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Lipid Nanoparticles: A Comprehensive Assessment of Liver Enzymes and Immunopathology Markers in Nonhuman Primates

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INTRODUCTION

Lipid nanoparticles (LNPs) represent a significant advancement in drug delivery technology as carriers for non-viral targeted therapies. Their ability to encapsulate and transport a variety of molecules across cell membranes while protecting them from degradation has opened new avenues in gene therapies and editing. However, evaluating the safety and efficacy of these new products is paramount, and while the Food and Drug Administration (FDA) provides regulatory guidance, a paucity of published data makes it challenging to design preclinical studies that allow for sampling and monitoring of parameters.

Nonhuman primates are a desirable test system for evaluating toxicity because they are a preclinical model closely resembling humans. This review aims to bridge these gaps in knowledge and contribute to the safe and effective use of LNPs in gene editing and therapy.

METHODS

We reviewed clinical observations, serum chemistry, coagulation, and histopathologic results in 14 studies performed from 2021 to 2024 at Altasciences Preclinical Seattle using 43 unique LNP formulations administered to 196 cynomolgus macaques. The parameters presented were selected based on the most pronounced changes noted during the first week post-dose.

Animals were of Cambodian (85.7%) or Mauritius origin (14.3%). At initiation of dosing, Cambodian animals ranged from 2.0 to 5.8 years of age and 1.6 to 5.6 kilograms, while Mauritius animals ranged between 6.2 to 8.5 years of age and 4.3 to 11.9 kilograms. Animals were naïve to test article administration prior to dosing initiation, and data for repeat administrations were suppressed for comparison. Sex, age, and body weight variances were minimal and, therefore, combined for the purpose of this presentation. A total of 79 females (40.3%) and 117 males (59.7%) were distributed across 14 studies, with 4 non-terminal and 10 terminal. Within the terminal studies, only 8 included histopathologic evaluation, predominantly of liver tissue or other organs of the immune system.

Formulations were administered via intravenous infusion, ranging from 30 to 60 minutes in duration, and dose levels varied from 0.1 to 5 mg of nucleic acid per kilogram, including DNA, mRNA, or siRNA payloads. Pretreatment or cotreatment was administered in 35.7% of the studies, with no significant improvement observed compared to animals not treated with immunomodulators. Treatments were heterogenous and included Diphenhydramine at 2 to 5 mg/kg and/or Dexamethasone at 2 to 4 mg/kg via intramuscular injection 30 to 60 minutes pre-dose, with Dexamethasone at times extended for up to 3 weeks post-dose (28.6%); two studies combined Dexamethasone and Diphenhydramine treatments (14.3%), and one additionally combined of Tocilizumab infusion (7.1%) at 12 mg/kg.

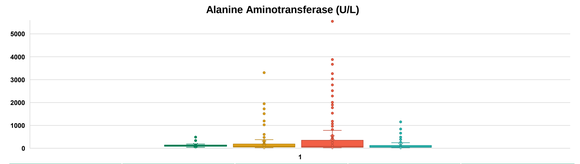
MORTALITY AND CLINICAL PRESENTATION

Severe clinical presentations that required veterinary intervention were uncommon, often presenting within 72 hours post LNP administration, and included dehydration, low body temperature, hunched posture, inappetence, decreased activity, and petechiation or ecchymosis. Moderate to marked increases in inflammatory markers (C-reactive protein, globulins, cytokines, and complement), prolonged coagulation times, and decreased fibrinogen, mainly when associated with dehydration and azotemia, presented across studies and test articles in correlation to progression and worsening of the clinical presentation, at times, resulting in unscheduled mortality. Early veterinary intervention (within 24 to 48 hours post-dose) with subcutaneous or intravenous fluids, produce, and heat supplementation likely contributed to the recovery of the surviving animals.

Unscheduled mortality was observed in 2 of 196 animals (1%) and related to increased inflammatory markers, clinical presentation of severe whole-body petechiation, dehydration, and prolonged coagulation times, with potential contribution of immunosuppression from the pretreatment received. The cause of death were attributed to kidney failure or disseminated intravascular coagulation.

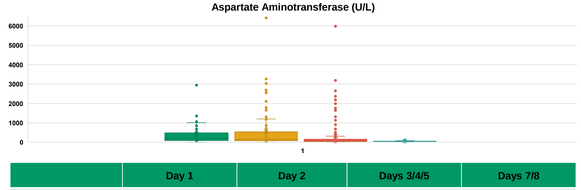
Table 1. Clinical pathology parameters

Alanine Aminotransferase (U/L)



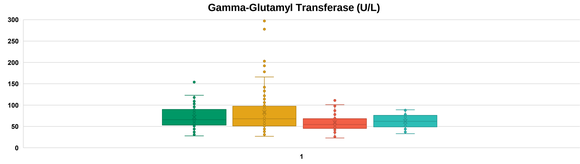
	Day 1	Day 2	Days 3/4/5	Days 7/8
n	33	95	197	116
Average	134.1515	241.2947	463.8376	133.4138
Median	108	95	85	65
Std Dev	92.91499	463.1178	864.235	183.4849
Maximum	490	3305	5551	1153
Minimum	42	25	21	19

Aspartate Aminotransferase (U/L)



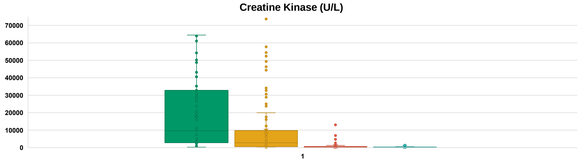
	Day 1	Day 2	Days 3/4/5	Days 7/8
n	71	95	196	116
Average	375.9014	500.3263	291.5204	48.82759
Median	202	145	63.5	43
Std Dev	433.5215	902.6552	686.3412	22.21761
Maximum	2947	6416	5988	148
Minimum	56	32	23	22

Gamma-Glutamyl Transferase (U/L)



	Day 1	Day 2	Days 3/4/5	Days 7/8
n	71	71	55	34
Average	71.22535	82.91549	59.4	62.73529
Median	65	68	54	62
Std Dev	25.27573	52.24031	19.93749	15.12435
Maximum	154	297	116	89
Minimum	28	27	23	33

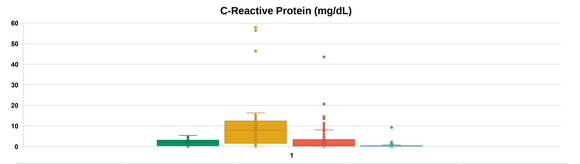
Creatine Kinase (U/L)



	Day 1	Day 2	Days 3/4/5	Days 7/8
n	66	91	197	116
Average	18076.88	9206.033	657.198	301.8879
Median	8603.5	2766	266	173.5
Std Dev	18368.11	15069.45	1310.328	323.4339
Maximum	64476	73693	13004	1886
Minimum	230	62	71	45

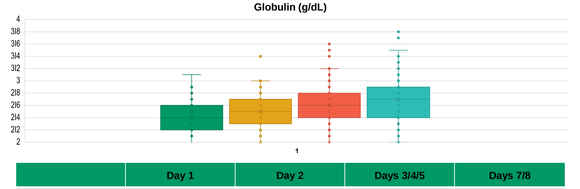
Day 1 Day 2 Days 3,4, or 5 Days 7 or 8

C-Reactive Protein (mg/dL)



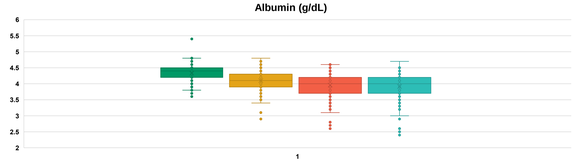
	Day 1	Day 2	Days 3/4/5	Days 7/8
n	66	66	139	60
Average	2.1	9.743939	3.453957	0.596667
Median	2.15	8.65	1.1	0.2
Std Dev	1.499538	10.88926	5.546996	1.250758
Maximum	5.4	58	43.6	9.3
Minimum	0.1	0.1	0.1	0.1

Globulin (g/dL)



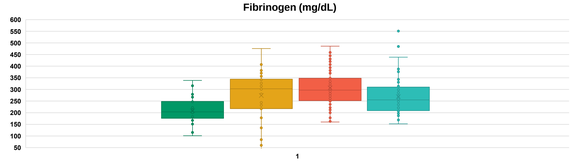
	Day 1	Day 2	Days 3/4/5	Days 7/8
n	72	96	198	117
Average	2.398611	2.49375	2.601515	2.689744
Median	2.4	2.5	2.6	2.7
Std Dev	0.244657	0.30325	0.320608	0.358249
Maximum	3.1	3.4	3.6	3.8
Minimum	1.9	1.6	1.7	2

Albumin (g/dL)



	Day 1	Day 2	Days 3/4/5	Days 7/8
n	71	95	197	116
Average	4.328169	4.098947	3.962437	3.911207
Median	4.4	4.1	4	4
Std Dev	0.295288	0.317401	0.356156	0.439718
Maximum	5.4	4.8	4.6	4.7
Minimum	3.6	2.9	2.6	2.4

Fibrinogen (mg/dL)



	Day 1	Day 2	Days 3/4/5	Days 7/8
n	33	33	107	60
Average	208.303	272.3939	307.0654	265.9333
Median	201	302	297	254
Std Dev	55.97013	105.409	80.16213	72.67757
Maximum	339	476	718	551
Minimum	101	40	160	152

Day 1 Day 2 Days 3,4, or 5 Days 7 or 8

HISTOPATHOLOGY

In animals that survived to scheduled necropsy, histopathologic findings in the liver were of increased severity up to 48 hours post-dose when compared to animals euthanized 7 to 40 days post LNP administration, with evidence of reversibility in recovery animals for studies in which a recovery subset were included. Findings were additionally observed in the spleen and kidneys, but a reduced tissue list for several of these studies could potentially conceal additional effects in target or off-target organs, including the potential for reproductive tract effects and germline editing.

Liver	Spleen	Kidneys
Vascular inflammation	Decreased cellularity of the white pulp	Tubule mineralization
Hepatocyte and Kupffer cell hypertrophy	Increased lymphoid cellularity of the follicle	Interstitial mononuclear cell infiltration
Hepatocyte necrosis and degeneration		Hyaline cast
Hepatocellular vacuolation		

CONCLUSION

Creatine kinase increases were more evident on Days 1 and 2, indicating muscle perturbation likely caused by prolonged restraint during scheduled activities despite the absence of macroscopic correlates and the limited microscopic evaluation of skeletal muscle in the studies. Variations in liver parameters were predominantly observed between Days 2 and 5 and showed poor correlation with clinical presentation or microscopic liver findings. This gap in understanding of the mechanisms of liver enzyme increase is noteworthy. Inflammatory markers correlated more strongly with severe presentations and adverse outcomes, underscoring their importance for monitoring and risk mitigation. The review found no benefits to combining immunosuppressive regimens with LNP administration, and these regimens were further associated with increased mortality. Hematologic parameter comparisons were hindered by the absence of control groups and inconsistent sampling schedules, affecting red cell mass, reticulocyte counts, and red cell distribution width. Future preclinical studies should focus on identifying relevant inflammatory markers and determining optimal monitoring time points. Comprehensive tissue microscopic evaluation and additional insights into tissue editing, including germline and reproductive tract considerations, are also essential.

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