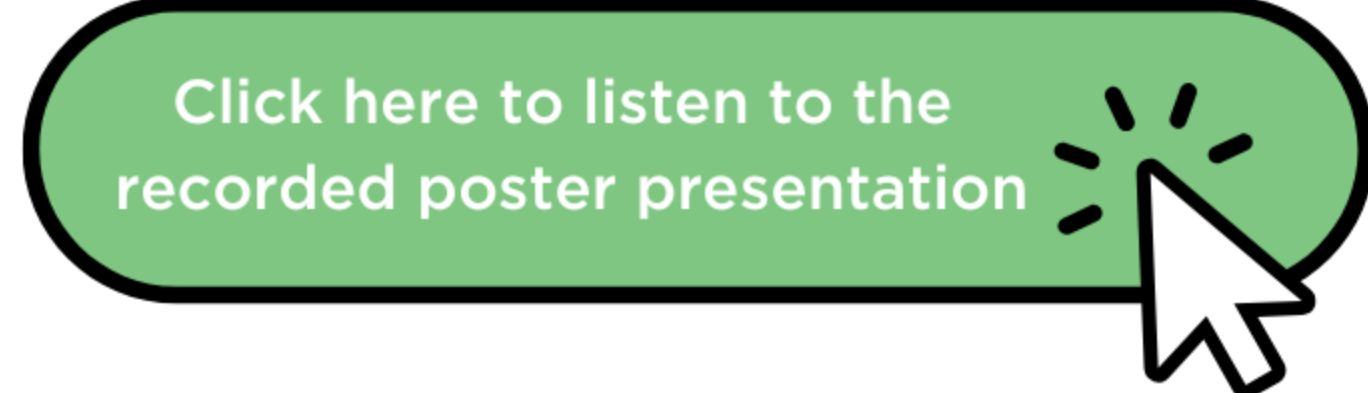


Toxicologic Profiles of Lipid Nanoparticle (LNP) Used as Delivery Systems for Nucleic Acid Therapeutics in Cynomolgus Monkeys: Insights From Cross-Study Analysis of Various LNP Formulations

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BACKGROUND AND PURPOSE

- Lipid nanoparticles (LNPs) are lipid-based nanocarriers formulated without an encapsulated therapeutic payload (empty LNP; 10-100nm), commonly used as delivery vehicles and formulation controls for nucleic acid therapeutics, chemotherapy agents, and other drugs. A recent review by Wang (2024, Vaccines)¹ summarized that nonclinical findings associated with **LNP-based medicines** are primarily characterized by **innate immune activation and inflammatory responses, and by distribution to off-target organs (predominantly the liver and spleen)** via engagement of the mononuclear phagocyte system (MPS), including Kupffer cells and tissue macrophages.
- While LNP drug products (LNP+RNA; **Table 1**) have been extensively evaluated in clinical and nonclinical programs, **nonclinical safety data characterizing empty LNPs (Figure 1) in nonhuman primates remain limited** and infrequently described in the literature or regulatory assessment reports, representing a knowledge gap in translational understanding.
- To address this gap, we performed a cross-study evaluation of empty LNP-related findings in cynomolgus monkeys (CMs), establishing a vehicle historical control database (HCD) to enable contextual interpretation of LNP-encapsulated candidates in Altasciences toxicity studies.

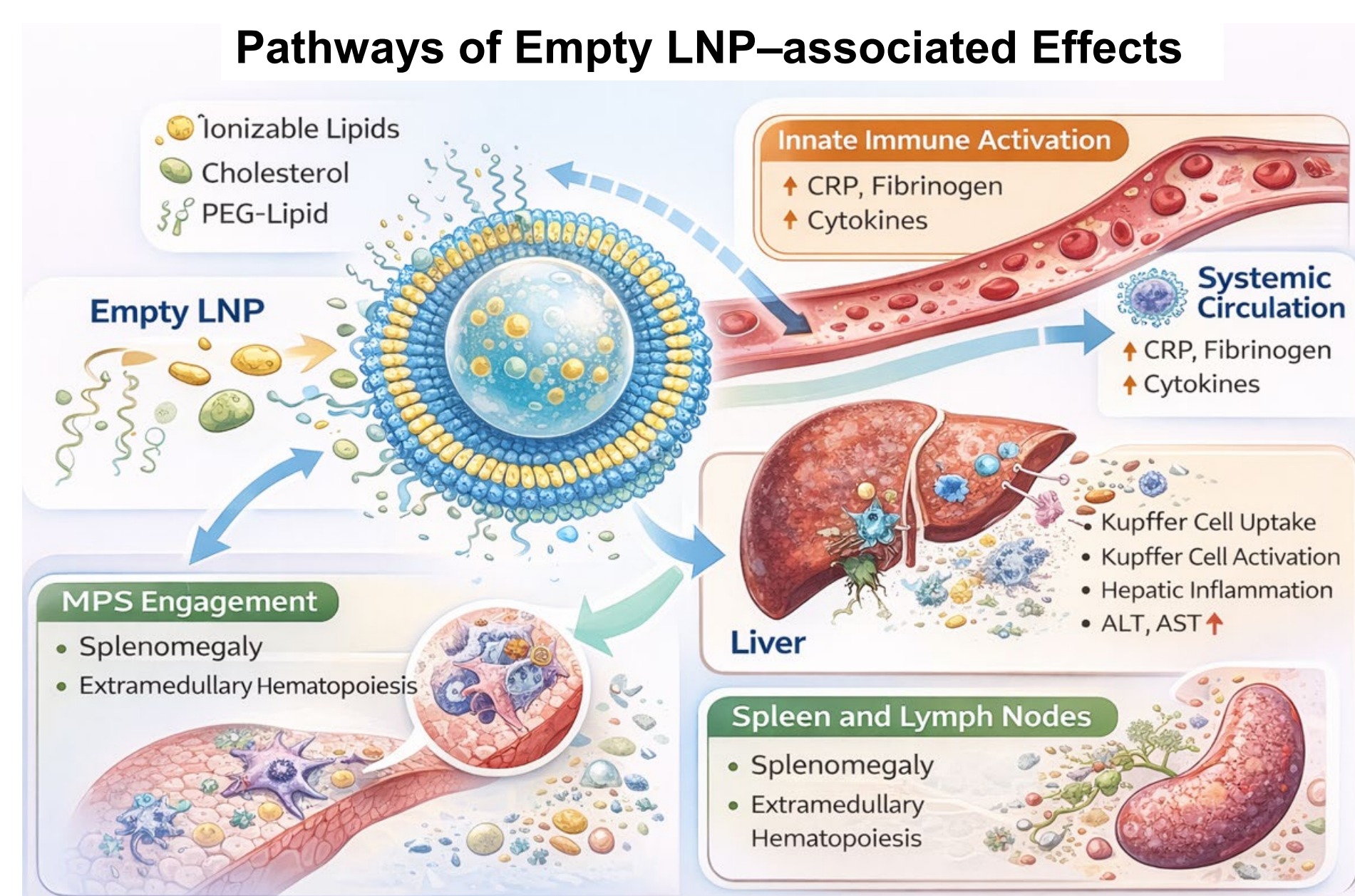


Figure 1. Schematic overview of empty LNP-induced hepatic and immune effects. LNPs share a conserved lipid architecture, consisting of 4 key components: ionizable cationic lipid (nucleic acid binding and endosomal escape), PEGylated lipids (stability and reducing aggregation), cholesterol (structural integrity), and phospholipids (membrane stabilization). Following administration, LNP uptake by the MPS (particularly Kupffer cells) drives **transient innate immune activation and lipid-associated hepatocellular stress**². The magnitude and pattern of these responses are LNP formulation- and dose-dependent, with variability largely attributable to differences in ionizable lipid chemistry.

METHODS

- Certara's SEND Explorer was utilized to extract pathology-focused data from Altasciences (Seattle) multiple CMs LNP toxicology studies (2022-2025) and integrated into a cross-study toxicity profile. Data were derived from single- and repeat-dose studies of empty LNP formulations, differing in ionizable lipid chemistry and dosing regimen (up to 8 mg/kg; IV or IM).
- Microscopic findings in major target organs, including liver, spleen, lymphoid tissues, and bone marrow, were evaluated in conjunction with clinical pathology endpoints (hematology, serum chemistry, acute-phase proteins: C-reactive protein, fibrinogen, and albumin). Organ weights and macroscopic observations were also evaluated.

- This poster highlights a short-term (4-day) case study of **empty LNP-related transient hepatic and immune findings (Figure 2)** and demonstrates the value of **integrating the HCD** to support contextual interpretation of nonclinical toxicity for LNP-based therapeutics.

RESULTS

Table 1. Regulatory Approved LNP-based Medicines: Characteristics, Human Safety, and NHP Toxicology^{3,4}

Product (Generic)	Brand Name	Company	Approval Year / Agency	Indication	Target	ROA	Payload Class	Payload Action	Ionizable Cationic Lipid	Key Human AEs	Nonclinical (NHP) Toxicity ^a
Patisiran	Onpatro [®]	Alnylam	FDA/EMA (2018); PMDA (2019)	hATTR amyloidosis	Transthyretin	IV	siRNA	Gene silencing	DLin-MC3-DMA	Infusion-related reactions (e.g., flushing, chills); gastrointestinal symptoms; fatigue	Transient liver enzyme elevations and hepatocellular vacuolation at supratherapeutic exposure; liver and spleen involvement consistent with MPS uptake of IV-dosed LNPs
Tozinameran	Comirnaty [®]	Pfizer/BioNTech	FDA/EMA (2021); MHRA (2020)	COVID-19	SARS-CoV-2 Spike	IM	mRNA	Antigen expression	ALC-0315	Local and systemic reactogenicity (injection-site pain, fever, fatigue); rare post-marketing myocarditis	Transient innate immune activation and inflammatory responses consistent with vaccine pharmacology; no persistent target-organ toxicity or adverse histopathology
Elasomeran	Spikevax [®]	Moderna	FDA (2022); EMA/MHRA (2021)	COVID-19	SARS-CoV-2 Spike	IM	mRNA	Antigen expression	SM-102	Similar local/systemic reactogenicity; rare post-marketing cardiac inflammatory events	Transient innate immune and inflammatory responses; no persistent or dose-limiting organ toxicity observed in repeat-dose studies
mRNA-1345	mRESVIA [®]	Moderna	FDA/EMA (2024)	RSV LRTD prevention	RSV F glycoprotein	IM	mRNA	Antigen expression	SM-102	Local and systemic reactogenicity; generally, well tolerated	Expected innate immune activation with favorable tolerability; no unexpected target-organ toxicity reported in toxicology studies
ARCT-154	Kostaive [®]	Arcturus/CSL/Meiji	MHLW (2023); EMA (2025)	COVID-19 (sa-mRNA vaccine)	SARS-CoV-2 Spike & replicase	IM	sa-mRNA	Self-amplifying antigen expression	ATX-126	Reactogenicity consistent with COVID-19 mRNA vaccines; acceptable clinical safety profile	Transient innate immune signals without persistent organ toxicity; supportive nonclinical immunogenicity and tolerability data

Note: ^aReported nonclinical findings in NHPs include transient serum transaminase (ALT/AST) elevations, hepatic inflammatory changes, increases in acute-phase proteins, and extramedullary hematopoiesis, consistent with systemic innate immune activation and engagement of the MPS, including liver. These findings are generally acute, reversible, and formulation-dependent, and are influenced by ionizable lipid chemistry and LNP dose.

Integrated Analysis of Empty LNPs Effects (hepatic and immune) in CMs (case example)

2A. Anatomic Pathology

- The liver is a primary site of LNP distribution and clearance and a common location for LNP-associated nonclinical findings. Single-cell hepatocellular necrosis and changes in immune organ cellularity were observed at a higher incidence than in origin- and age-matched CM HCD (N=83; Altasciences).

Representative microscopic changes (incidence):

Liver:
 Single cell necrosis; hepatocyte (2/3) vs. 0/83
 Mixed inflammatory cells infiltration; sinusoids; perivascular (2/3) vs. 24/83

Spleen:
 Increased cellularity; neutrophilic; red pulp (3/3) vs. 0/83

Lymph nodes:
 Mandibular: Decreased cellularity; lymphocytes; follicles (2/3) vs. 0/83
 Increased mineralization (1/3) vs. 0/83

Mesenteric: Decreased cellularity; lymphocytes; follicles (3/3) vs. 0/83

Bone marrow:
 Decreased cellularity; erythroid precursors (2/3) vs. 0/83

2B. Clinical Pathology

Key results include:

- Increased serum ALT and AST, in association with **hepatotoxicity (i.e., single cell necrosis; 2A)**.
- Increased fibrinogen and C-reactive protein with decreased albumin, indicative of an inflammatory response.
- Decreased reticulocyte and platelet counts, associated with reduced bone marrow cellularity
- vs. Altasciences origin- and age-matched reference intervals (RI: 2.5-97.5th percentile)

Serum transaminase (mean ± SD)

- ALT (U/L): BL 31±13 vs. (Day 4) 58±29 (20-113)
- AST (U/L): BL 35±8 vs. (Day 4) 87±43 (24-113)

Acute-phase proteins (mean ± SD)

- CRP (mg/dL): BL 0 vs. (Day 4) 25.4±20.4
- Fibrinogen (mg/dL): BL 206±43 vs. (Day 4) 444±93 (188-383)
- Albumin (g/dL): BL 4.6±0.1 vs. (Day 4) 3.3±0.1 (3.7-4.9)

Hematology (mean ± SD)

- RETIC (10⁶/μL): BL 0.076±0.023 vs. (Day 4) 0.016±0.003 (0.030-0.130)
- PLT (10³/μL): BL 475±85 vs. (Day 4) 229±14 (283-621)

Insights from Altasciences Pathologists: Transient Nature of Empty LNP-Related Hepatic and Immune Effects

- Based on our direct experience evaluating LNP drug candidates, empty LNP-related hepatic and immune effects were early-onset and reversible, with findings typically initiated as early as Day 1, increased over the subsequent 3–5 days, and entered recovery by approximately Day 8, with complete resolution by Day 22. This pattern was observed across studies with varied designs and necropsy time points, capturing both acute and subchronic effects.
- Our accumulated knowledge provides critical context for interpreting empty LNP findings in nonclinical studies, further supports LNP drug candidates' development as well as toxicological evaluation of emerging LNP platforms, which are expected to have broad applications beyond vaccines.

2C. Organ Weight

- Liver weights increased (spleen weights within historical range) relative to origin- and age-matched CM HCD (Altasciences).

Liver w/GB (mean ± SD)

- Weight (absolute; g): 98.40 ± 3.44 vs. **73.67 ± 20.42**
- Weight/TBW (%): 3.00 ± 0.93 vs. **1.90 ± 0.32**
- Weight/Brain (%): 147.42 ± 3.59 vs. **101.00 ± 28.00**

Spleen (mean ± SD)

- Weight (absolute; g): 5.02 ± 1.01 vs. **5.69 ± 2.01**
- Weight/TBW (%): 0.15 ± 0.03 vs. **0.15 ± 0.05**
- Weight/Brain (%): 7.52 ± 1.42 vs. **7.65 ± 2.19**

Figure 2. Toxicologic Profiles of Empty LNP in CMs. Microscopic examination (2A), clinical pathology evaluation (2B), and organ weight (2C) results are presented. No macroscopic abnormalities were observed in the liver, spleen, lymph nodes, or bone marrow at necropsy. Cytokines and complement data will be presented separately.

CONCLUSIONS AND PERSPECTIVE FOR FUTURE LNP PRODUCT DEVELOPMENT

- Our data demonstrated that the empty LNPs in CMs produced a consistent **pattern of "transient" hepatic and immune-related changes** that align with current scientific understanding, regulatory, and published nonclinical data, reflecting payload-independent vehicle effects.
- With the growing number of LNP programs at Altasciences, continued accumulation of empty-LNP historical control data (HCD) is enabling more informed interpretation of adaptive responses. This vehicle-specific HCD provides a critical baseline to distinguish payload-related effects and supports standardized, data-driven nonclinical safety assessment for future LNP therapeutics.

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