Enhanced Biodegradable Lipids Reduce Liver Exposure and Improve Tolerability of mRNA-LNP While Maintaining Potency at Clinically Relevant Dose Levels

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Abstract

mRNA-LNP have shown great potential in the field of hepatic gene editing, and as more intravenously administered mRNA-LNP therapeutics advance into clinical evaluation, the need for highly potent LNP with broad therapeutic index and mRNA activity at clinically relevant dose levels remains of critical importance. Several clinical trials of mRNA-LNP have reported transient elevations in liver transaminases, a marker of liver injury. To reduce the liver toxicity of LNP, we rationally designed ionizable lipids that would significantly lower lipid exposure to the liver and hepatic effects while maintaining or improving potency.

A series of novel ionizable lipid analogs were synthesized, modifying various structural features such as tail branching and ester orientation, based on one of our lead lipids as the parent compound. These novel lipids were formulated into LNP using a standard composition and assessed for plasma and liver pharmacokinetics (PK), activity and tolerability in mice and non-human primates (NHP). Our benchmark (BM) ionizable lipid for these studies is comprised of symmetrical branched tails with the branch point occurring at the second carbon downstream from a "carbonyl-first" ester (beta-branched tails). By changing the lipid tail structure, we produced LNP with faster liver clearance in mice. LNP124 with linear tails was associated with the lowest liver C_{max} and AUC, with a C_{max} of 16 ng/g that is 17-fold lower than the BM at 273 ng/g. Moving the tail branchpoint further away from the ester to the gamma position, or combining linear and beta-branched tails yielded intermediate C_{max} levels ~40 ng/g. Although considerable improvements in liver tolerability were achieved with these lipid analogs (ALT ~10x lower than BM in NHP), there was also some loss of potency. The expression of an IgG reporter mRNA formulated with these analogs was ≤0.5x of the BM in both mouse and monkey at 0.3 mg/kg. Further structural alterations to the ester orientation rescued the activity and maintained the gains achieved in tolerability. Analogs with "oxygen-first" esters (LNP145 and LNP157) had 4-9x lower mouse liver C_{max} values and activity (IgG level) was 1-1.2x of BM respectively. Liver transaminases for these analogs remained low in monkeys and IgG expression was improved relative to BM (1.6-1.8x at 0.3 mg/kg). Liver PK for LNP145 and LNP157 in monkey showed a similar trend and rank order compared to the mouse data, with LNP157 displaying faster liver clearance in both species. In a mouse PCSK9 editing model at a sub-saturating dose of 0.15mg/kg, LNP157 produced 16% indels compared to 21% indels with BM, illustrating the ability of this LNP to maintain sufficient liver activity in addition to enhanced liver clearance.

By exploring the structure and liver PK relationship of ionizable lipids and how this relates to tolerability and activity, we have discovered two new biodegradable lipids and LNP (LNP145 and LNP157) with reduced liver exposure, improved liver tolerability and equivalent or improved potency at clinically relevant dose levels in monkeys.

1 Ionizable Lipid Tail Structure Influences Liver Pharmacokinetics

LNP	Tail Structure	Liver $t_{1/2}$ (h)	Liver Cmax (µg/g)	Liver AUC_{last} (h*µg/g)
Benchmark (BM)	β-branched	40	273	7369
LNP124	Linear	12	16	120
LNP125	γ-branched	23	44	619
LNP126	α-branched	87	168	13545
LNP139	Linear + β-branched	24	37	678

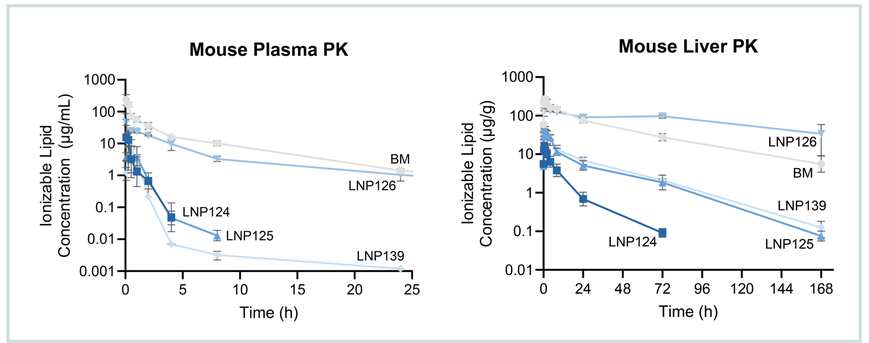
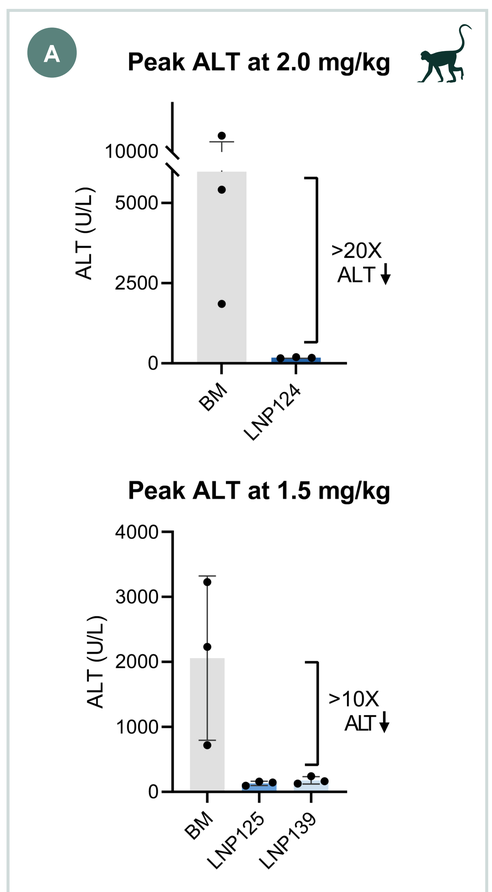
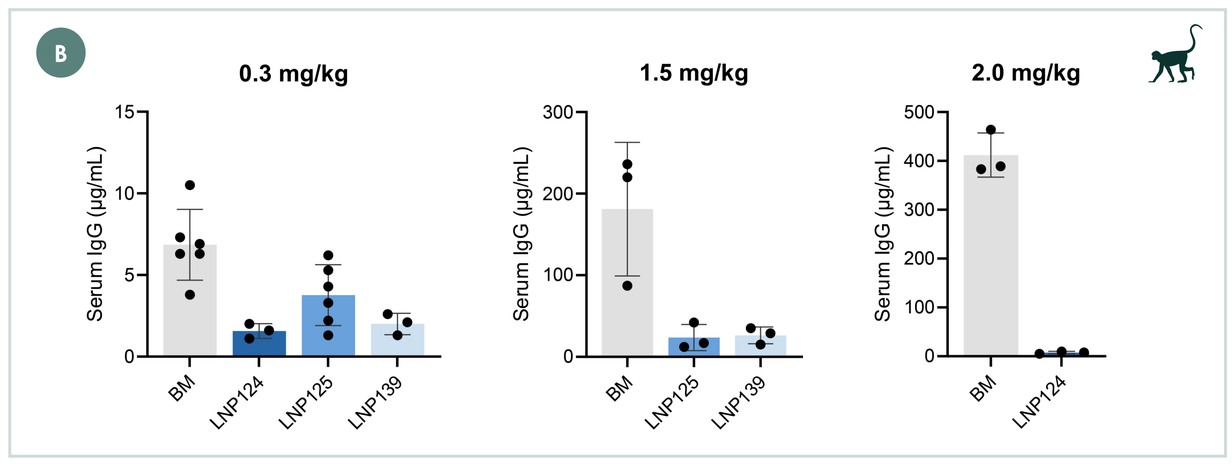


Fig. 1. Ionizable lipid pharmacokinetics. To determine how the tail structure of the ionizable lipid influences liver exposure, we synthesized a series of ionizable lipids with various branch points in the acyl chain. The parent compound, designated as benchmark (BM) in these studies, contains β-branched tails and is one of our lead lipids currently in clinical trials. Plasma and liver PK profiles were generated in mice following IV bolus injection of 1 mg/kg mRNA-LNP and show that lipids with linear tails have the lowest liver exposure. Lipids with γ-branched tails have an intermediate liver exposure that is similar to mixed linear+β-branched tails.

2 First Generation of Biodegradable Lipids Reduce Hepatic Toxicity but Impair Activity





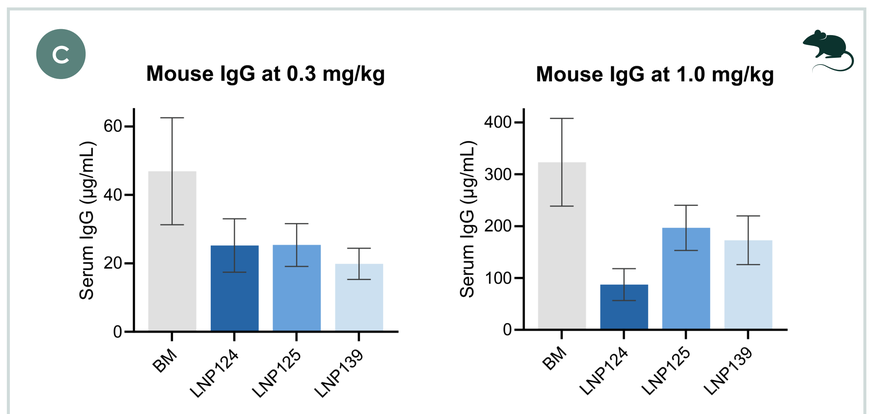


Fig. 2. Tolerability and activity of biodegradable lipids. Hepatic toxicity of LNP124, 125 and 139 was evaluated in NHP at 2.0 or 1.5 mg/kg by measuring ALT levels in serum 2 days post-IV infusion of LNP **(A)**. LNP formulated with this set of biodegradable lipids have dramatic ALT reductions compared to the benchmark lipid. However, activity of these new LNP is also reduced, as shown by ≤ 0.5X expression of a human IgG reporter mRNA in monkeys **(B)** and mice **(C)** when compared to the benchmark.

3 Ester Orientation Improves the Activity of Biodegradable Lipids

LNP	Ester Orientation	Liver $t_{1/2}$ (h)	Liver Cmax (µg/g)	Liver AUC_{last} (h*µg/g)
BM	Carbonyl-first	40	273	7369
LNP145	Oxygen-first	24	70	1494
LNP157	Oxygen-first	3	29	160

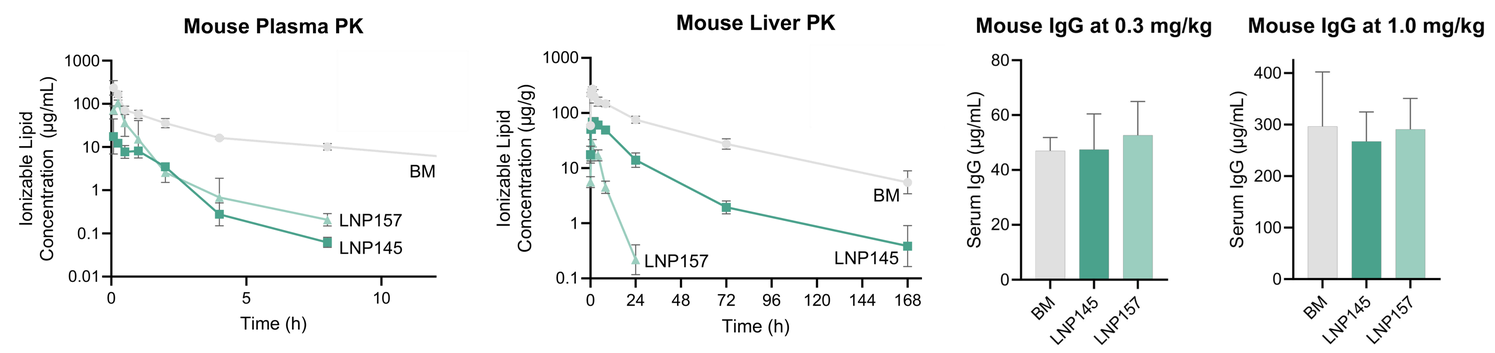


Fig. 3. Pharmacokinetics and activity of biodegradable lipids with alternate ester orientation. We sought to maintain the tolerability gains that were achieved with the tail-branching modifications, but to improve upon the LNP potency by further modifying the ester orientation in the ionizable lipid tail. Plasma and liver PK profiles in mice dosed at 1 mg/kg show that LNP145 and 157, with γ-branched tails and "oxygen-first" esters, have more rapid liver clearance than the benchmark. Unlike LNP125, IgG reporter expression is maintained at the same level as the benchmark at 0.3 and 1.0 mg/kg in mice.

4 New Biodegradable Lipids Improve Tolerability and Activity in Primates

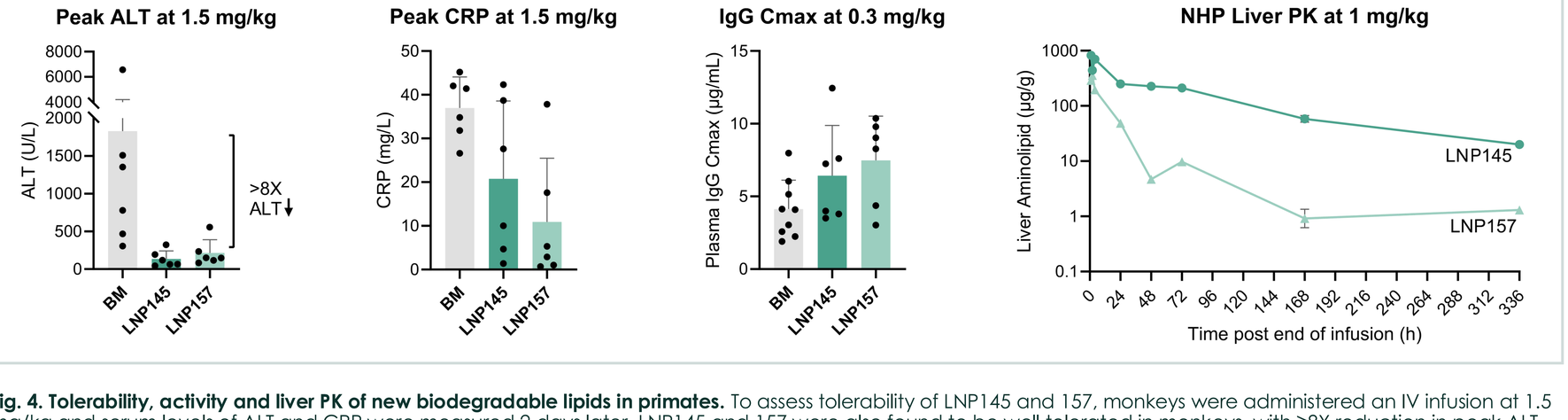


Fig. 4. Tolerability, activity and liver PK of new biodegradable lipids in primates. To assess tolerability of LNP145 and 157, monkeys were administered an IV infusion at 1.5 mg/kg and serum levels of ALT and CRP were measured 2 days later. LNP145 and 157 were also found to be well tolerated in monkeys, with ≥8X reduction in peak ALT, similar to the first generation of biodegradable lipids. The new LNP induced lower CRP levels, indicative of less systemic inflammation. Activity of LNP145 and 157 as measured by reporter IgG expression was 1.6X and 1.8X higher than the benchmark LNP respectively at 0.3 mg/kg. The liver PK profile in monkeys was generated from a combination of biopsy and terminal liver samples collected at various timepoints after a 1 mg/kg IV infusion (n=1 or 2 per timepoint). Clearance of the new lipids from monkey liver showed the same trend as in mice, with LNP157 exhibiting lower liver exposure than LNP145.

5 Biodegradable Lipids Enable Hepatic Gene Editing

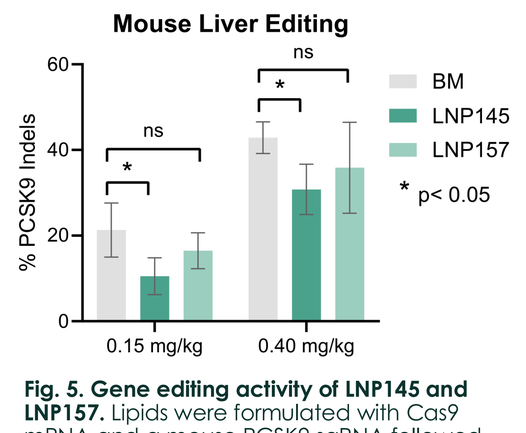


Fig. 5. Gene editing activity of LNP145 and LNP157. Lipids were formulated with Cas9 mRNA and a mouse PCSK9 sgRNA followed by IV injection in C57BL/6J mice. Editing was analyzed by amplicon sequencing of liver samples collected 4 days post-dose. The potency of LNP157 hepatic gene editing is not significantly different from the benchmark (t-test; p>0.25).

Summary

1. Ionizable lipids with linear and γ-branched tails are associated with rapid liver clearance and improved tolerability but have low activity.
2. Changing the ester orientation to "oxygen-first" in lipids with γ-branched tails retained the benefits of improved liver PK while also increasing activity.
3. LNP145 and LNP157 are new LNP with enhanced liver clearance that show improved liver transaminase levels while maintaining high potency at doses < 1 mg/kg.
4. LNP145 and LNP157 are active in a gene editing model.
5. Enhanced biodegradable lipids may be advantageous when repeat LNP dosing is anticipated over a short timeframe (i.e. in vivo CAR-T). Rapid clearance could prevent lipid accumulation in target tissues and reduce any potentially related toxic effects.

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