Vivofectamine LNPs enable effective and durable genome editing in the liver

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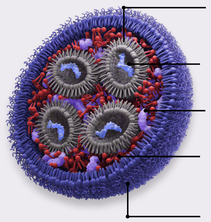
Abstract

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* in research samples. Here, we utilize Invitrogen™ Vivofectamine™ LNPs to achieve high-efficiency, durable genome editing in the liver of mice. We demonstrate efficacious performance in multiple mouse models including Cas9-mediated knockout of TTR, and adenine base editor–mediated knockout of PCSK9. We also track durability of editing in a tdTomato reporter model.

LNP characteristics

Vivofectamine LNP solutions for delivery to the liver contain an optimized ratio of ionizable lipid and helper lipids. They are used to create LNPs for *in vivo* delivery of mRNA or mRNA plus synthetic guide RNA (sgRNA) via the intravenous route. LNP reagents usually consist of the following components:



Ionizable cationic (core) lipid
- Nucleic acids complexation
- Membrane fusion/endosomal release

Nucleic acid (payload)
- mRNA

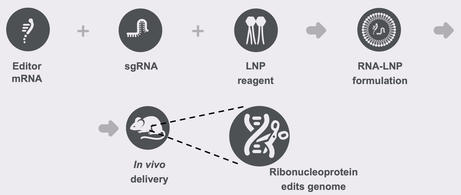
Cholesterol
- Integrity
- Endosomal release

Phospholipid
- Complex support
- Provides highly stable structure

PEGylated lipid
- Hydrophilic surface
- Steric hindrance
- Circulation half life

Using LNPs for double payload delivery *in vivo*

Editor mRNA (Cas, base editor, or prime editor) and sgRNA are co-encapsulated in the LNPs and delivered *in vivo* systemically for genome editing in the liver.

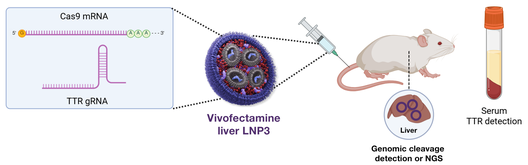


Formulation and physicochemical characterization

Editor mRNA and sgRNA were encapsulated in liver LNP3 from the Invitrogen™ Vivofectamine™ chemically diverse LNP library. A microfluidic approach at a ratio of 4:1 reactive amine to phosphate (N:P) was used for the encapsulation. Characterization results from three LNPs encapsulating different payloads are shown in the table.

Encapsulated RNA	Size (nm)	Polydispersity index (PDI)	Encapsulated efficiency (%)
Cas9 mRNA + TTR sgRNA	92.2	0.14	99.4
Cas9 mRNA + tdTomato sgRNA	85.3	0.14	92.7
ABE mRNA + PCSK9 sgRNA	62.6	0.13	90.1

Efficient genome editing of the *TTR* locus



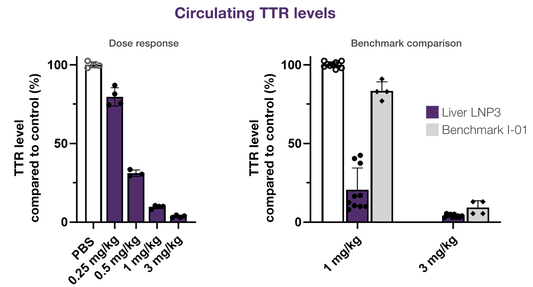
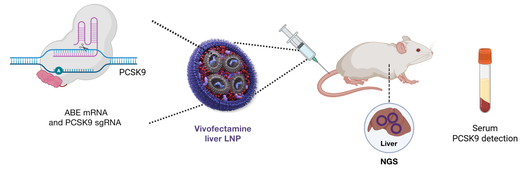


Figure 1. Co-encapsulated Cas9 mRNA and TTR sgRNA reduced serum TTR in mice in a dose-dependent manner. The RNAs were co-encapsulated at a 1:1 (wt:wt) ratio. Serum was collected 1 week after delivery, and TTR levels were measured using ELISA. Liver LNP3 surpassed clinical benchmark I-01 used in clinical-stage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

Efficient base editing of the *PCSK9* locus



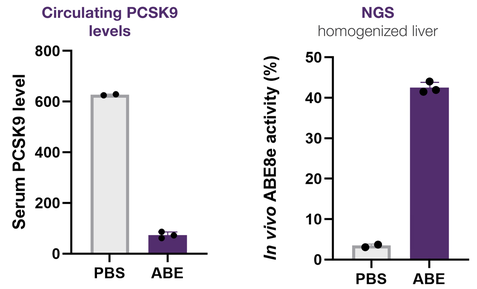


Figure 2. Co-encapsulated adenine base editor (ABE) mRNA and PCSK9 sgRNA effectively edited the PCSK9 locus in mice. The RNAs were co-encapsulated in liver LNP3 at a 1:1 (wt:wt) ratio, and a total 1.25 mg/kg dose of RNA was delivered to mice. Genome editing was analyzed after 1 week in the homogenized liver sample using next-generation sequencing (NGS). PCSK9 serum levels were measured using ELISA.

Tolerance at a high 3 mg/kg dose

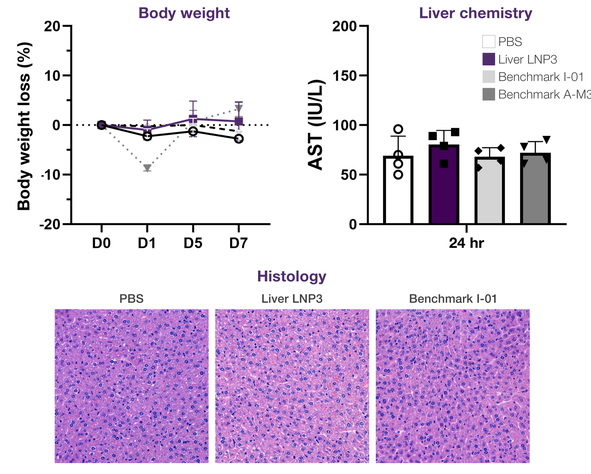
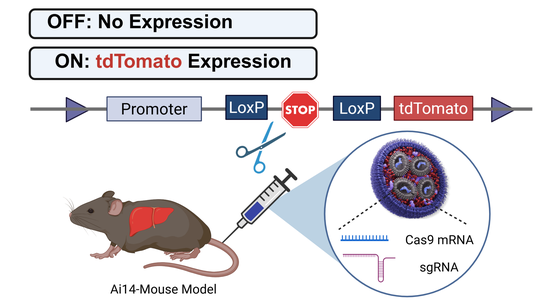


Figure 3. A 3 mg/kg dose of RNA-LNP is well tolerated in mice. A 3 mg/kg dose of RNA (Cas9 mRNA and TTR sgRNA, 1:1 wt) was delivered to mice. Aspartate aminotransferase (AST) levels were analyzed in serum 24 hours after injection, and liver samples were taken for hematoxylin and eosin (H&E) analysis after 7 days. Benchmarks I-01 and A-M3 are used in clinical-stage or commercial liver genome editing or siRNA therapies.

Visualization of editing in a tdTomato reporter model



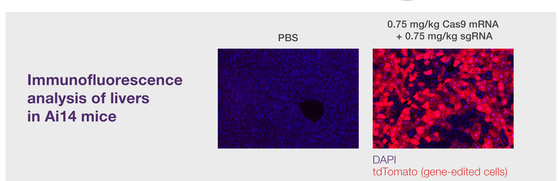


Figure 4. Genome editing with Cas9 mRNA and STOP cassette sgRNA was evident 1 week post-delivery in cryosectioned mouse livers. An Ai14 tdTomato reporter mouse model was used to visualize the delivery. The RNAs were co-encapsulated at a 1:1 (wt:wt) ratio. Edited (tdTomato-positive) cells were visualized 1 week after delivery in cryosectioned livers using immunofluorescence and confocal microscopy.

Durable (>1 year) genome editing

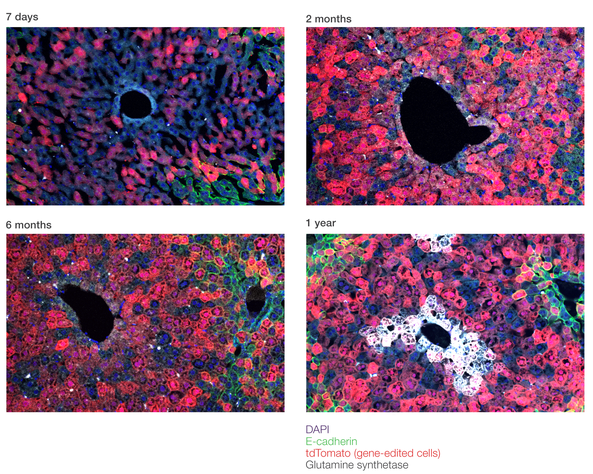


Figure 5. A 1-year time course confirmed lasting genome editing with Cas9 mRNA and STOP cassette sgRNA in cryosectioned mouse livers. Edited (tdTomato-positive) cells were visualized at different time points after delivery in cryosectioned livers using immunofluorescence and confocal microscopy. Counterstaining with E-cadherin and glutamine synthetase was used to visualize different liver zones.

Confirmation of editing by spatial microscopy

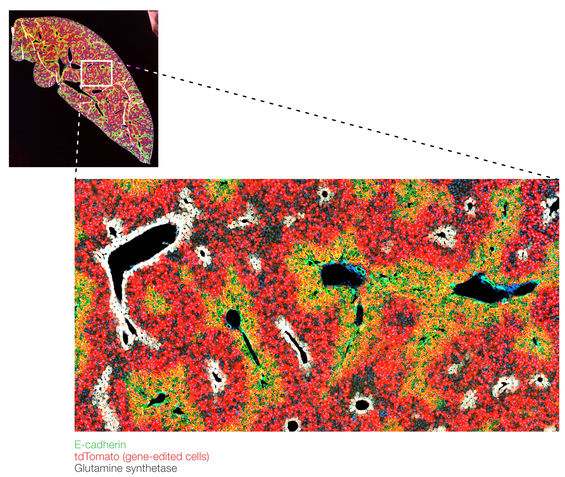


Figure 6. Spatial imaging verifies editing throughout the whole liver lobe. The Invitrogen™ EVOS™ S1000 Imaging System was used to visualize editing in the whole mouse liver lobe. The tiled scan is shown in the upper left, and an enlarged part in lower right.

Key findings

- Vivofectamine LNPs demonstrate highly efficient genome editing in the liver of mice using both Cas9 and base-editing systems
- Durable genome editing of >1 year is confirmed by microscopy of cryosectioned livers
- High doses of Vivofectamine LNPs are well tolerated in mice

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Acknowledgement: Illustrations in figures were created with BioRender.com.

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