# Vivofectamine LNP delivery solutions for RNA vaccines

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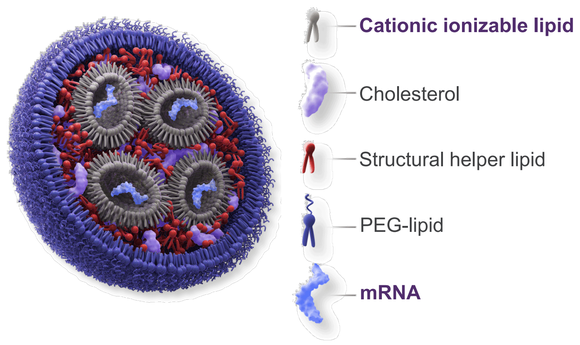
## Vivofectamine chemically diverse LNP library

The rapidly expanding utilization of mRNA as a therapeutic modality for vaccination has presented the field with the challenge of innovating delivery methods. Our team has leveraged over 30 years of expertise in lipid-based delivery to develop lipid nanoparticle (LNP) solutions that can efficiently deliver mRNA *in vivo*. Here we report on the Invitrogen™ Vivofectamine™ ionizable lipid library, which can be used for RNA vaccine development with high immunogenicity and tolerability in research models. Our lipid library was designed using sophisticated multi-step organic synthesis to generate high chemical diversity relative to a combinatorial approach. Our leading candidates were selected from a library of over 6,000 synthesized lipids that went through iterative rounds of *in vitro* and *in vivo* screening. These LNPs demonstrate high efficacy and tolerability in a range of research applications, such as vaccine development, *ex vivo* and *in vivo* targeting for immune cells, and protein expression in liver.

## LNP structure




## Selection of our top lipids

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

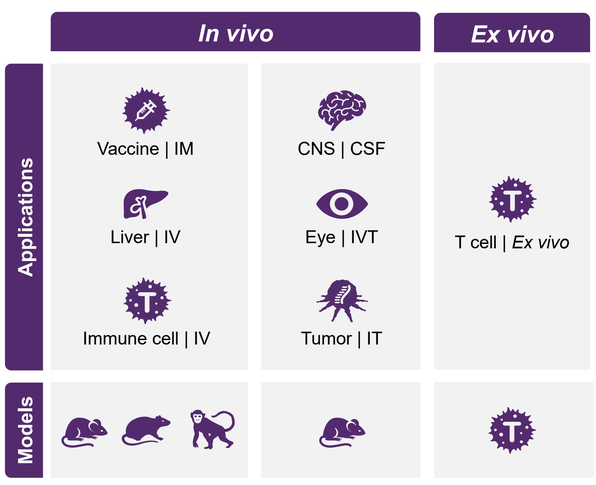
**2,000+ screened *in vitro***
- Stable LNP formation
- *In vitro* efficiency and toxicity evaluated

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

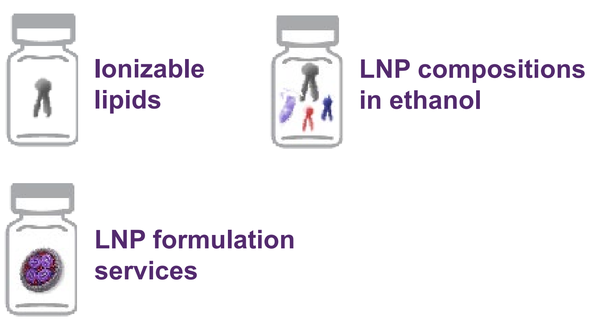
**Top 10+ application-specific lipids selected**
- Detailed biological and biophysical characterization
- Tested in large animals (rats and/or nonhuman primates)

## Application areas

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




## Types of offerings




## Competitive immunogenicity compared to clinical benchmarks in mice

Collaborator-generated data

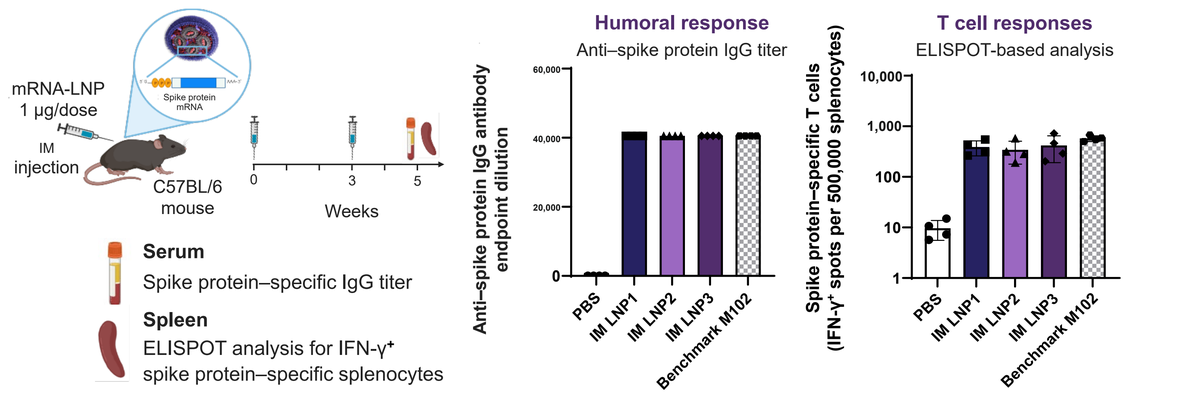



**Figure 1. Vivofectamine IM lipids demonstrate competitive immunogenicity relative to a clinical benchmark.** 1 µg of mRNA encoding the SARS-CoV-2 spike protein was encapsulated in LNPs and delivered via intramuscular (IM) injection to C57BL/6 mice. A booster dose was delivered on day 21. Serum samples were collected on day 35 after prime and boost doses for ELISA analysis. T cell responses were detected by ELISPOT in isolated splenocytes after stimulation with S1 and S2 peptides. The benchmark control was an ionizable lipid used in an FDA-approved mRNA vaccine.

## Tolerability: well-tolerated repeated dosing regimen in rats

Collaborator-generated data

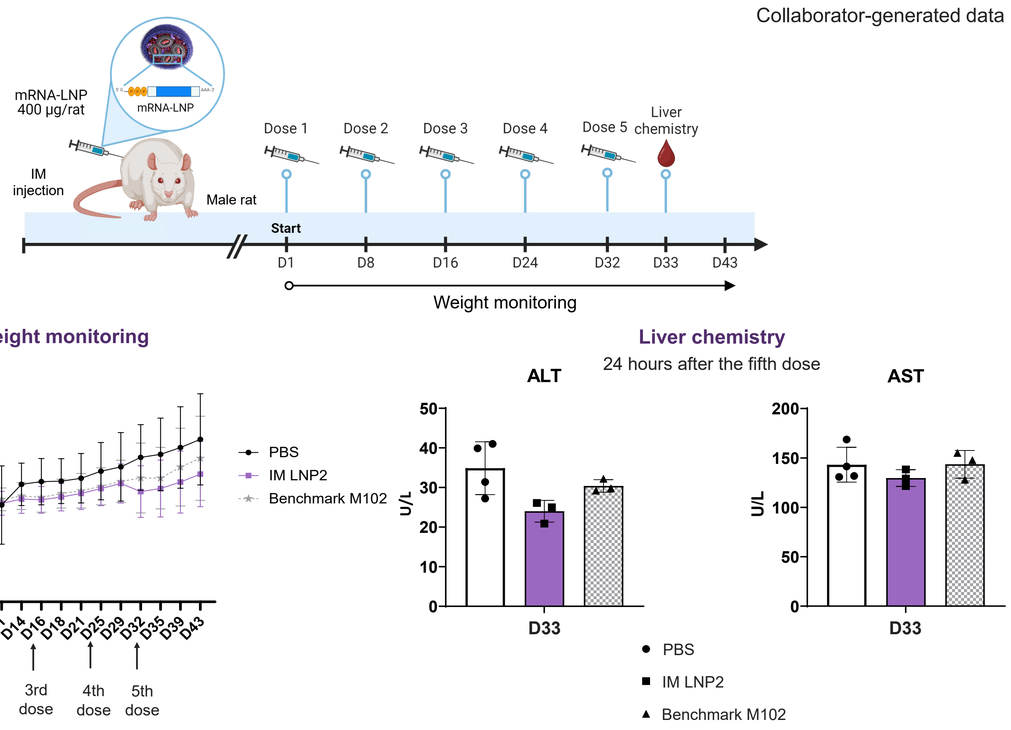



**Figure 2. Vivofectamine IM lipids are well-tolerated in a repeated dosing regimen in rats.** 400 µg of mRNA was encapsulated in LNPs and delivered via IM injection to rats. Additional doses were administered on days 8, 16, 24, and 32. Serum samples were analyzed 24 hours after the fifth injection for liver enzymes. The benchmark control was an ionizable lipid used in an FDA-approved mRNA vaccine.

## High systemic protein expression level and good tolerability profile in NHP model

Collaborator-generated data

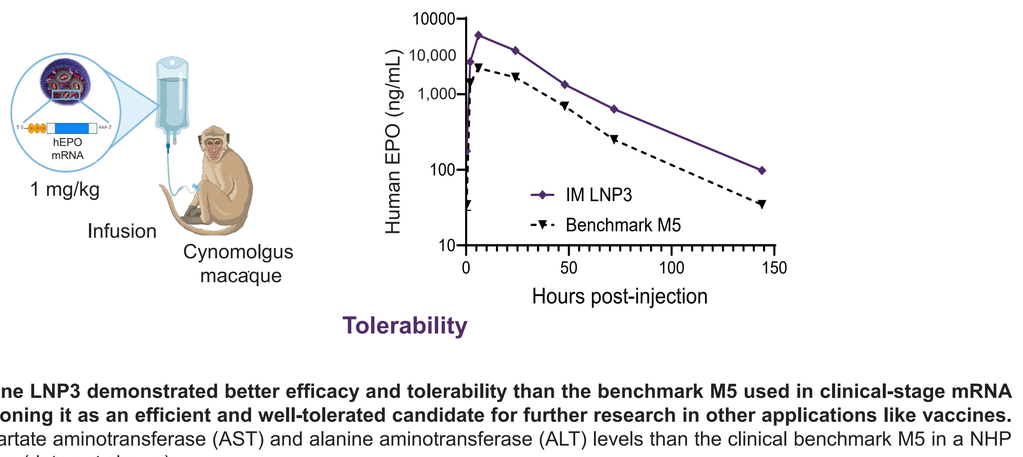



**Figure 3. Vivofectamine LNP3 demonstrated better efficacy and tolerability than the benchmark M5 used in clinical-stage mRNA liver therapies, positioning it as an efficient and well-tolerated candidate for further research in other applications like vaccines.** LNP3 led to lower aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels than the clinical benchmark M5 in a NHP model at a 1 mg/kg dose (data not shown).

## Cryopreserved mRNA-LNPs are stable.

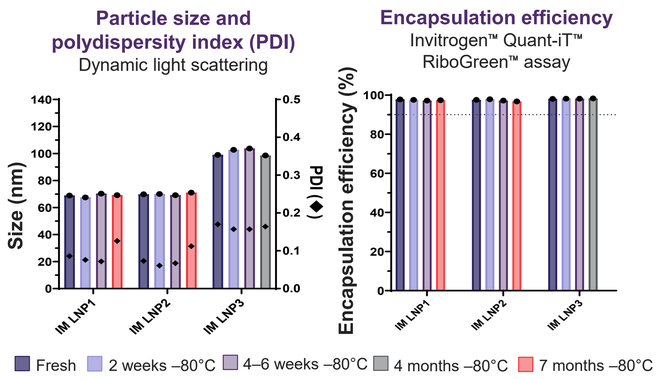



**Figure 4. Cryopreserved IM LNPs demonstrate consistent particle size, PDI, and encapsulation efficiency after 7 months of cryopreservation.**

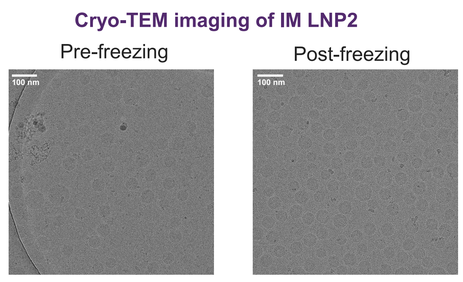



**Figure 5. mRNA-LNPs preserve particle morphology after cryopreservation.** For cryo-TEM characterization, LNPs were imaged on the Thermo Scientific™ Glacios™ 2 Cryo Transmission Electron Microscope (cryo-TEM) with the Thermo Scientific™ Falcon™ camera.

## Cancer vaccine proof of concept

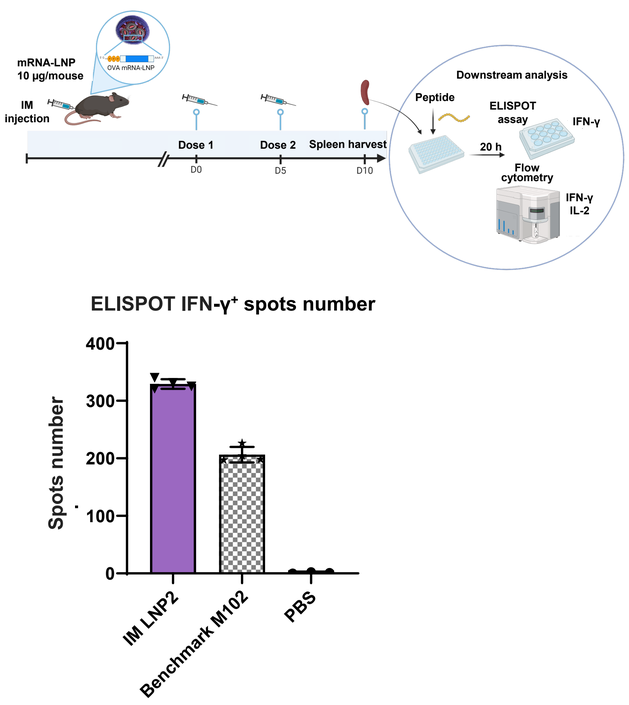



**Figure 6. Vivofectamine lipids demonstrate promising performance for cancer vaccine development.** 10 µg of mRNA encoding ovalbumin (OVA) encapsulated in LNPs was delivered via IM injection to C57BL/6 mice. Spleens were collected on day 10 after prime and boost injections, and isolated splenocytes were rechallenged by peptide. T cells secreting IFN-γ and IL-2 were quantified using ELISPOT and intracellular flow cytometry.

## Summary


- Vivofectamine LNPs induce similar levels of immunogenicity when compared to a clinical benchmark in mice.
- Vivofectamine LNPs induce robust humoral and T cell responses in research models in mice, making them suitable for development of prophylactic vaccines and cancer vaccines.
- Vivofectamine LNPs are well-tolerated in repeated dosing regimens in animal research models.
- LNP performance has been confirmed across multiple animal models, including mice, rats, and NHPs.

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