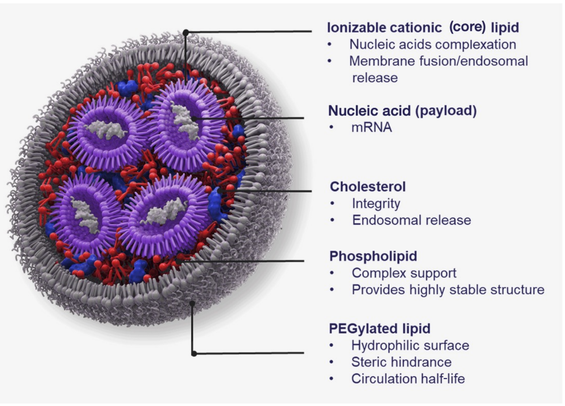
Vivofectamine LNP library for *in vivo* RNA delivery to the liver from mouse to NHP

Neha N. Parayath, Wen Yang, Koshi Kunimoto, Peter Lam, Virginia Aragon, David Papp, Priyanka Chaudhary, Juanita Martinez, Tyson Vonderfecht, Joel Jessee, Jason Potter, Evgenia Verovskaya

Thermo Fisher Scientific, 5781 Van Allen Way, 92008 Carlsbad, CA, United States

Introducing the Vivofectamine chemically diverse LNP library

The rapidly expanding utilization of mRNA as a therapeutic tool has presented the field with the challenge of innovating delivery methods. We leveraged over 30 years of lipid-based delivery expertise to develop a diverse set of lipid nanoparticle (LNP) solutions that can efficiently deliver mRNA *in vivo* and *ex vivo*. Here, we report on the Invitrogen™ Vivofectamine™ chemically diverse ionizable lipid library designed with sophisticated multi-step organic synthesis to generate a broader range of chemical diversity compared to a combinatorial approach. These LNPs demonstrate high efficacy and tolerability in a range of research applications, including protein expression in liver, vaccines, and targeting of immune cells.



Screening and selection of top lipids

6,000+ ionizable lipids synthesized
- Chemically diverse for various application needs
- Biodegradable bonds for tolerability

2,000+ screened *in vitro*
- Stable LNP formation
- *In vitro* efficiency and toxicity evaluated

500+ screened *in vivo*; 15 in NHPs
- *In vivo* efficacy, organ targeting, and tolerability tested
- Comparison to clinical benchmarks

Top 10+ application-specific lipids selected
- Detailed biological and biophysical characterization
- Tested in large animals (rats and/or NHPs)

Application areas

Our lipid library has been tested for performance and tolerability in research applications, including vaccination and liver delivery. We have also demonstrated the feasibility of using these lipids in research studies for CNS, eye, tumor, lung, and immune cell delivery.

	In vivo		*Ex vivo*
Applications	Vaccine | IM	CNS | CSF	T cell | *Ex vivo*
	Liver | IV	Eye | IVT	
	Immune cell | IV	Tumor | IT	
Model			

Formulation and physicochemical characterization

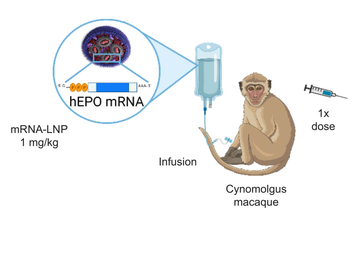
mRNA-LNP complexes were generated using a microfluidic approach and frozen in cryobuffer. Analysis was done post-formulation and at **4 months after one freeze-thaw cycle**. The results from three LNPs are shown as an example.

LNPs	Size (nm)		Polydispersity index		Encapsulation efficiency (%)	
	Fresh	Thawed	Fresh	Thawed	Fresh	Thawed
LNP1	66.9	67.8	0.10	0.09	97.4	97.8
LNP2	83.1	84.9	0.05	0.06	98.9	98.9
LNP3	99.0	98.6	0.17	0.16	98.1	98.3

Liver LNPs show exceptional tolerability profiles and high levels of protein expression in rats and NHPs.

Efficacy

Collaborator-generated data



Levels of circulating hEPO protein

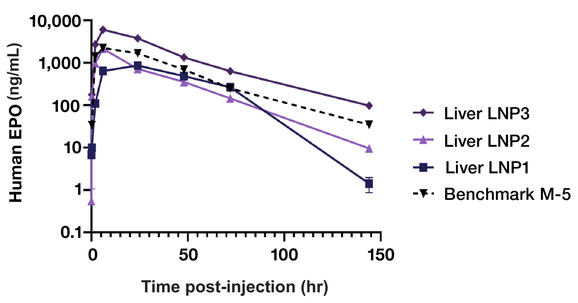
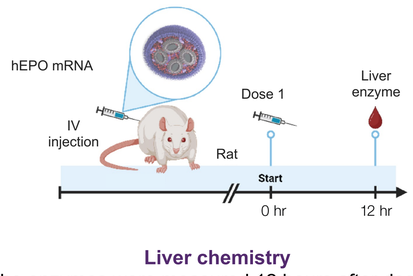


Figure 1. LNP-encapsulated human erythropoietin (hEPO) mRNA elicited protein production in nonhuman primates (NHPs). A 1 mg/kg dose of encapsulated hEPO mRNA was delivered to NHPs via infusion, and EPO levels in serum were analyzed at different time points. Liver LNP3 demonstrated **better efficacy than benchmark M-5** used in clinical-stage mRNA liver therapies.

Tolerance at a high dose

Collaborator-generated data



Liver chemistry
The enzymes were measured 12 hours after dosing.

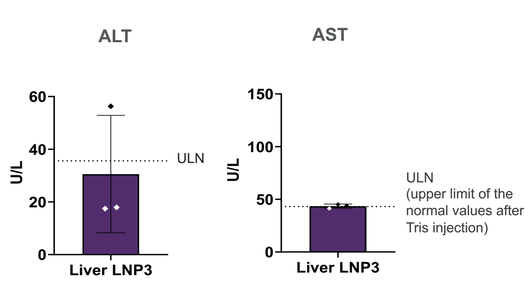
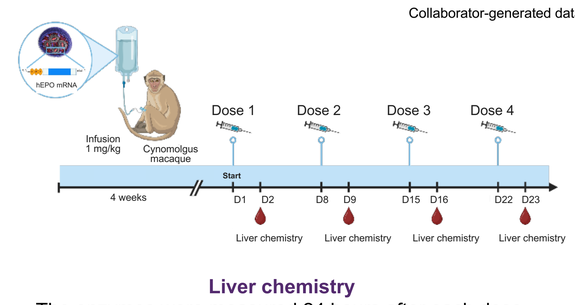


Figure 2. LNP-encapsulated hEPO mRNA was well-tolerated in rats at high doses. Liver LNP3–encapsulated hEPO mRNA was delivered to rats systemically at a high **9 mg/kg dose**, and liver enzyme expression in serum (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels) was analyzed 12 hours later. The dotted line (ULN) indicates the range of values observed in control rats injected with Tris.

Repeated dosing

Collaborator-generated data



Liver chemistry
The enzymes were measured 24 hours after each dose.

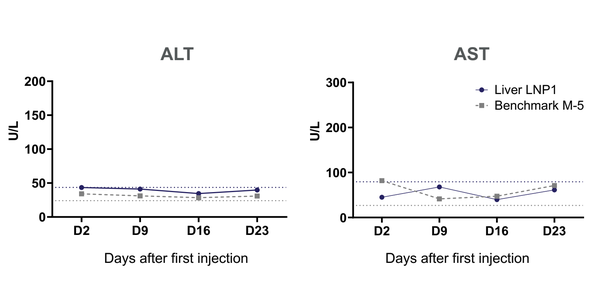
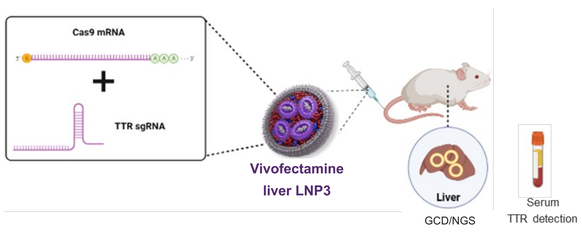


Figure 3. LNP-encapsulated hEPO mRNA was well-tolerated in NHPs after repeated dosing. A 1 mg/kg dose of encapsulated hEPO mRNA was delivered **weekly for 4 weeks**. Liver enzyme levels in serum were analyzed 24 hours after each dosing and compared to clinical benchmark LNP M-5. Dotted lines show baseline (pre-dosing) alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in respective NHPs.

Liver LNPs demonstrate highly efficient *in vivo* genome editing in mice.

Efficient TTR locus genome editing



Circulating TTR levels

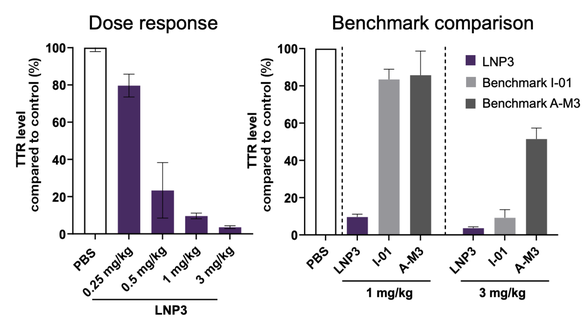
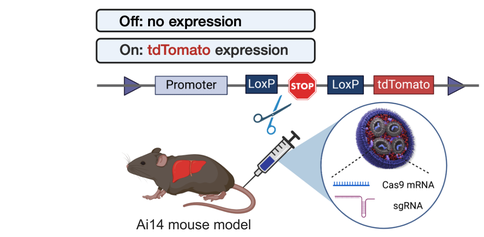


Figure 4. Co-encapsulated Cas9 mRNA and TTR sgRNA reduced serum TTR in rats in a dose-dependent manner. The RNAs were co-encapsulated at a 1:1 (wt:wt) ratio. Serum was collected 1 week after delivery, and TTR levels were measured using ELISA. **Liver LNP3 surpassed clinical benchmarks I-01 and A-M3** used in clinical-stage or FDA-approved liver-directed genome editing and siRNA therapies.

Editing visualization in reporter mice



Immunofluorescence analysis of livers in Ai14 mice

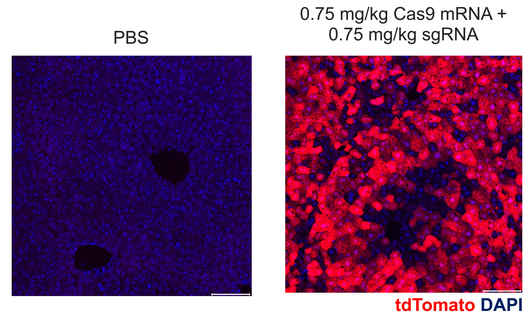
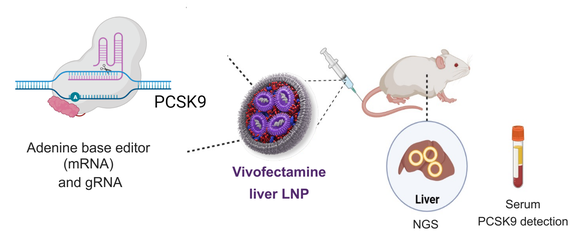
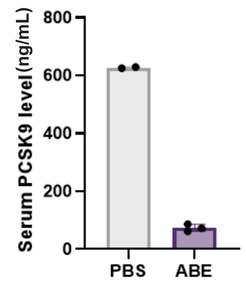


Figure 5. Genome editing with Cas9 mRNA and STOP cassette sgRNA was evident 1 week post-delivery in cryosectioned mouse livers. An Ai14 tdTomato reporter mouse model was used to visualize the delivery. The RNAs were co-encapsulated at a 1:1 (wt:wt) ratio. **Edited (tdTomato-positive) cells were visualized** 1 week after delivery in cryosectioned livers using immunofluorescence and confocal microscopy.

Efficient base editing of *PCSK9* locus



Circulating PCSK9 levels



NGS
homogenized liver

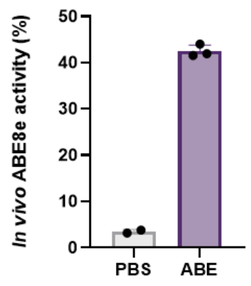


Figure 6. Co-encapsulated base editor mRNA and PCSK9 sgRNA effectively edited the *PCSK9* locus in mice. The RNAs were co-encapsulated in Liver LNP3 at a 1:1 (wt:wt) ratio, and a total **1.25 mg/kg dose of RNA** was delivered to mice. Genome editing was analyzed after 1 week in the homogenized liver sample using an NGS approach. PCSK9 serum levels were measured using ELISA.

Effective protein knockdown

siRNA-mediated FVII knockdown

Circulating FVII levels

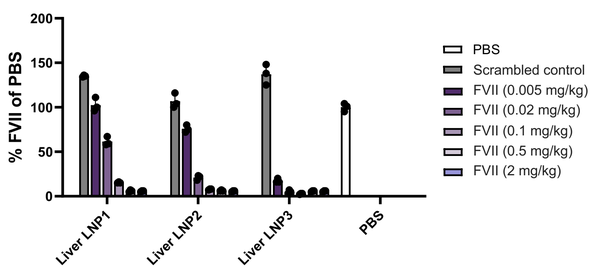


Figure 7. Encapsulated siRNA successfully knocked down Factor VII (FVII) in mice. Invitrogen™ Ambion™ *In Vivo* Factor VII siRNA was encapsulated and delivered systemically in doses ranging from 0.005 mg/kg to 2 mg/kg. The levels of circulating FVII were analyzed using a chromogenic assay (Biophen) 24 hours after delivery. Invitrogen™ *Silencer*™ Select Negative Control No. 1 siRNA was used as a scrambled control.

Summary

- ✓ Vivofectamine liver LNP3 demonstrates **better efficacy than benchmark M-5** used in clinical-stage mRNA liver therapies in NHPs.
- ✓ Vivofectamine liver LNPs **are well-tolerated in rats and NHPs** at high and/or repeated doses.
- ✓ Vivofectamine liver LNPs demonstrate **highly efficient genome editing in mice** using both Cas9 and base editing systems.
- ✓ Vivofectamine liver LNPs demonstrate **effective FVII knockdown** in mice using Ambion *In Vivo* Factor VII siRNA.

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at **thermofisher.com/vivofectamine**

invitrogen