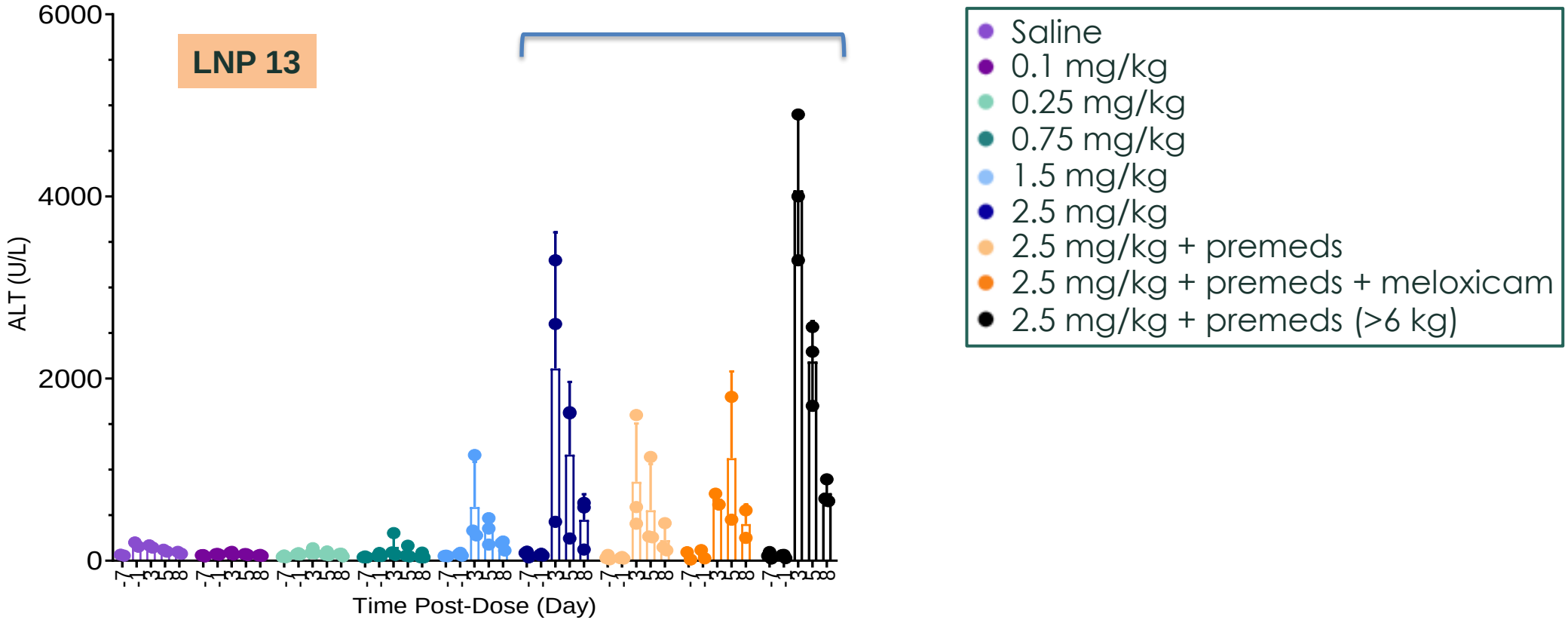
1 h iv infusion



~3 kg or
6-8 kg

Tolerability

Impact of Animal Size and Concomitant Medications on ALT

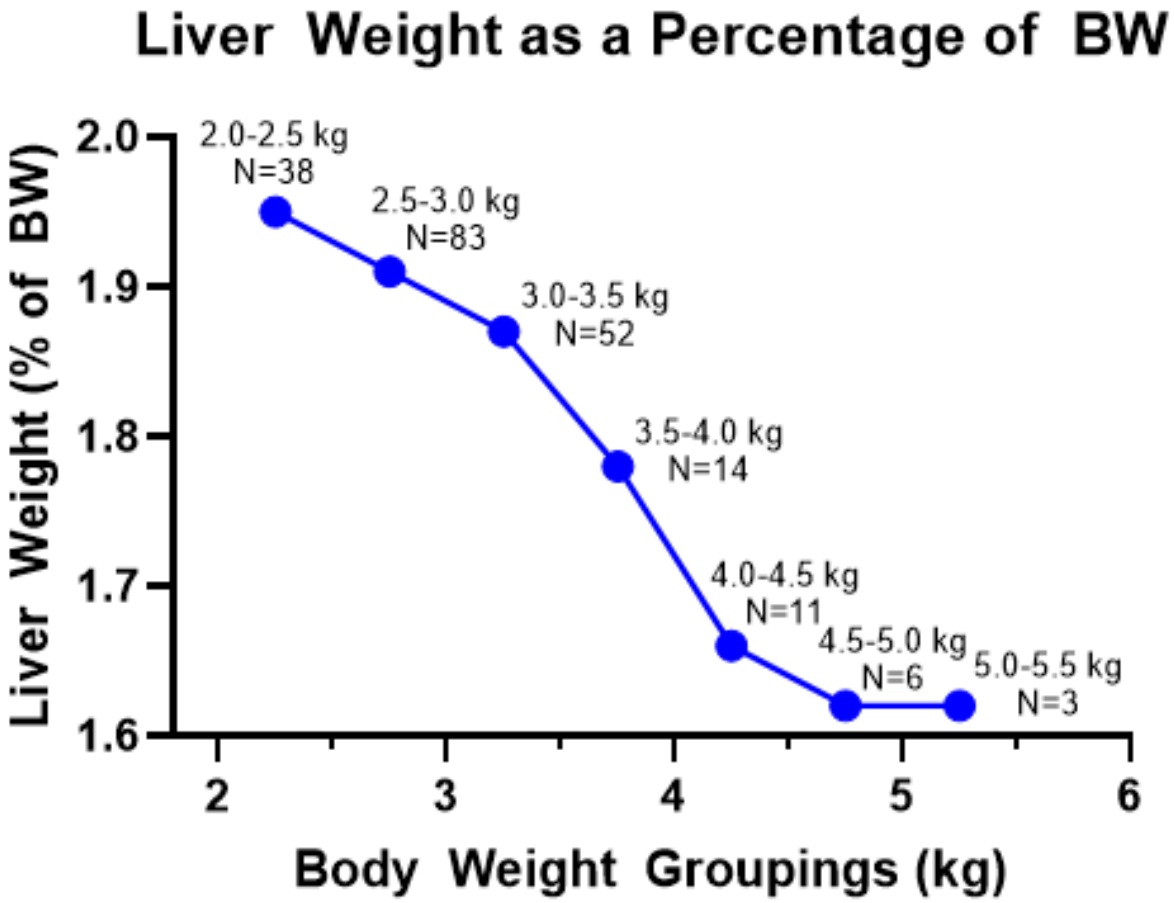


- ALT/AST elevated at ≥ 1.5 mg/kg on Day 3, near/nearing baseline on Day 8
- Highest ALT/AST elevations observed in no premed group and large NHPs (>6 kg)

ACUITAS THERAPEUTICS

Tolerability

Increased Liver Exposure in Larger Monkeys



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

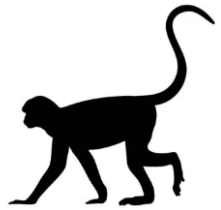
- **Example:**
  - Assuming 75% of dose to liver
    - **2.25 kg monkey** @ 2 mg/kg
      - Total dose = 4.5 mg
      - Liver dose = 3.4 mg
      - Liver dose by weight = **72.5 mg/kg liver**
    - **5.25 kg monkey** @ 2 mg/kg
      - Total dose = 10.5 mg
      - Liver dose = 7.9 mg
      - Liver dose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

~27% Liver Exposure Increase in Large vs Small Monkeys

ACUITAS THERAPEUTICS

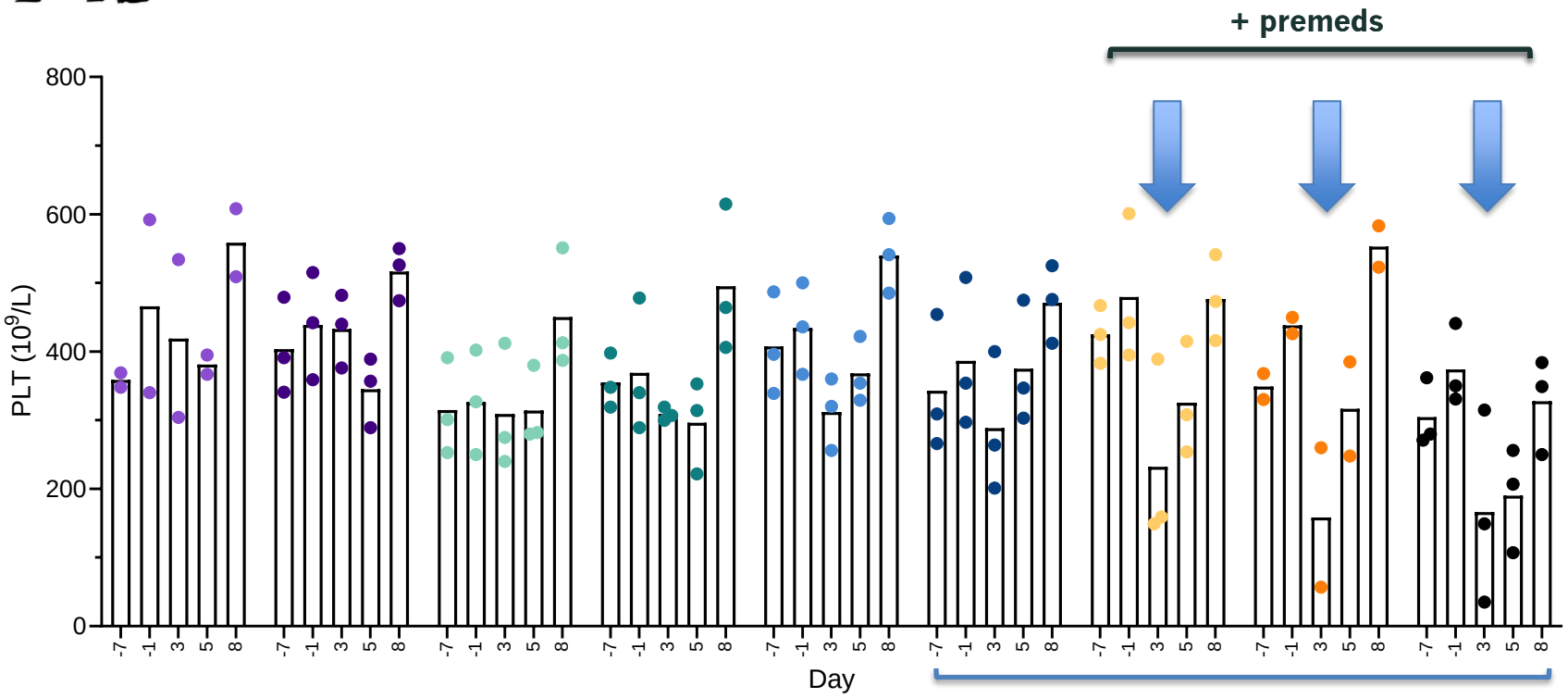
M. fascicularis

1 h iv infusion



Tolerability

Impact of Animal Size and Concomitant Medications on Platelets



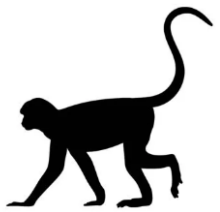
- Saline
- 0.1 mg/kg
- 0.25 mg/kg
- 0.75 mg/kg
- 1.5 mg/kg
- 2.5 mg/kg
- 2.5 mg/kg + premeds
- 2.5 mg/kg + premeds + meloxicam
- 2.5 mg/kg + premeds (>6 kg)

- 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

ACUITAS THERAPEUTICS

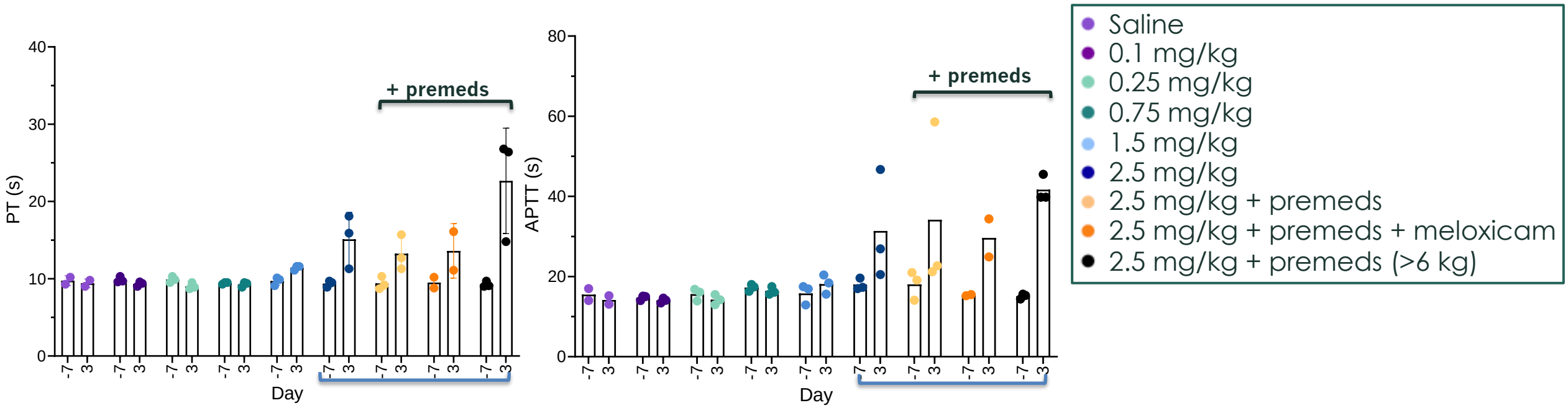
M. fascicularis

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

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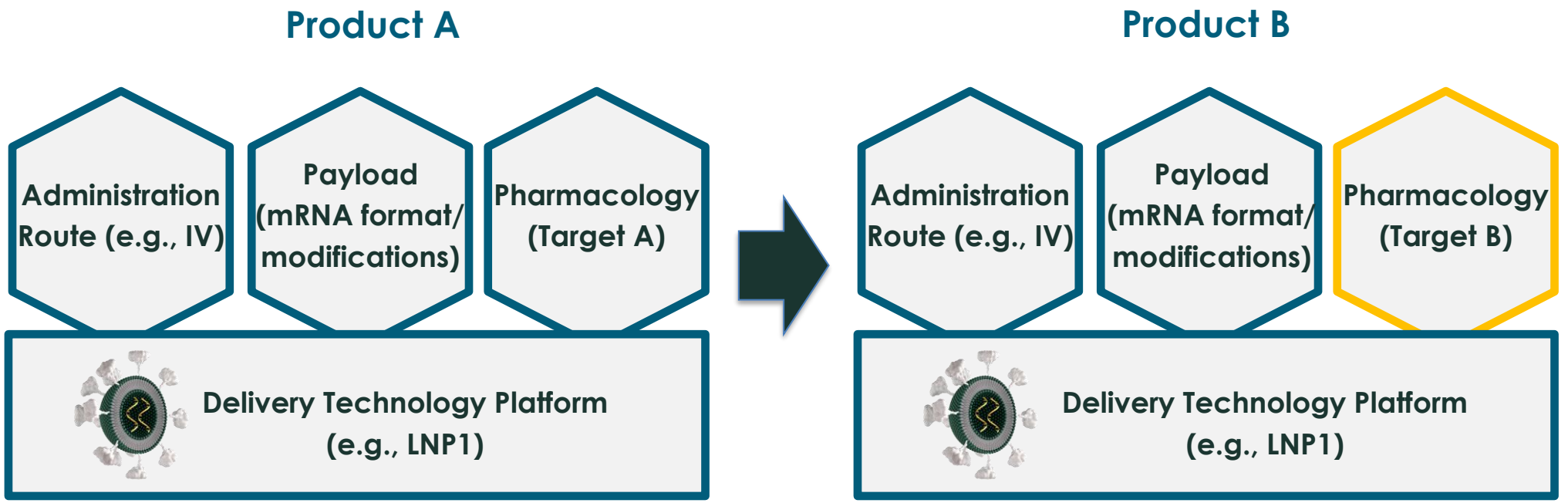
Leveraging an LNP Delivery Platform



Standard characterization package

Streamlined package

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

ACUITAS THERAPEUTICS

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**

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