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Enabling *In Vivo* Gene Editing with Proprietary Lipid Nanoparticles (LNPs)

Rammohan Devulapally, Ph.D.
Director, Nonviral Delivery
Life Edit Therapeutics, Inc

Formulation & Delivery U.S. 2024 Conference
San Diego, October 16-17, 2024

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Outline

1. Life Edit Proprietary LNP1 Development

    - Process Development and Characterization
    - Biodistribution
    - Immunogenicity

2. LNP1 EPO Study in NHP

3. Proprietary Gene Editing Delivered by Life Edit LNPs

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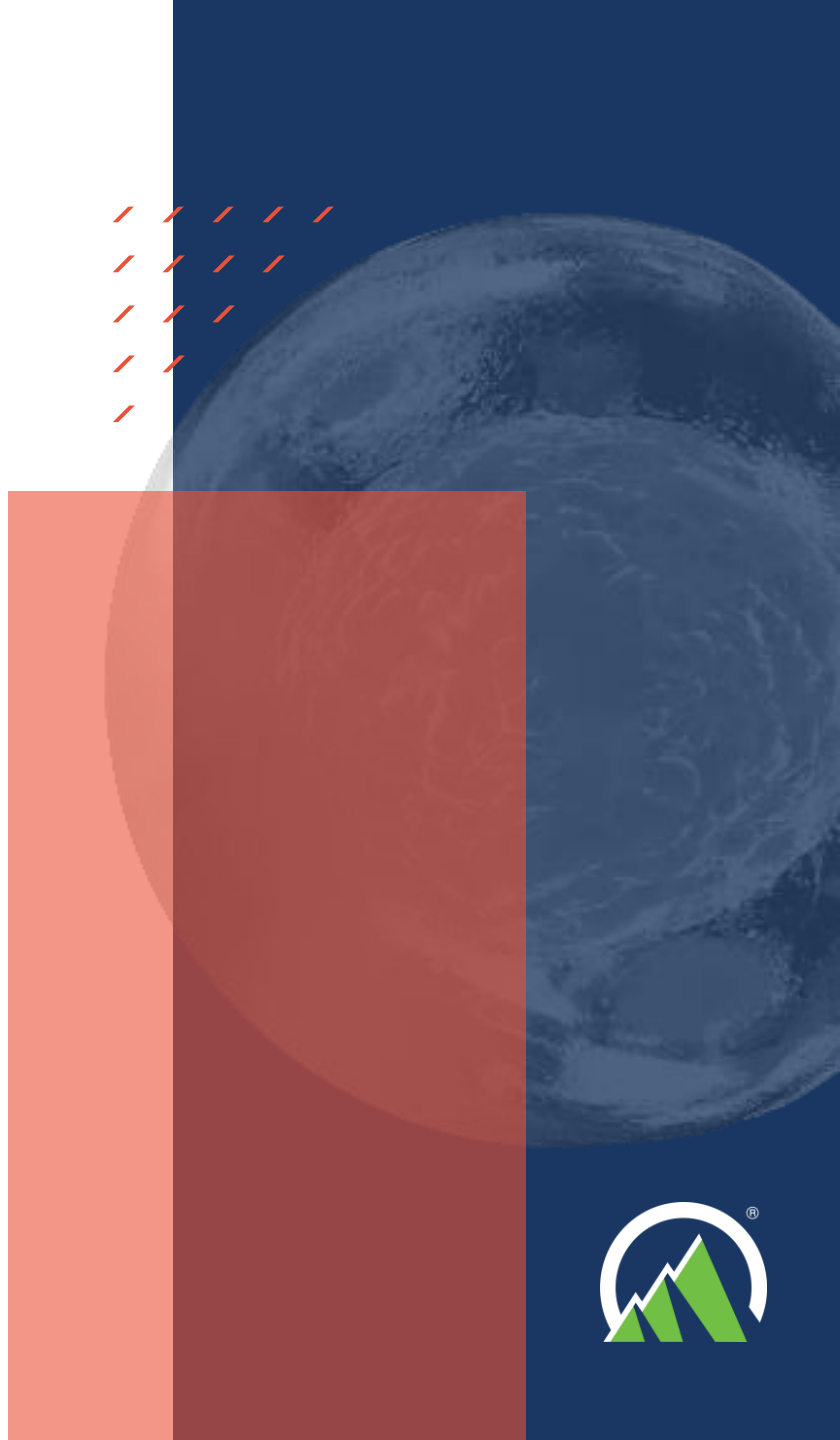
Proprietary LNP1 Development



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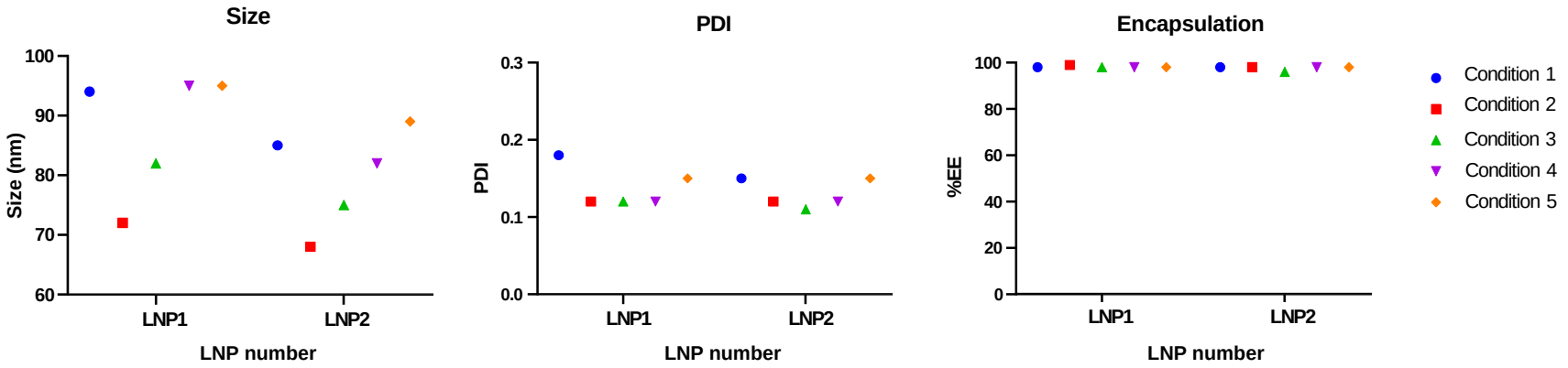
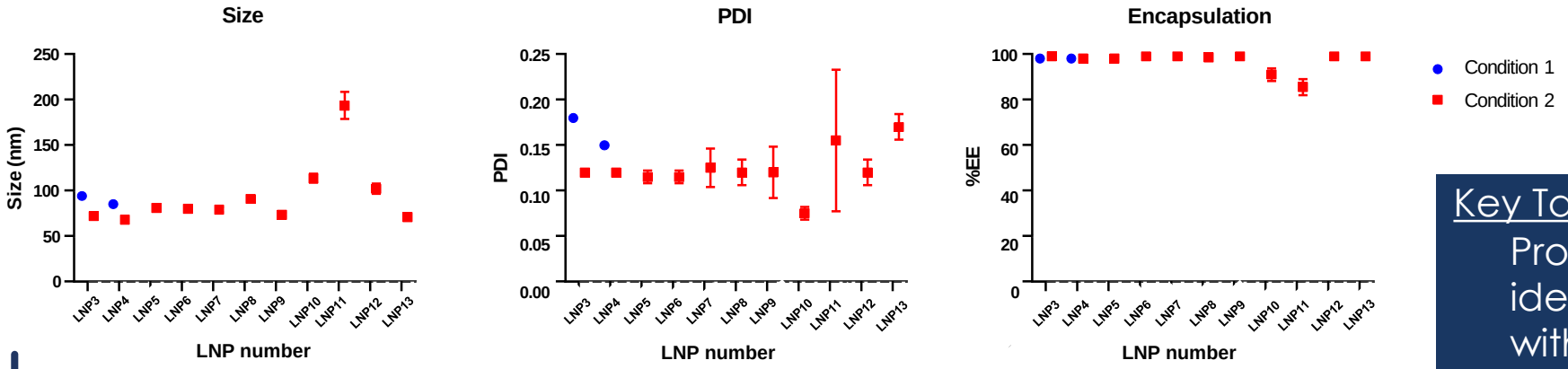
LNP optimization with Life Edit ionizable lipid libraries

Process optimization to achieve smaller LNP size



Optimization is reproducible across the LNP library



<u>Key Takeaway</u>

Process-optimized LNPs have ideal size and polydispersity with a high encapsulation efficiency of payload for systemic delivery

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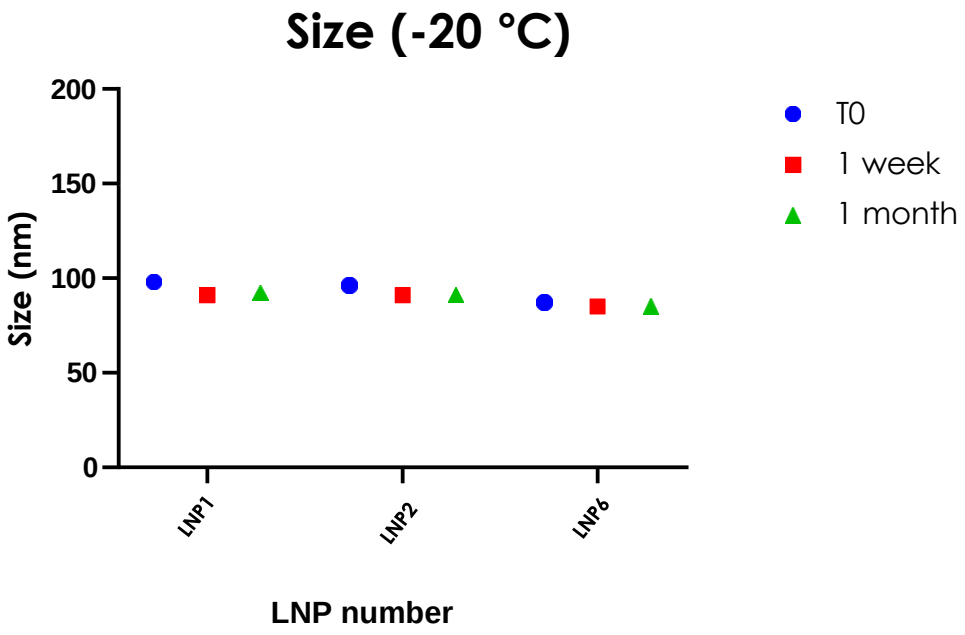
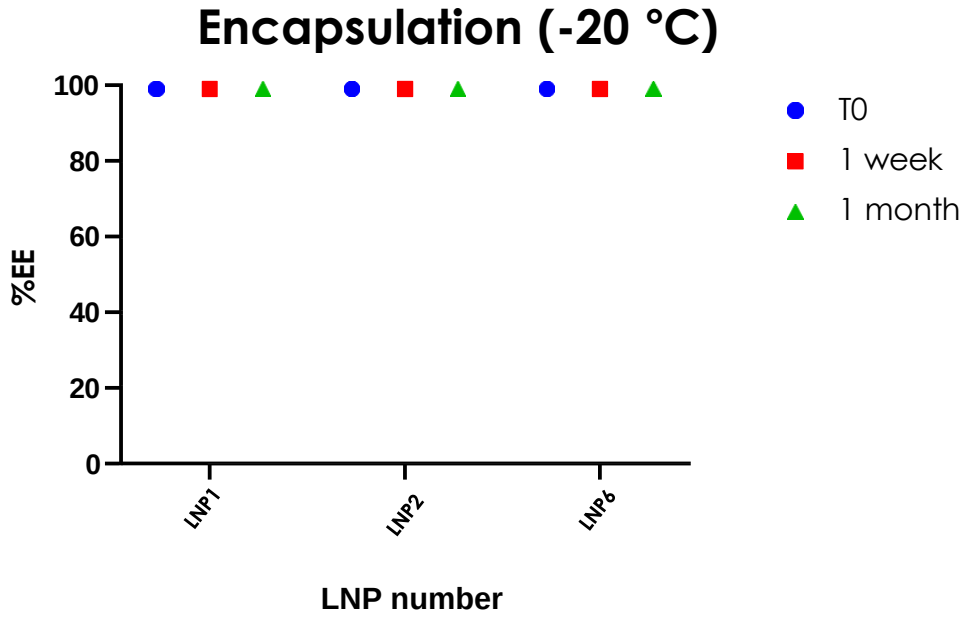
* PDI = polydispersity index, EE = encapsulation efficiency

Life Edit LNP stable at - 20 °C





Key Takeaway

Cryopreservation-optimized LNPs showed stability at -20°C with retention of size and encapsulation efficiency of the payload

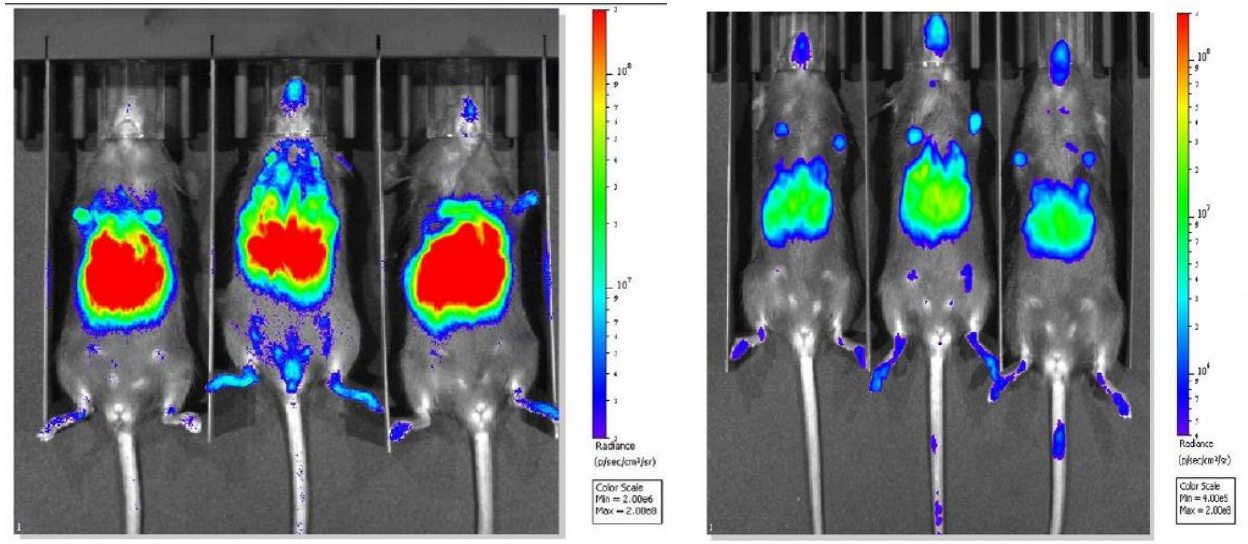
*LNPs cryopreserved using cryoprotectant

Life Edit LNP1 has a strong preference for delivery to the liver through LDLR

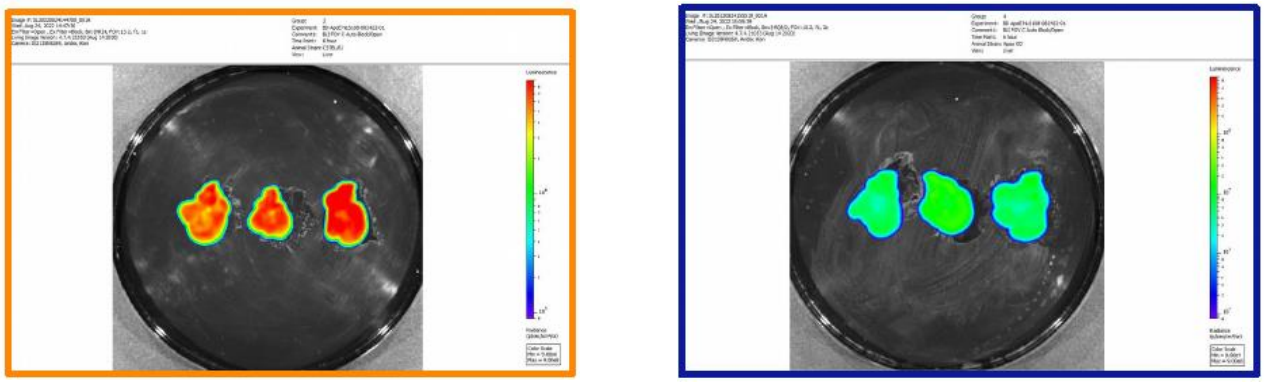
WT mice

ApoE-KO mice

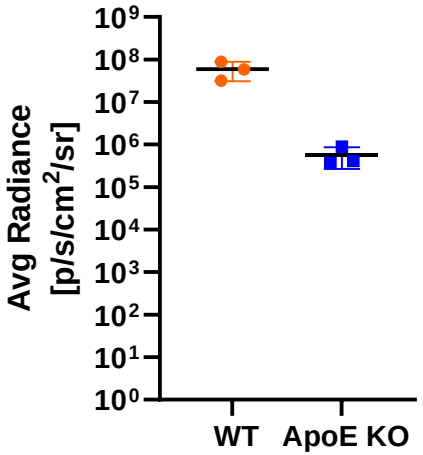
In vivo whole body



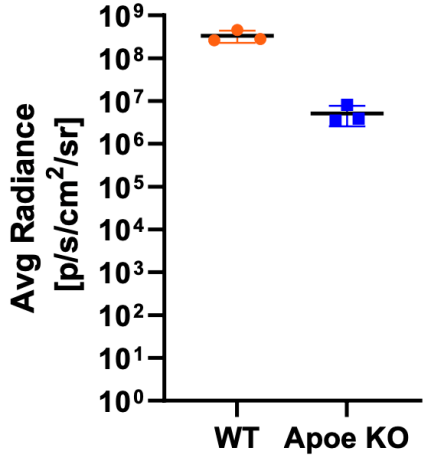
Ex vivo Liver



Whole Body Expression



***Ex vivo* Liver Expression**



Key Takeaways

- LNP targets hepatocytes by absorbing ApoE, which binds to LDLR expressed on hepatocytes
- Li7 does deliver RNA in the absence of ApoE, but delivery efficiency is decreased

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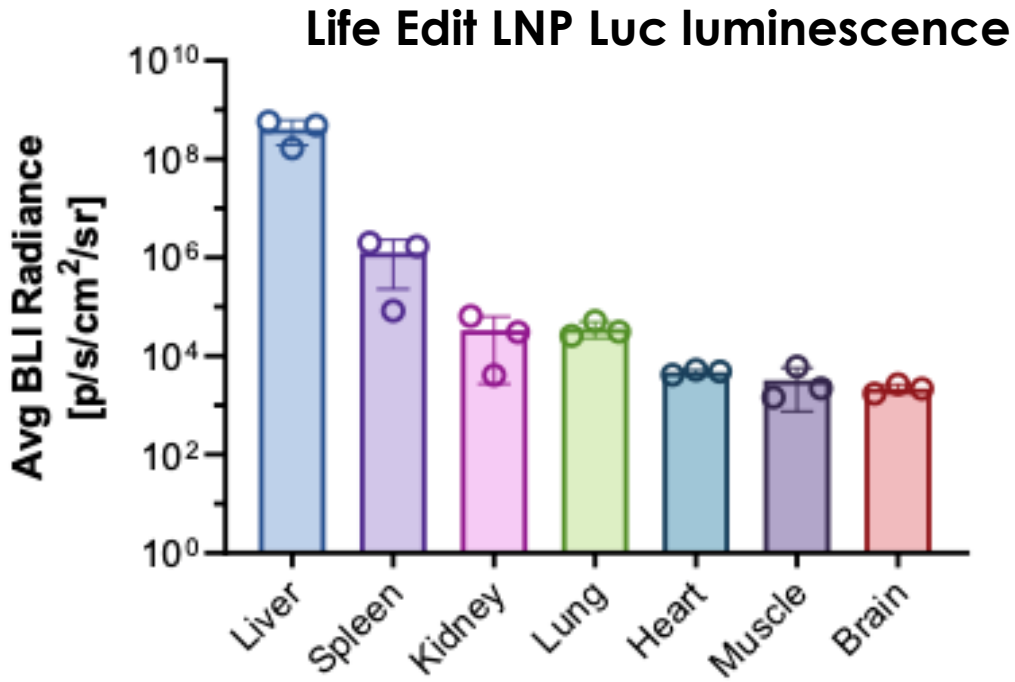
- Mouse strain: C57BL/6 and ApoE KO
- Dose: 1 mg/kg
- Life Edit LNP
- mRNA: fLuc + b-gal combination (1:1)
- Timepoint: 6 hours post IV administration

* Life Edit LNP1 contains Life Edit ionizable lipid and hydrolyzable PEG lipid.

Life Edit LNPs containing proprietary ionizable and PEG lipids drive preferential liver expression



Animal: Mouse
Delivery: Life Edit LNP1
Dose:1 mg/kg
Timepoint: 6 hours post-administration
Readout: Luciferase imaging (IVIS Lumina)

Key Takeaways

- LNP1 is distributed across different organs at 6 hours post-administration in mice
- LNP1 shows a strong preference for delivery to the liver

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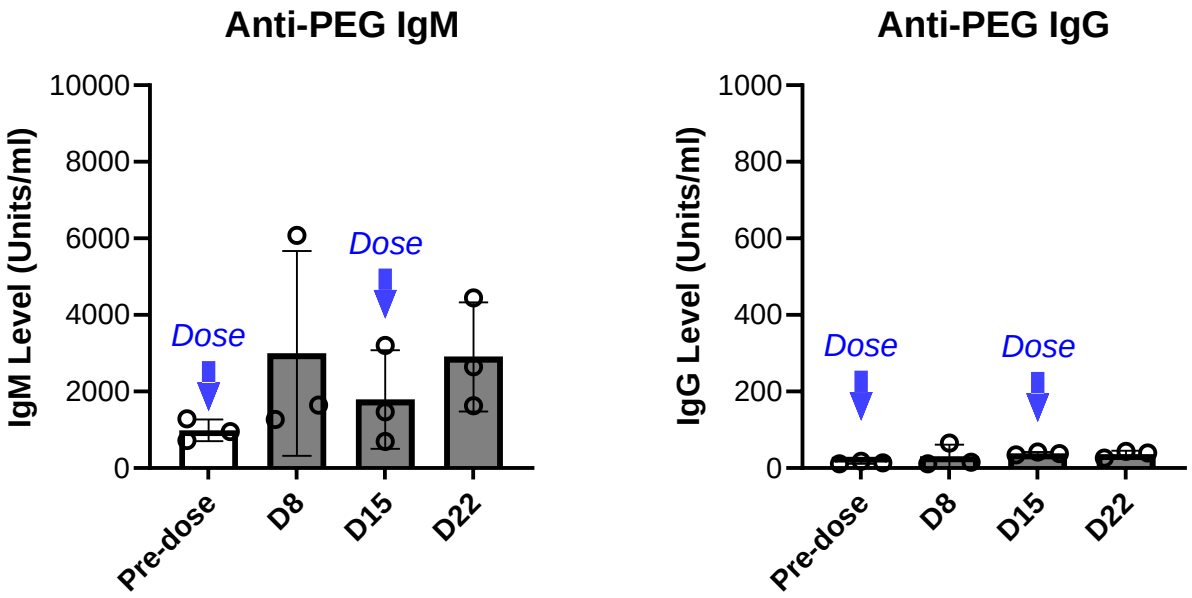
* Life Edit LNP1 contain Life edit ionizable lipid and hydrolyzable PEG lipid.

Novel PEG lipids display low immunogenicity with repeat dosing



Animal: Naïve female SD Rat
Delivery: Life Edit LNP1
Dose: 2 repeated IV injection, 1 mg/kg
mRNA: hEPO

Key Takeaway

Life Edit LNP1 containing hydrolyzable PEG lipid shows minor effects on anti-PEG antibodies

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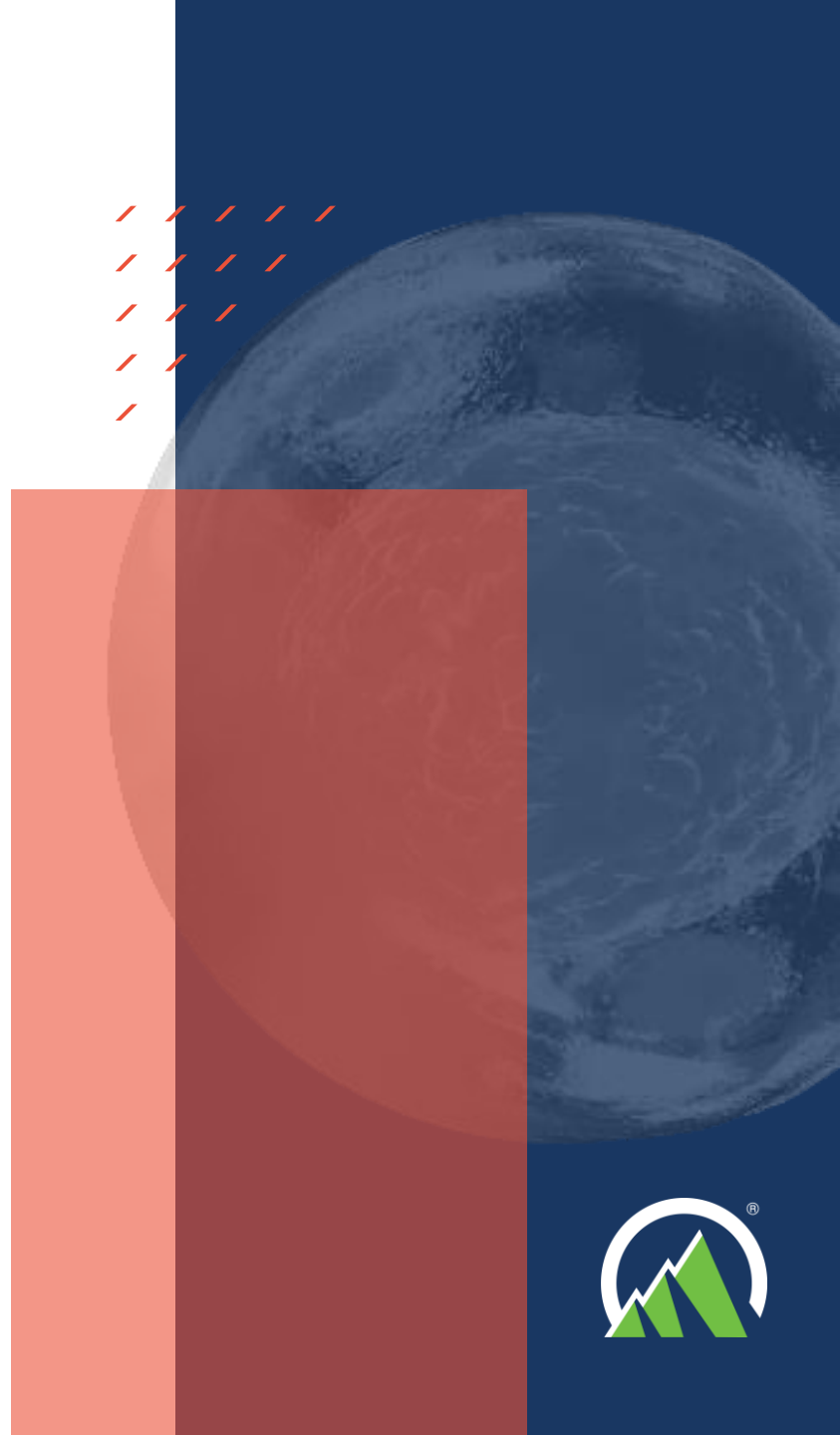
LNP1 EPO Study in NHP



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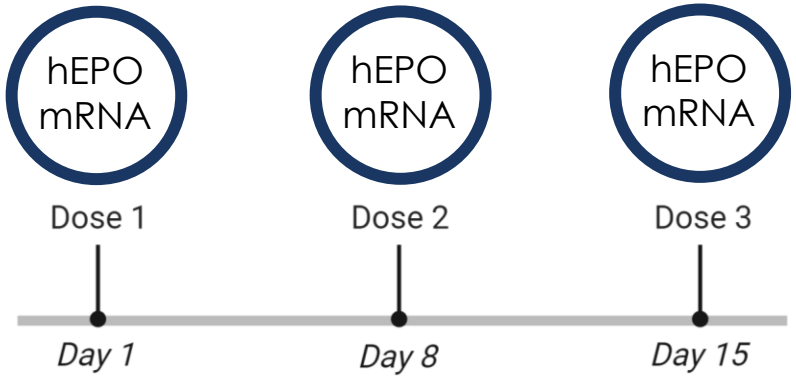
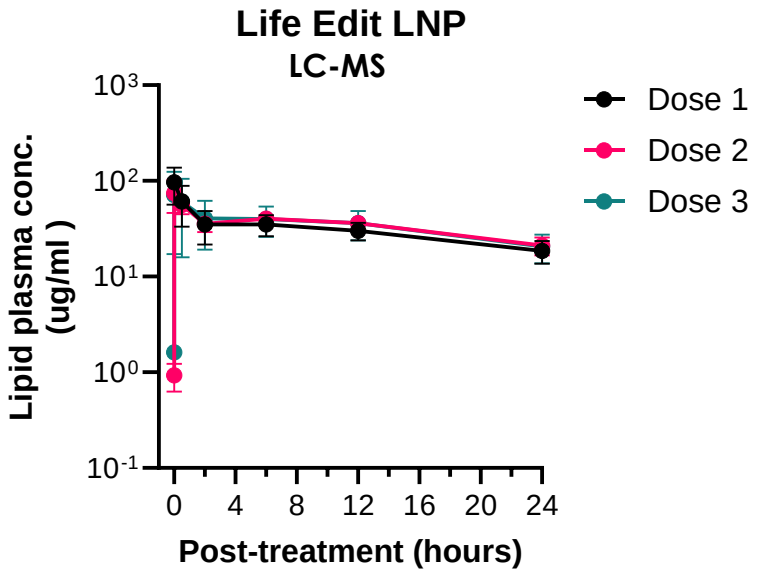
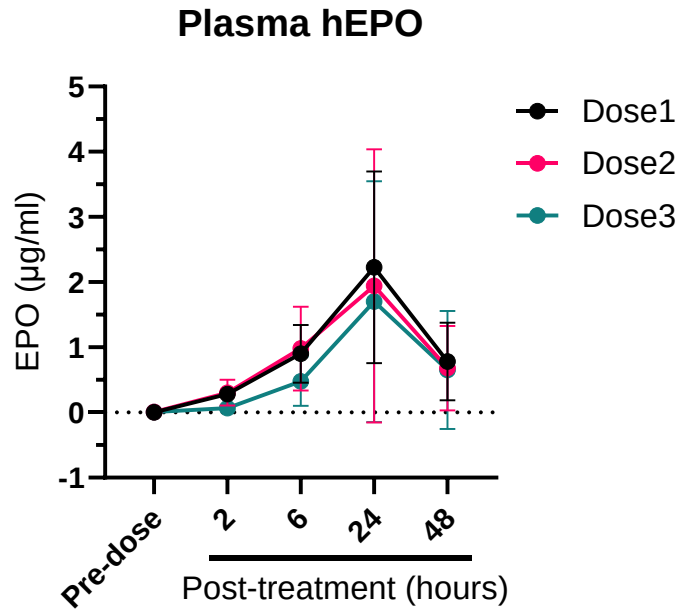
Life Edit LNP1: Sustained delivery of hEPO following Q3W in NHPs



Nonhuman primate
(n=3)
Repeated IV dose
at 0.3 mg/kg





Key Takeaways

- Comparable pharmacokinetic (PK) profiles with repeat dosing of LNP in NHP with similar exposure post each weekly dose
- Life Edit LNP1 expression reaches its peak shortly after injection and remains stable over 24 hours
- hEPO expression is increased gradually and reaches its peak at 24 hours post-treatment

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*No immunosuppression regimen was used in this study; ionizable lipid concentration by LC-MS

Life Edit LNP capabilities

- Versatile LNPs enable the delivery of small to very large RNA payloads
- Novel ionizable lipids have the potential to provide for a variety of tissue distributions
- Novel, cleavable PEG lipids provide tunable pharmacokinetics and potentially lower immunogenicity
- IV administrations are well tolerated with robust RNA expression

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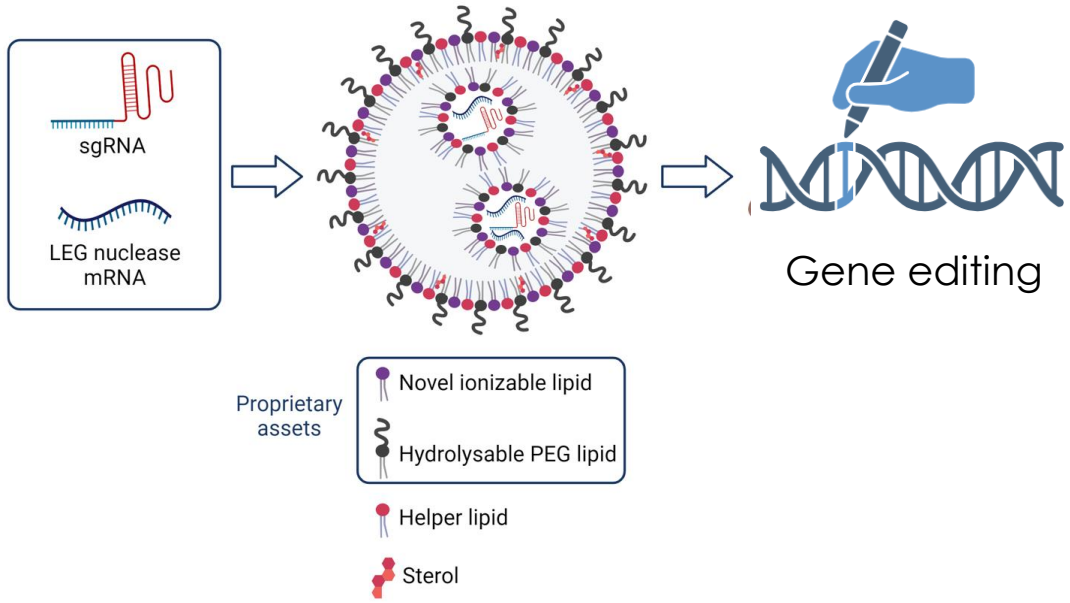
Proprietary Gene Editing Delivered by Life Edit LNPs



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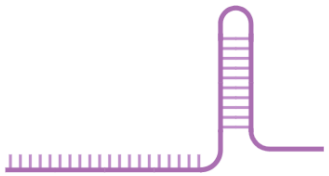
Optimization of LNP delivery for *in vivo* gene editing

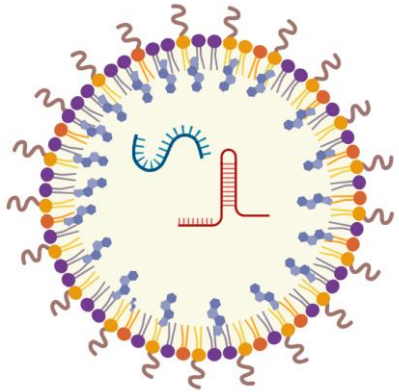
1. Guide RNA



2. mRNA

Efficient gene editing

3. LNP



All three components must work together to get efficient gene editing

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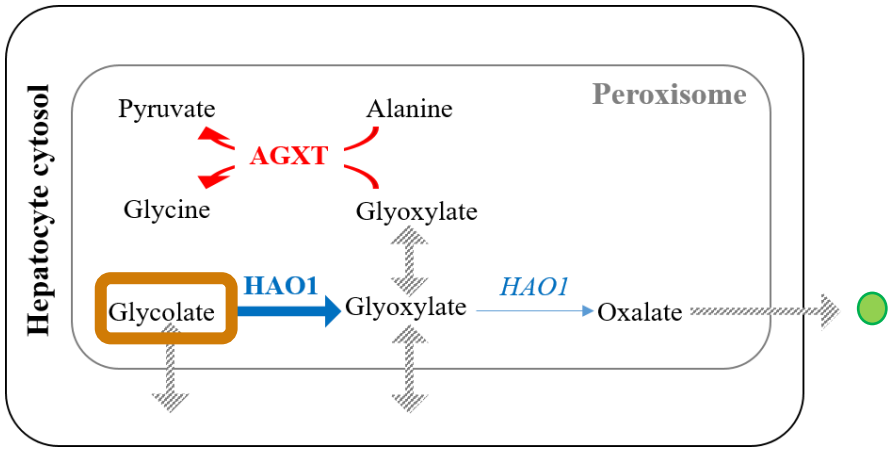
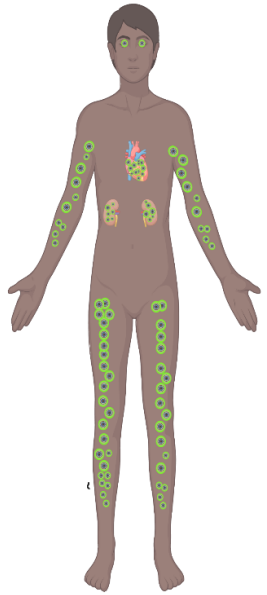
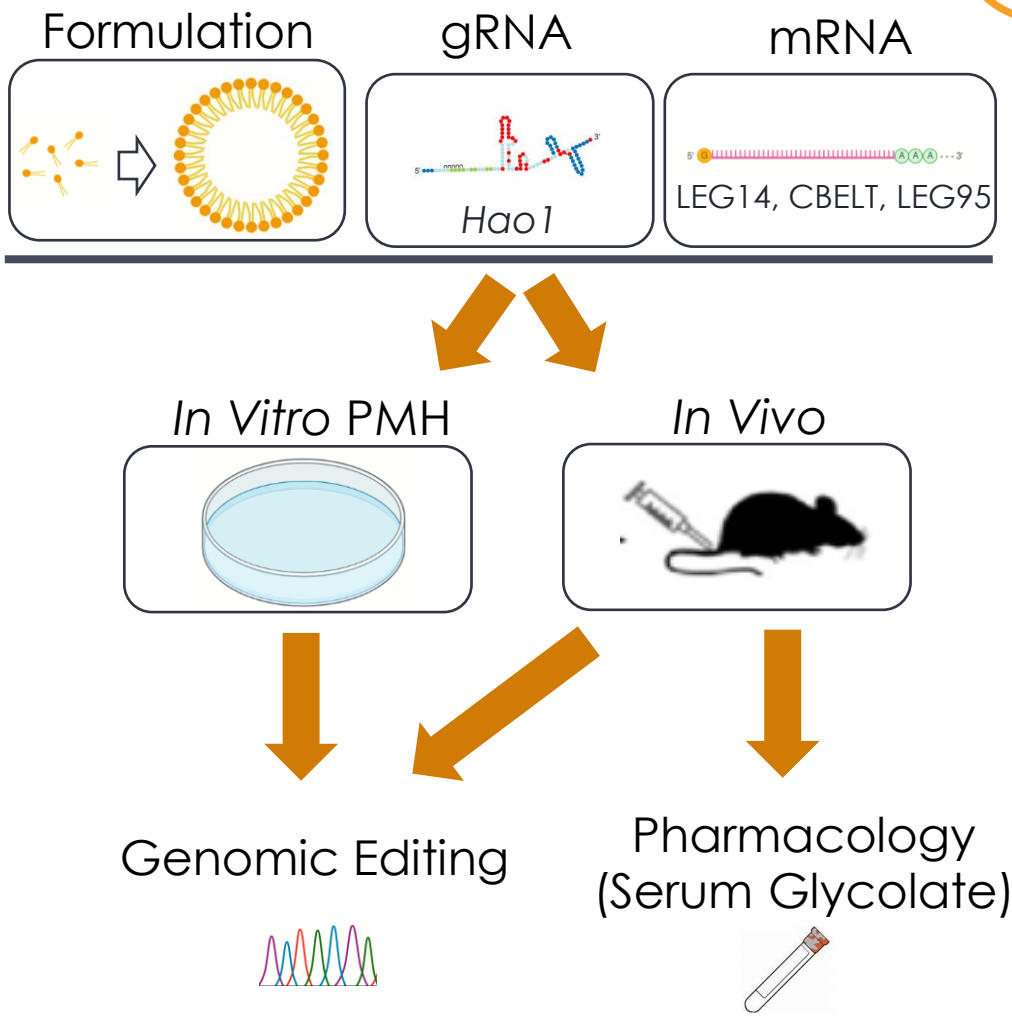
HAO1 as a genetic target for screening LNPs

Primary hyperoxaluria type 1 (PH1) is caused by loss-of-function mutations in the *AGXT* gene that lead to calcium oxalate deposits.

Targeting HAO1, an upstream enzyme, alleviates symptoms







- Nov 2020: FDA approved the first RNAi therapeutic Oxlumo (Lumasiran) for primary hyperoxaluria type 1 (PH1) developed by Alnylam

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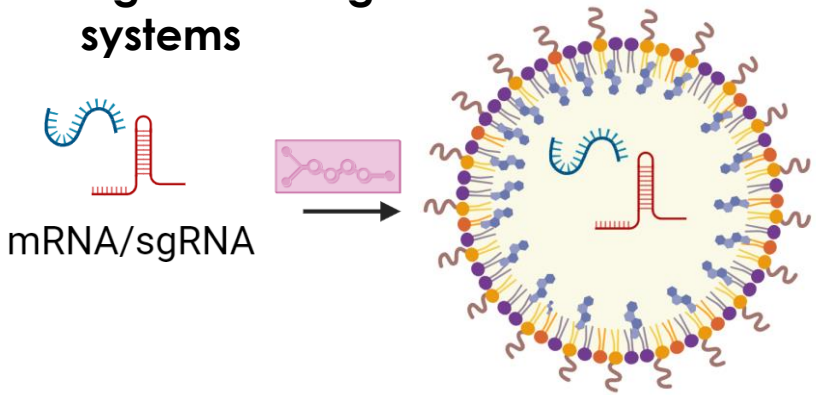
In vivo HAO-1 study flow-chart

Life Edit gene editing systems



Single IV dose*

C57BL/6 (n=4)

D1 — D8

LNP QC testing

1. *Size/PDI*
2. Zeta potential
3. Encapsulation efficiency
4. Endotoxin

Readouts

1. *Hao1 editing*
2. *Serum glycolate*
3. *Hao 1 protein*

* Murine model

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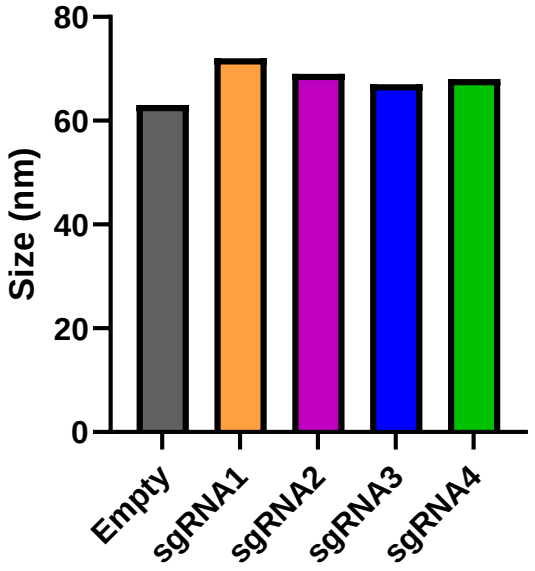
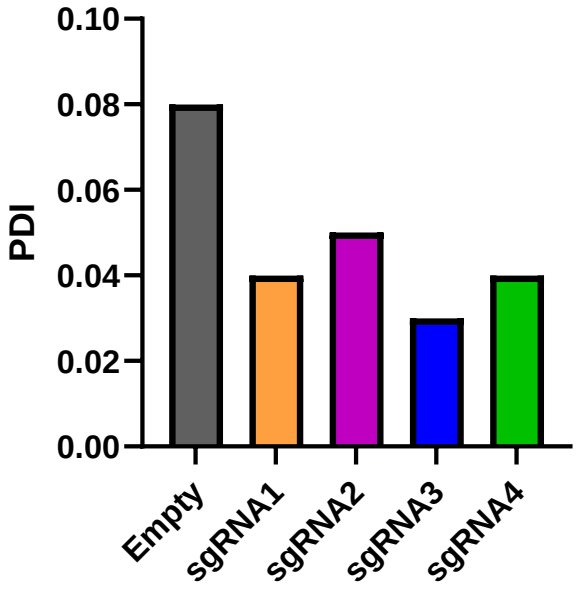
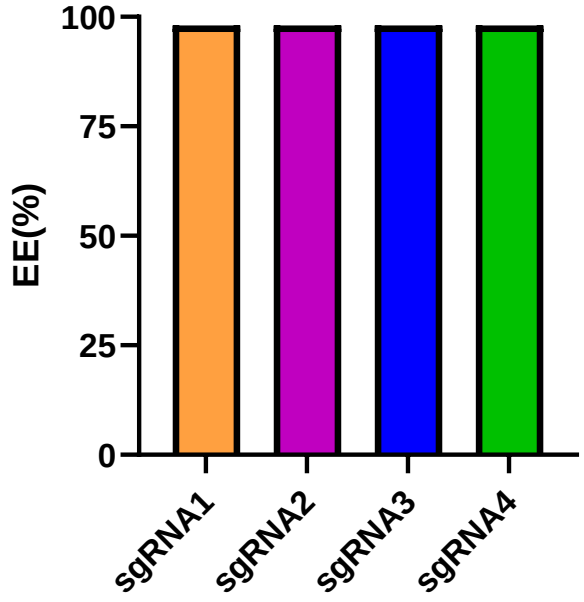
gRNA screening - characterization of LNPs

LNPs showed lower size, polydispersity (PDI), and higher encapsulation efficiency







Key Takeaways:

- Size: 60-75 nm, PDI<0.1, EE：98%
- All 4 sgRNAs show similar LNP physical characteristics

LNP source 1
gRNA 1 - 4
mRNA 1

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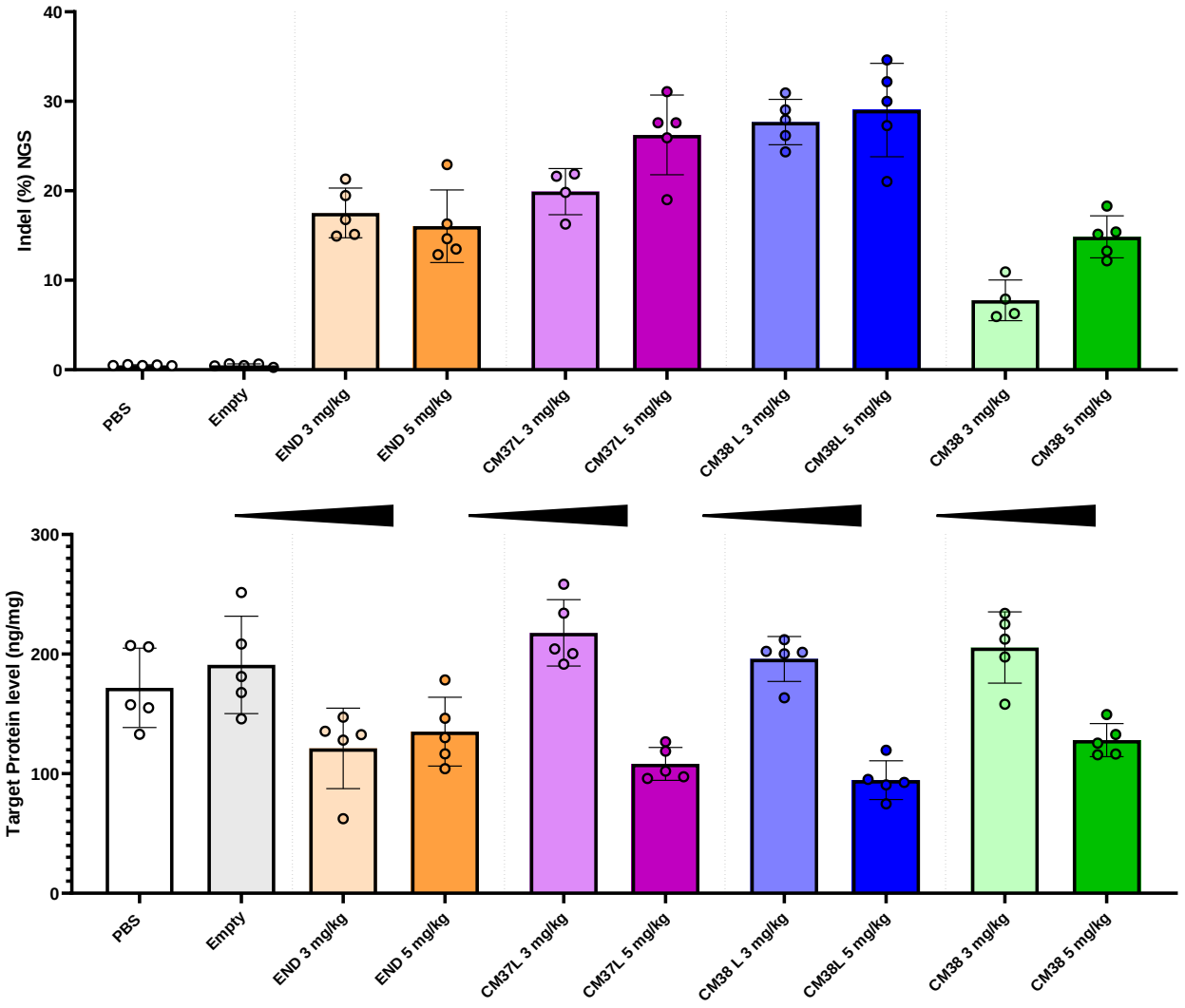
gRNA screening - *in vivo* editing

Genomic editing of *Hao1* and increase of protein level in female C57BL/6 mice following IV administration of LNPs loaded with Life Edit gene editing system using different gRNA modifications



Key Takeaways:

- Up to 28% editing from CM38L at 3 mg/kg
- **gRNA37L and CM38L outperform gRNA1 and 4**

LNP source 1, mRNA 1, gRNA 1 - 4
mRNA/gRNA ratio: 1:2
3 and 5 mg/kg dose

* Single dose *IV administration* in female C57BL/6 mice. All mice well tolerated the procedure with no overt changes in clinical observations

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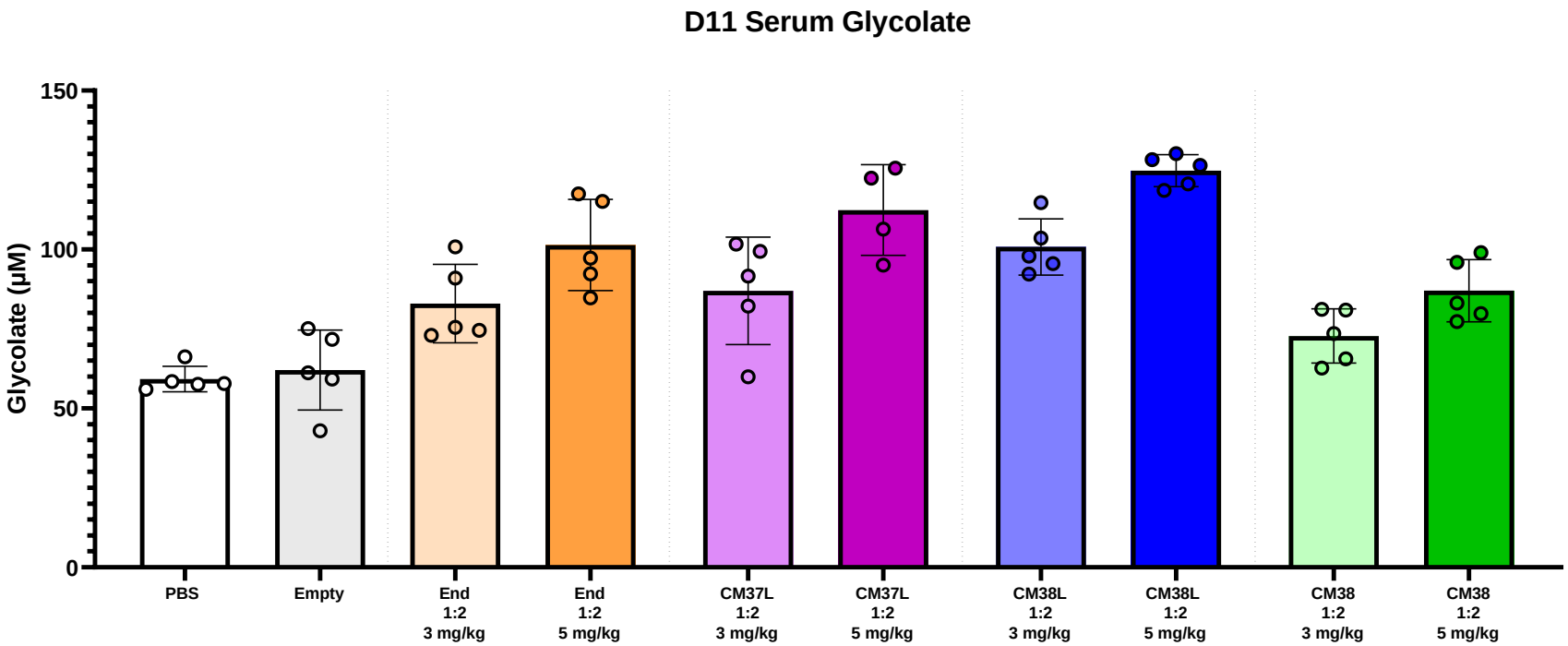
gRNA screening – *serum glycolate*

Increase of serum glycolate in female C57BL/6 mice following IV administration of LNP loaded with Life Edit gene editing system using different gRNA modifications



Key Takeaway

Glycolate increases 1.4-1.7x at 3 mg/kg and 1.7-2.1x at 5 mg/kg

LNP source 1, mRNA 1, gRNA 1 - 4
mRNA/gRNA ratio: 1:2
3 and 5 mg/kg dose

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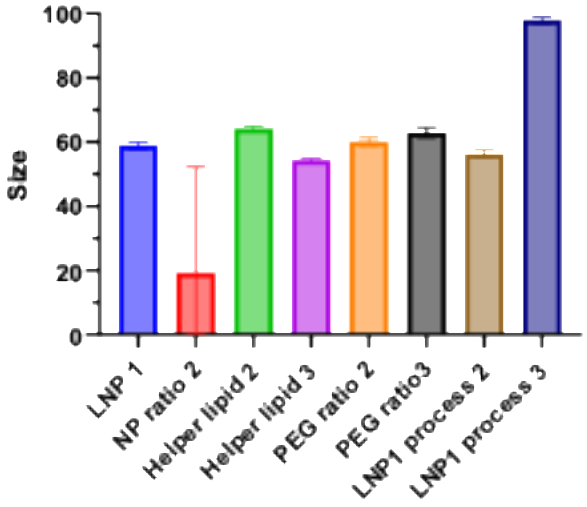
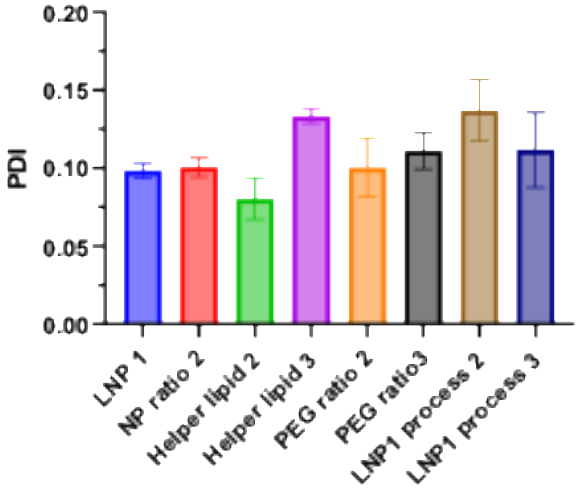
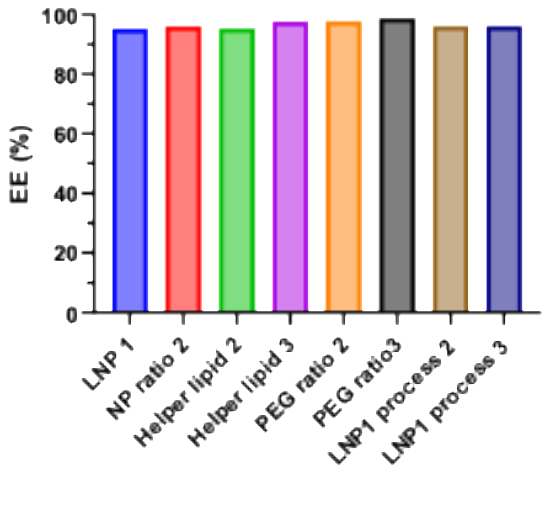
* Single dose *IV administration* in female C57BL/6 mice. All mice well tolerated the procedure with no overt changes in clinical observations

Hao1 editing with Life Edit LNPs

formulation/process screening for liver editing







Key Takeaways:

- 8 LNPs with different NP, helper lipids, PEG ratios and processes
- Size<100 nm, PDI<0.2, EE> 90%

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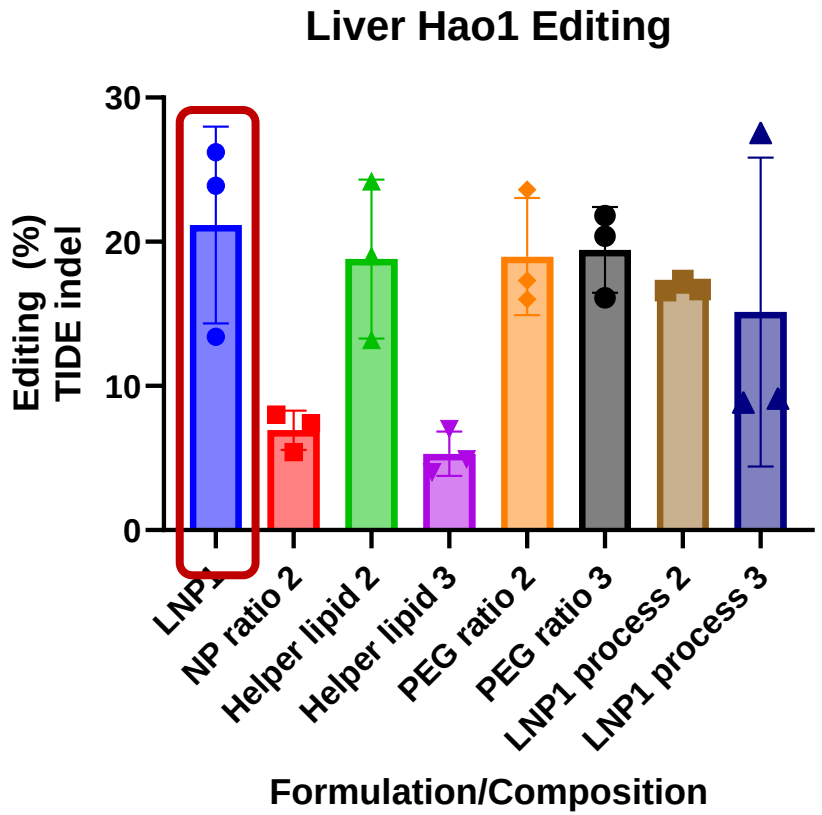
Hao1 editing with Life Edit LNPs

formulation/process screening for liver editing



Key Takeaways:

- Life Edit-LNP1 showed highest editing
- NP ratio and help lipids are critical for *in vivo* editing
- Process 1 is the optimal process

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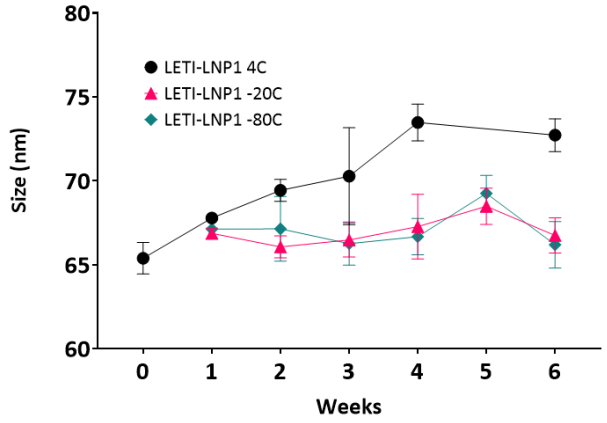
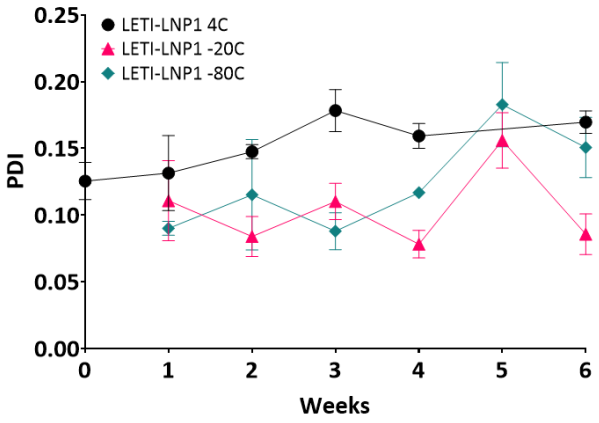
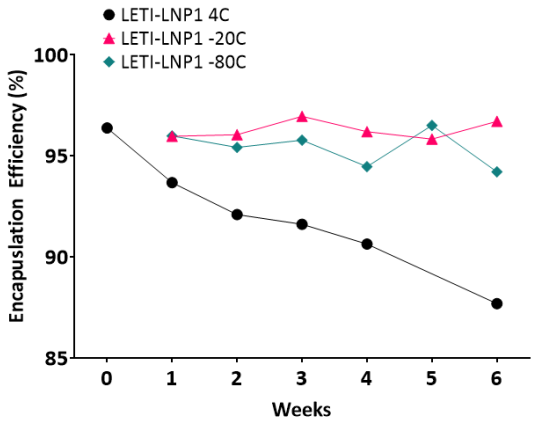
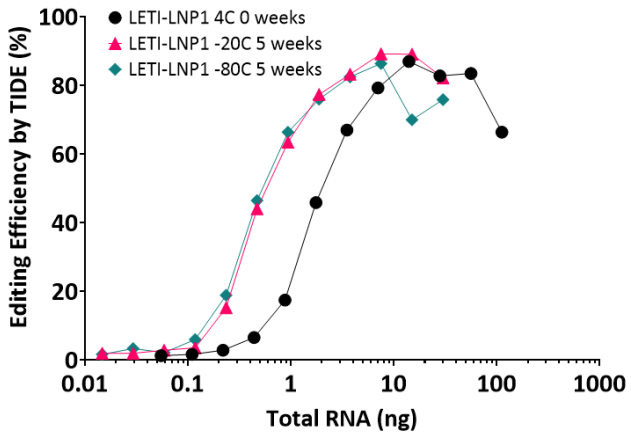
Development of Life Edit proprietary LNPs

freeze-thaw stability testing of lead candidate LNP1









Key Takeaways:

- LNP1 showed desirable LNP size and physiochemical properties
- High encapsulation efficiency
- Delivery efficiency remains upon storage at −20 ◦C and −80 ◦C

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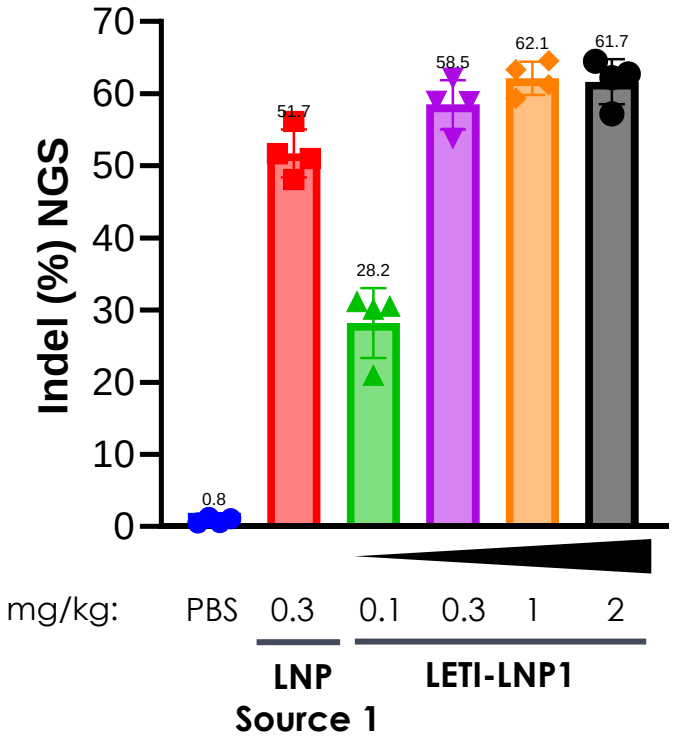
* Cryopreservatives added to LNPs stored at -20°C and -80°C

In vivo Hao-1 editing with Life Edit LNPs



Key Takeaways:

- Life Edit LNP1 outperformed LNP Source 1 at 0.3 mpk
- **Life Edit LNP1 achieved its maximum editing at 0.3 mpk**
- 28.2% editing at 0.1 mpk

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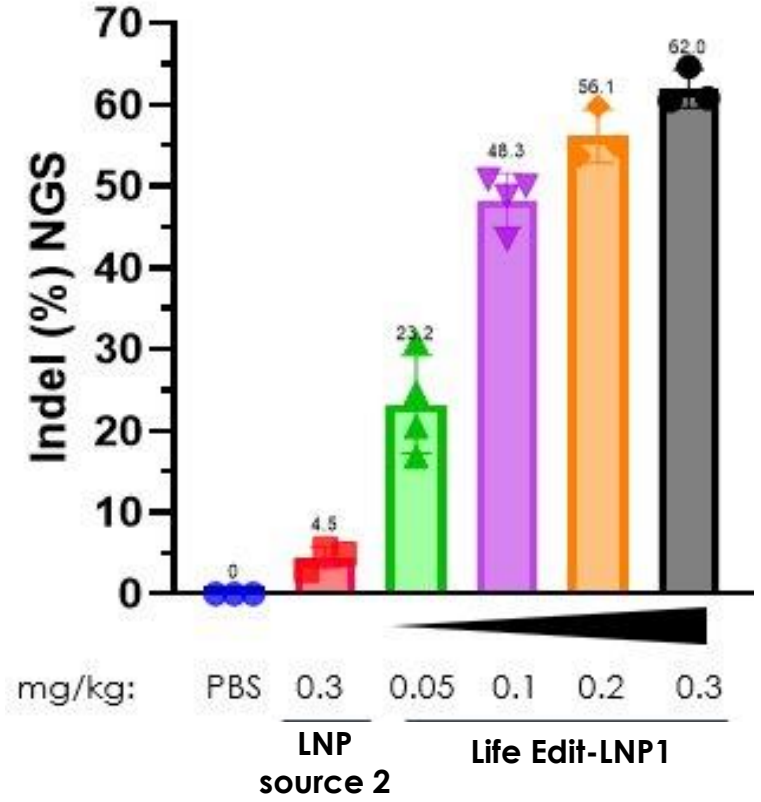
* Single dose *IV administration* in female C57BL/6 mice. All mice well tolerated the procedure with no overt changes in clinical observations

In vivo Hao-1 editing with Life Edit LNPs



Key Takeaways:

- Robust replication of editing of 0.3 mpk dose (57.7%)
- 20.3% editing from lowest dose tested (0.05 mpk)
- Steep inflection from 0.05 to 0.1 mpk doses

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* Single dose *IV administration* in female C57BL/6 mice. All mice well tolerated the procedure with no overt changes in clinical observations

Summary

Life Edit LNPs:

- Delivered Life Edit editing system leading to high *in vivo* editing at low dose
- Can deliver a variety of RNAs *in vivo*
- has ideal physiochemical characteristics, stable at −20oC
- Hydrolyzable PEG-lipid showed lower immunogenicity
- Showed tunable pharmacokinetics and potentially lower immunogenicity
- Suitable for repeat dosing

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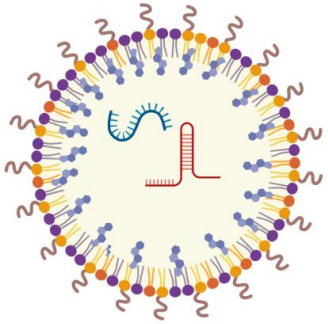
Acknowledgments



- **Preclinical Development**
  - Xiuying Li
  - Daniel Lee
  - Tyler McCullough
  - Nikki Frazier
  - Amber Valentino
  - Ritika Jaini
  - Sally Kim
  - Kathryn Woodburn
- **Technology Development, RNA**
  - David Wiley
  - Meng sun
  - Hui-Chia Yu-Kemp
  - Tim Schwochert
  - Jason Gibson
  - Josh Seo
- **Business Development**
  - Clare Murray
  - Jane Gajsiewicz
- **Gene Editing**
  - Clayton Morrison
  - Shahin Sendi
  - Phil Borden
- **IP team**
  - Aaron Nudelman
  - Anne Fleckenstein

We are actively hiring across all teams

https://www.lifeeditinc.com/careers#career-openings

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Thank You!

Questions?