Optimizing Lipid Nanoparticles for Clinical Applications of mRNA Therapeutics

Sean Semple
Vice President, Preclinical Research
Acuitas Therapeutics, Inc.

LNP Technology for mRNA Therapeutics

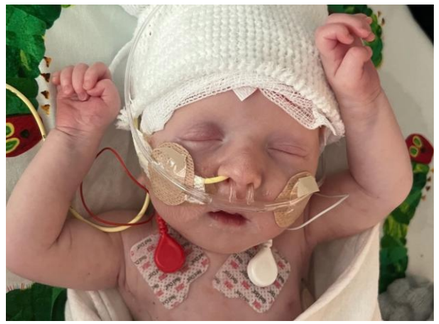
Clinically Validated World Firsts

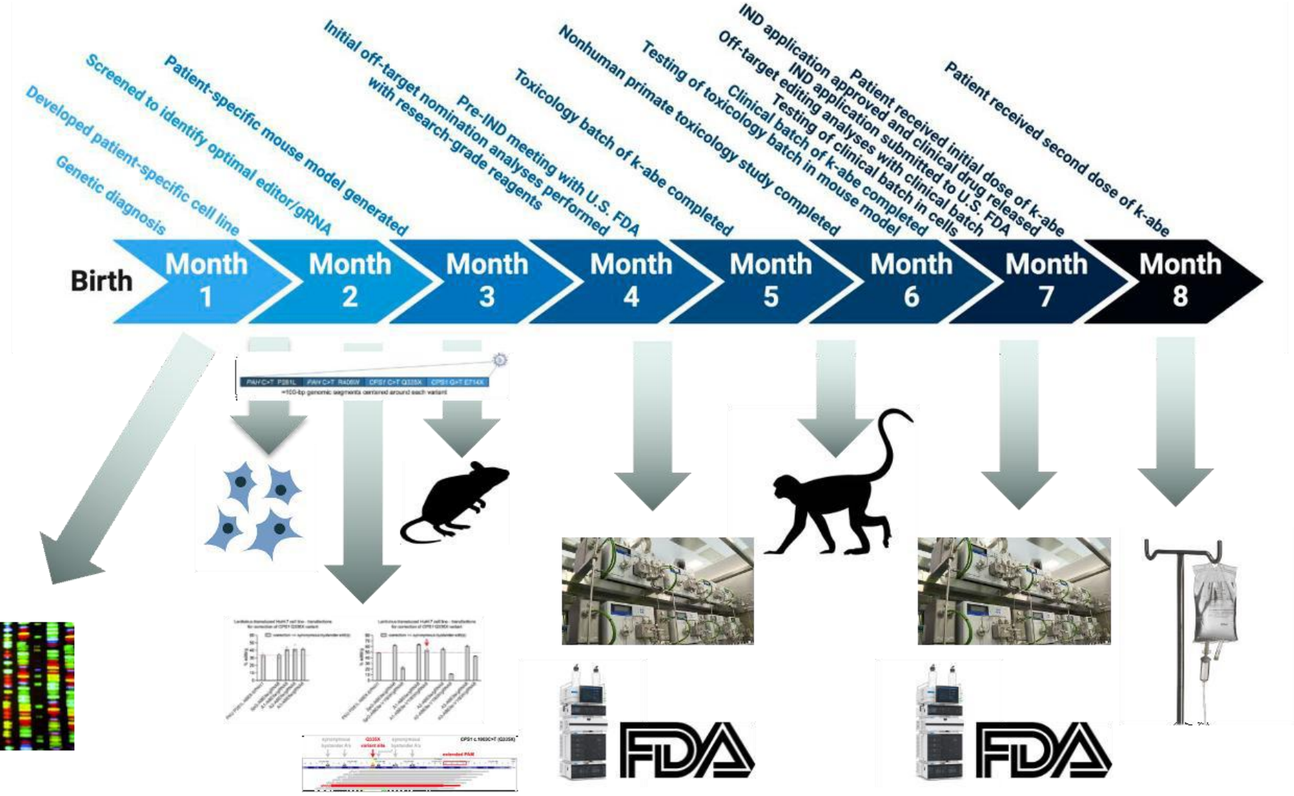
- Acuitas LNP formulation used in **ONPATTRO®** (Alnylam partnership)
  - First Approved RNAi product (2018)
  - Approved in Canada, US, EU, Japan & elsewhere

- Acuitas LNP formulation used in **Comirnaty®** (BioNTech/Pfizer partnership)
  - Emergency authorization in Canada, US, EU, UK and elsewhere (2020)
  - First approved mRNA therapeutic (2021)

LNP Technology for mRNA Therapeutics

Clinically Validated World Firsts



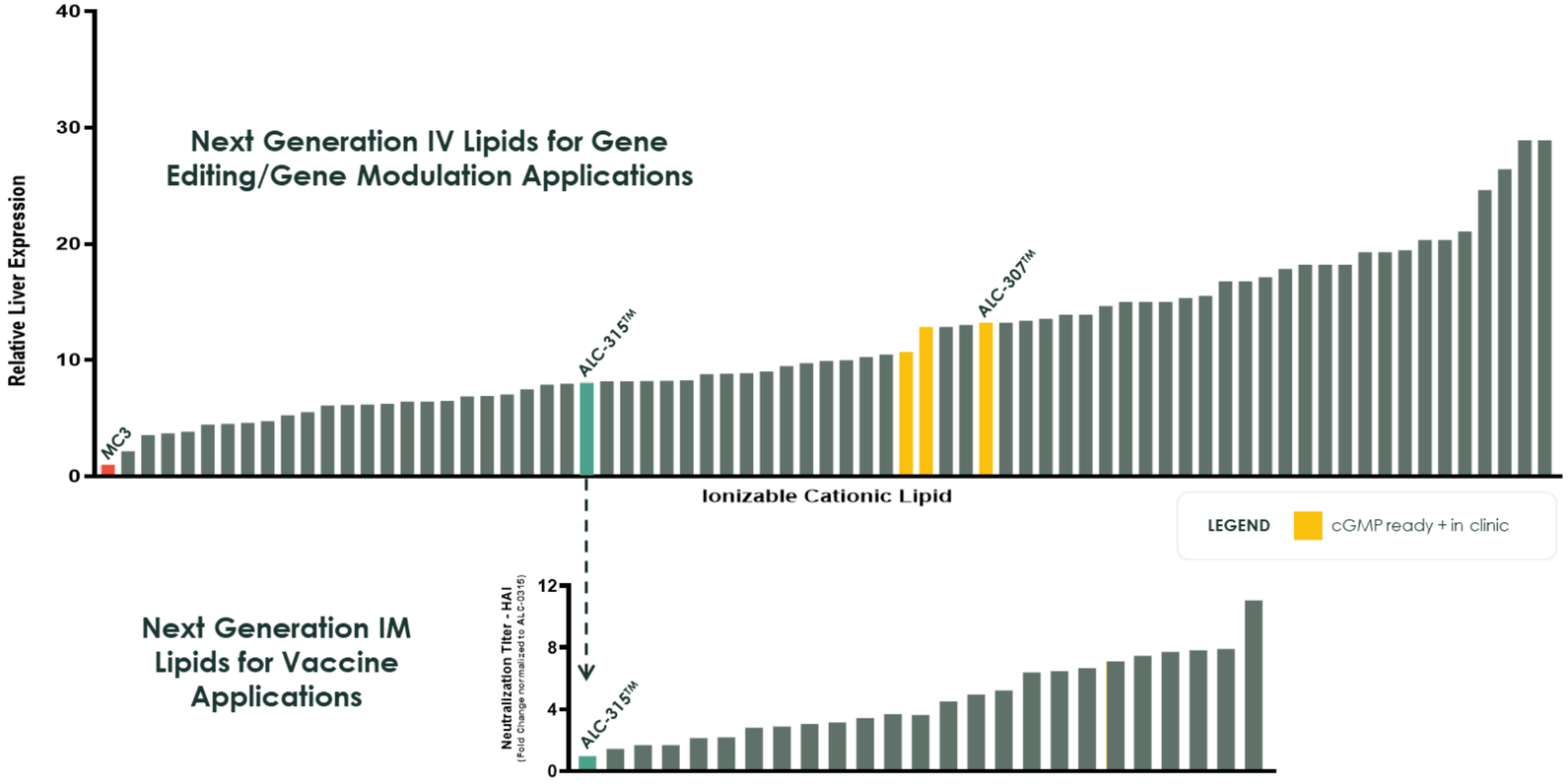


- Acuitas LNP formulation used in first personalized CRISPR therapy (2025)

Musunuru et al., 2025, NEJM
Photos from: https://www.chop.edu/centers-programs/genetherapy4inheritedmetabolicdisorders/future-personalized-medicine-here-kjs

LNP Technology for mRNA Therapeutics

Our Approach to Innovation

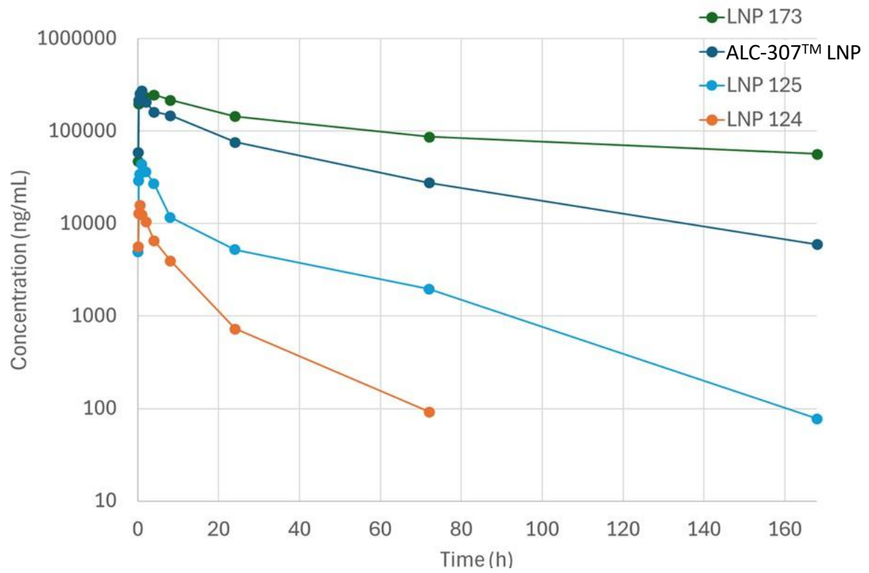


- Screening program combined with structure-activity relationship (SAR) analysis results in improved potency
- Enhanced potency and focus on therapeutic index enables partner programs across a broad range of applications

Optimizing Ionizable Lipids to Improve Therapeutic Index

Reducing Liver Exposure Improves Tolerability

Liver PK – 1 mg/kg mRNA-LNP



		Liver PK	Tolerability	Activity
		C_{max} (µg/mL)	ALT (U/L)	IgG Fold Change vs. Benchmark
		1.0 mg/kg	5.0 mg/kg	0.3 mg/kg
	PBS	---	19	---
α	**LNP 173**	248	32253	0.9
β	**ALC-307™ LNP**	273	691	1.0
γ	**LNP 125**	44	72	0.5
linear	**LNP 124**	16	36	0.5

- Directed adjustment of metabolic stability of ionizable lipid controls liver exposure and lowers ALT/AST in mice

Optimizing Ionizable Lipids to Improve Therapeutic Index

Reducing Liver Exposure and Preserving Potency in Mice

	Liver PK			Tolerability	Activity
	$t_{1/2}$ (h)	C_{max} (µg/mL)	AUC_{last} (h*µg/mL)	ALT (U/L)	IgG Fold Change vs. Benchmark
	1.0 mg/kg			5.0 mg/kg	0.3 mg/kg
ALC-307™ LNP	39.8	273	7369	691	1.0
LNP 141	27.1	183	2213	157	2.1
LNP 142	39.6	129	2042	151	0.8
LNP 140	44.1	124	2000	200	0.9
LNP 146	3.2	85	371	99	1.4
LNP 144	21.3	83	1636	96	1.2
LNP 145	23.9	70	1494	70	1.0
LNP 157	3.5	29	160	207	1.2

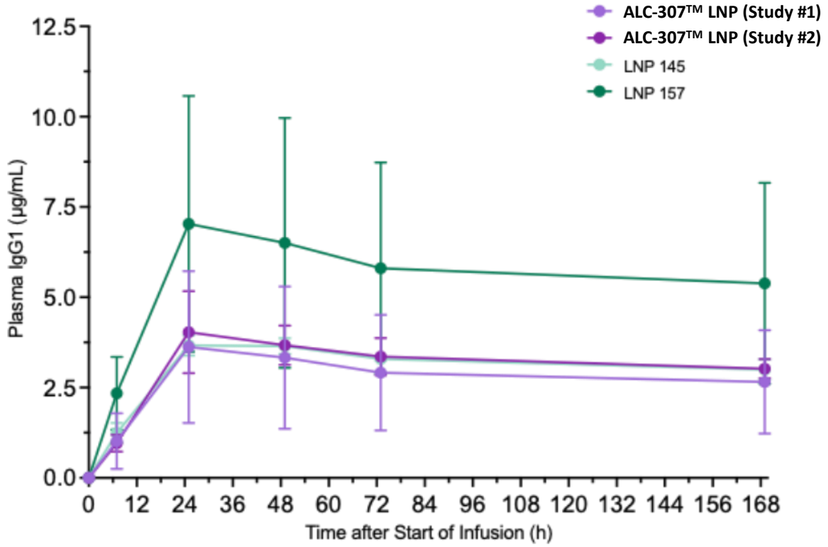
Evaluated for potency and tolerability in monkeys

- Further optimization of low ALT/AST lipid structures maintains low liver exposure and recovers activity in mice

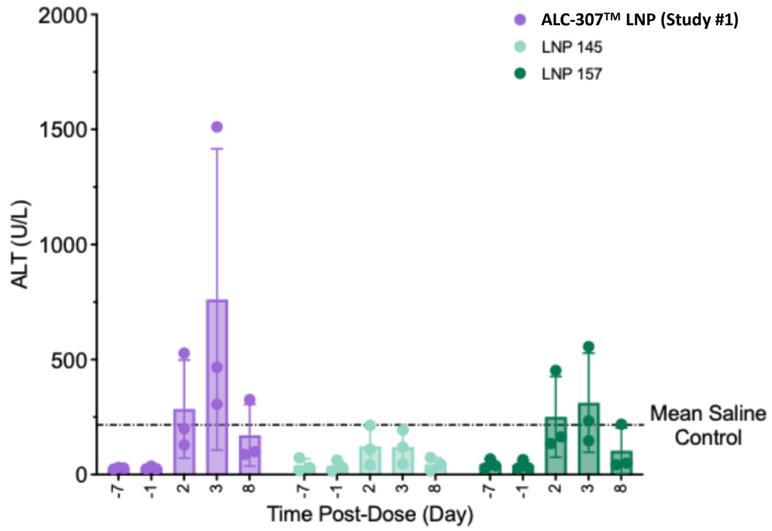
Optimizing Ionizable Lipids to Improve Therapeutic Index

Improving Liver Tolerability and Preserving Potency in Monkeys

Activity (IgG) – 0.3 mg/kg



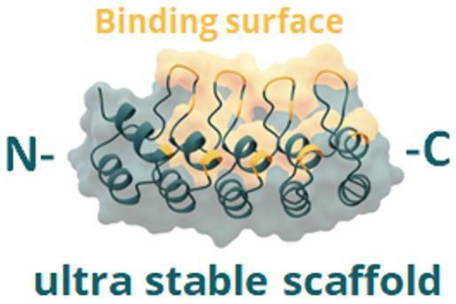
Tolerability (ALT) – 1.5 mg/kg

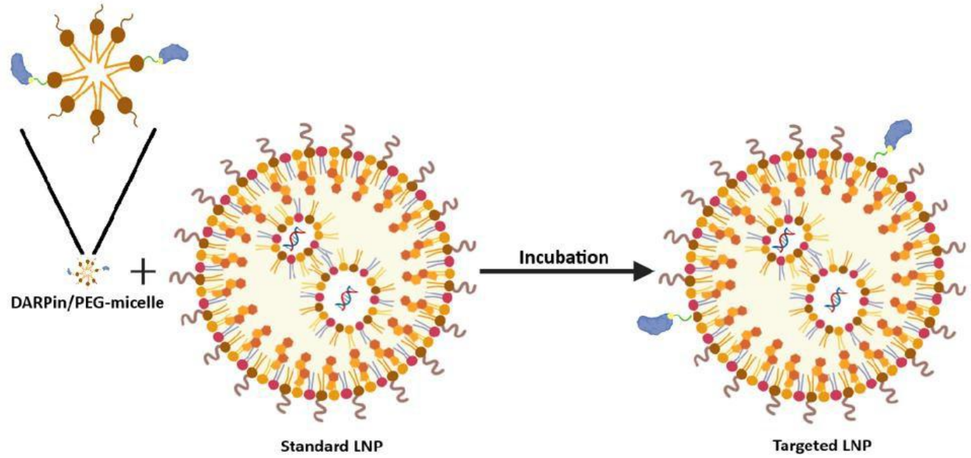


- Enhanced biodegradable (eBD) lipids identified with retained/increased potency, improved liver tolerability and an overall improved therapeutic index vs clinical benchmark

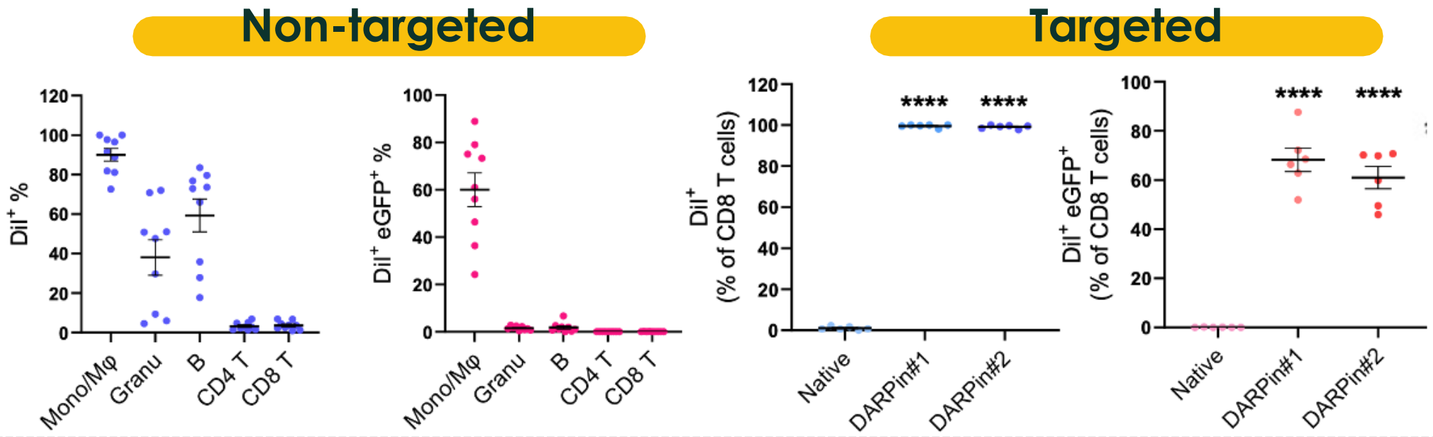
Delivery to T-lymphocytes with Targeted LNP

- Designed ankyrin repeat proteins (DARPins) for *in vivo* targeting of mRNA-LNP to T-lymphocytes





- Target cell binding and reporter gene expression for non-targeted and targeted LNP

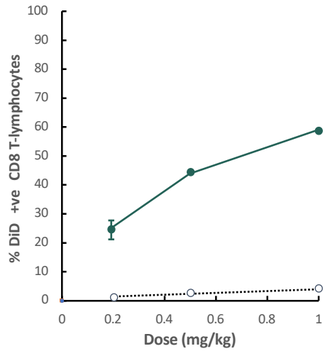


Delivery to T-lymphocytes with Targeted LNP

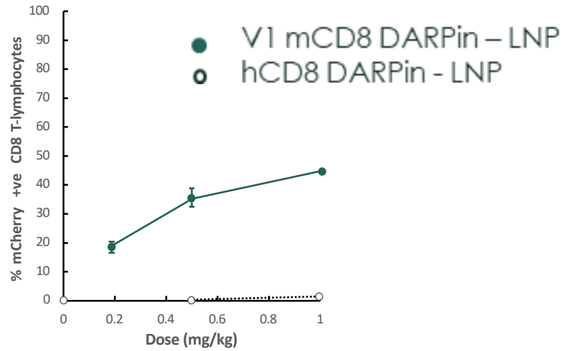
- Dose dependent, target-specific binding/uptake and transgene expression of CD8 DARPin-targeted mRNA-LNP

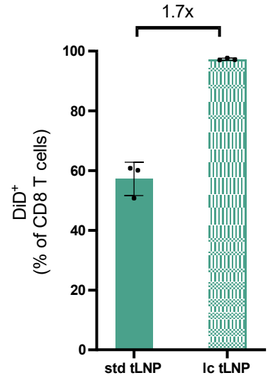
- Increased binding/uptake and expression of optimized long circulating (LC) LNP
- LC LNP expression in liver is ~10× lower vs. standard LNP

LNP Binding/Uptake

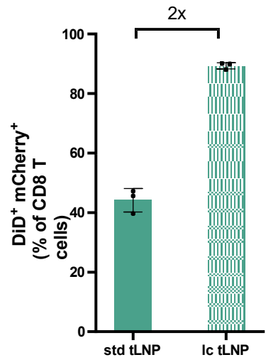


Reporter Expression

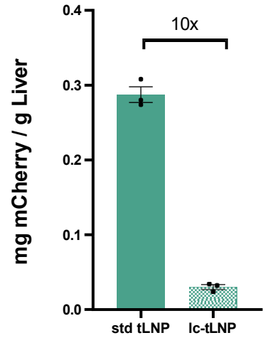




CD8+ T-cells

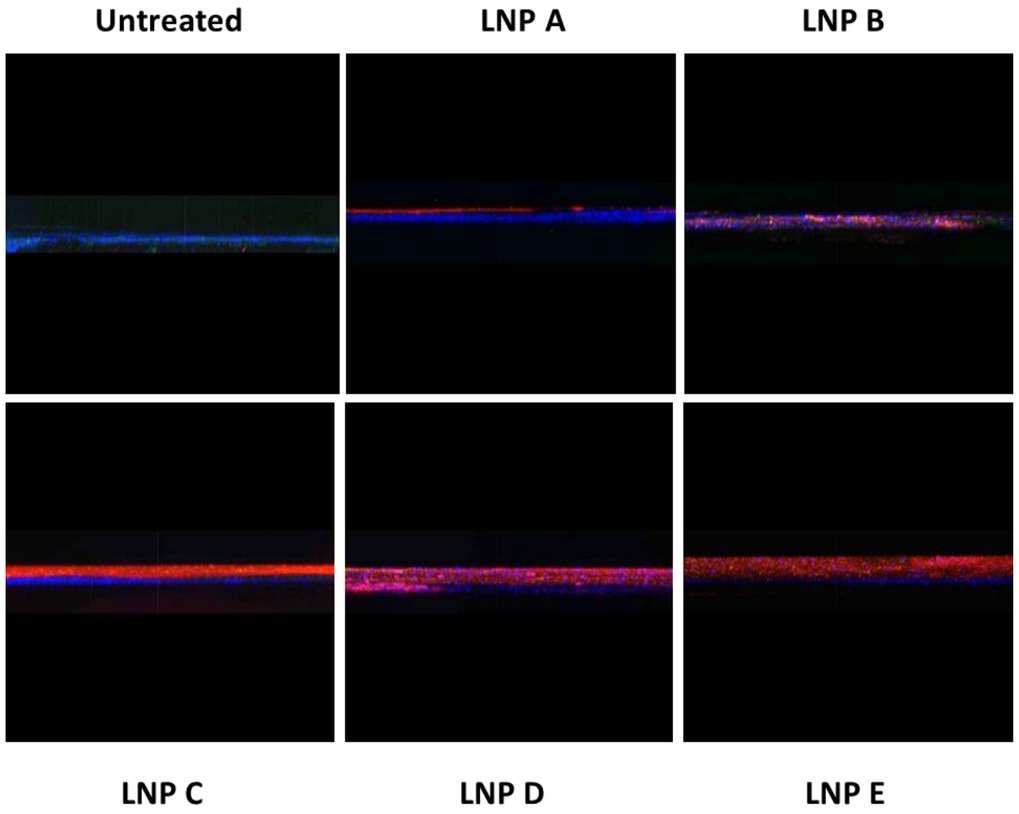


CD8+ T-cells



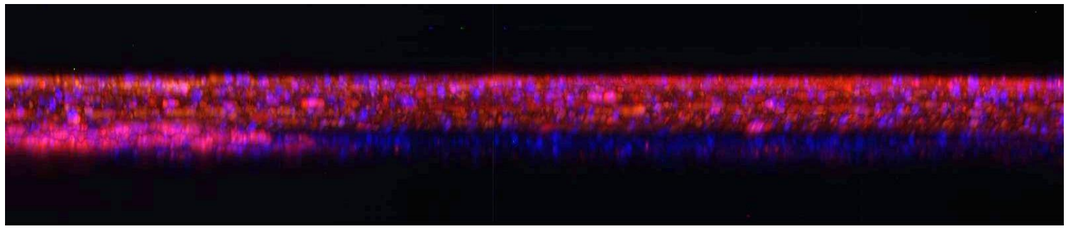
Liver

Delivery of mRNA-LNP to Lung Epithelial Cells

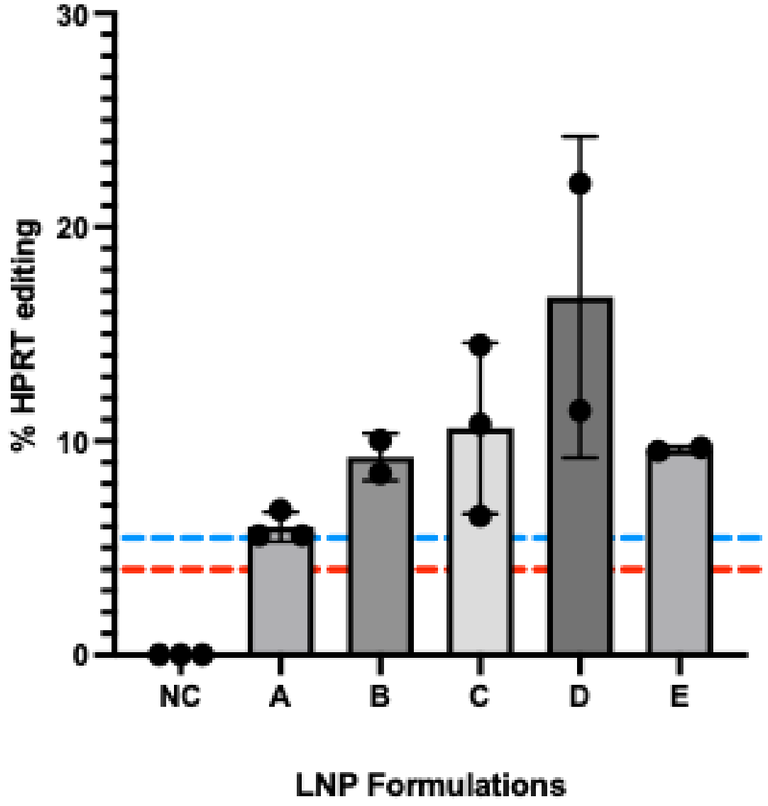


- 3D stack images of CF lung model treated with Acuitas LNPs
  - DiI-labeled LNPs
  - Hoechst stained nuclei

LNP D



Delivery of mRNA-LNP to Lung Epithelial Cells

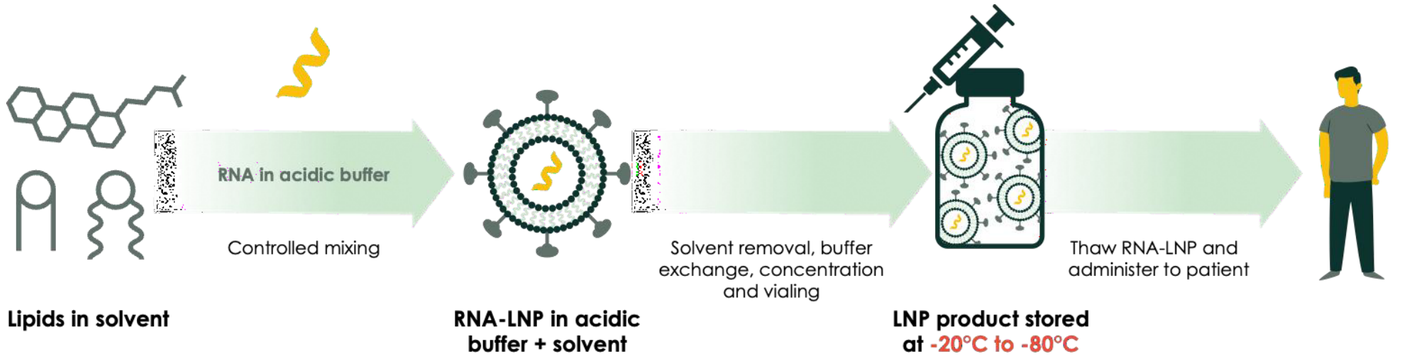


- Gene editing in 3D lung models
- Editing levels of all LNP exceed previous published benchmarks in this model

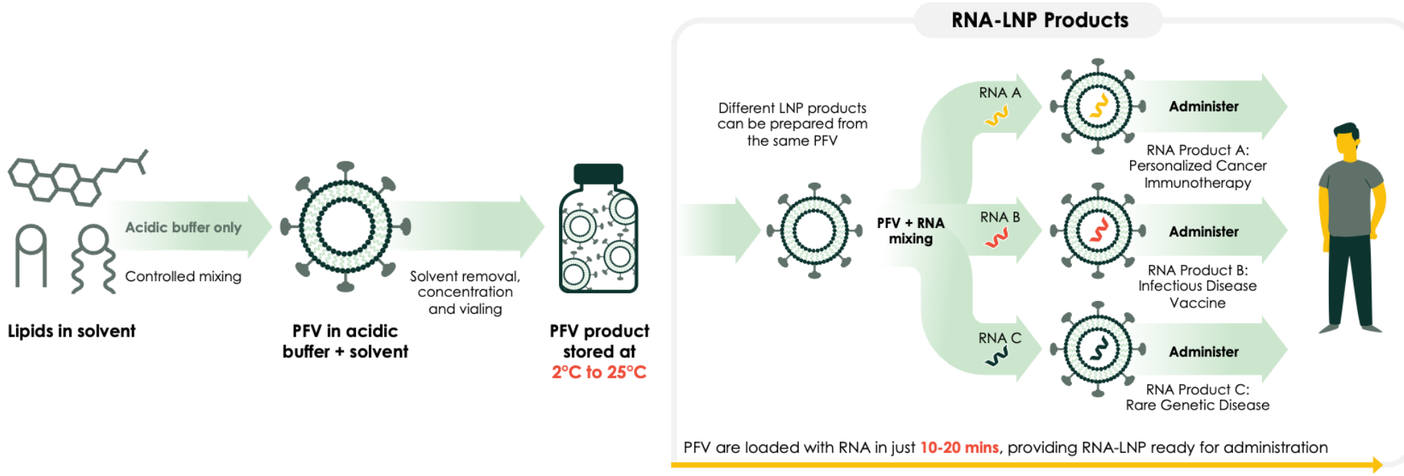
Alternative LNP Manufacturing

Pre-formed Vesicles (PFV)

Conventional Method

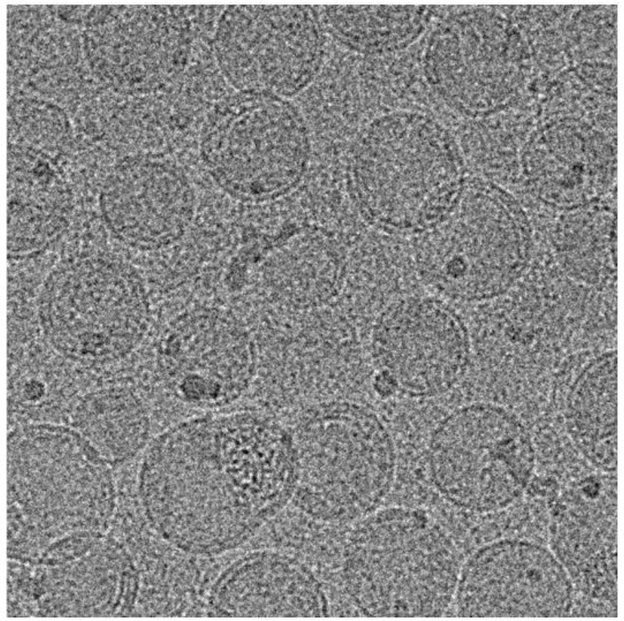


PFV Method

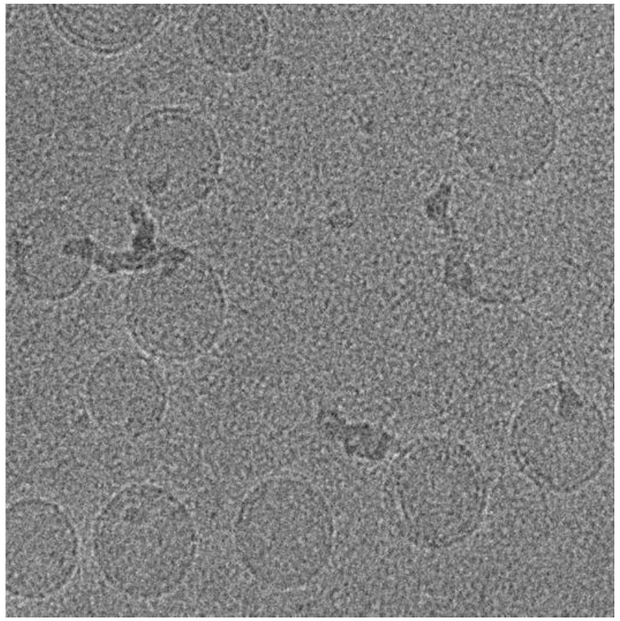


Alternative LNP Manufacturing

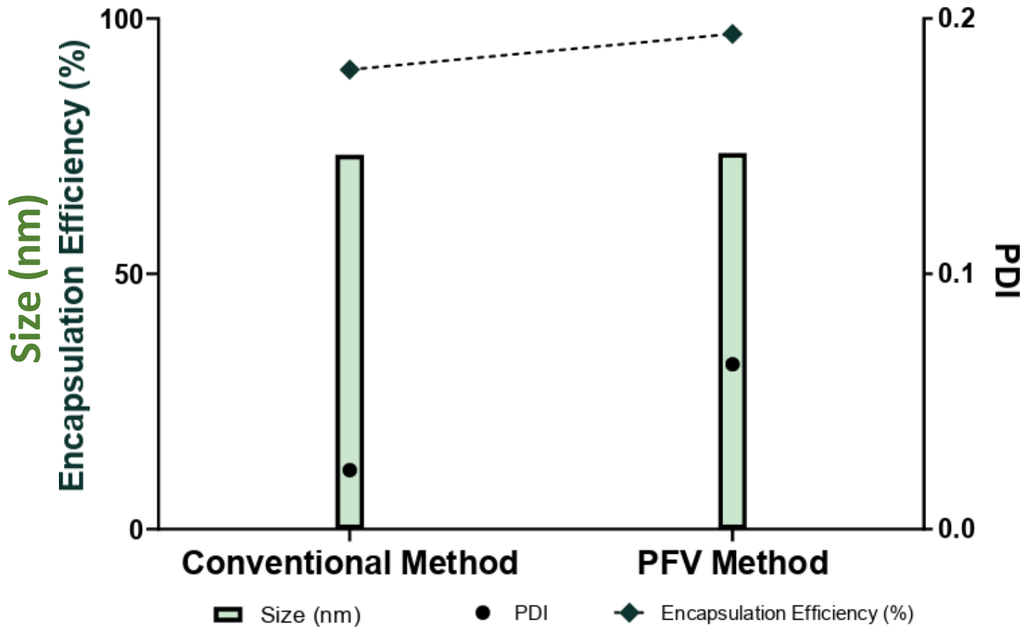
PFV: Equivalent Morphology and Physical Properties



Conventional Method



PFV Method

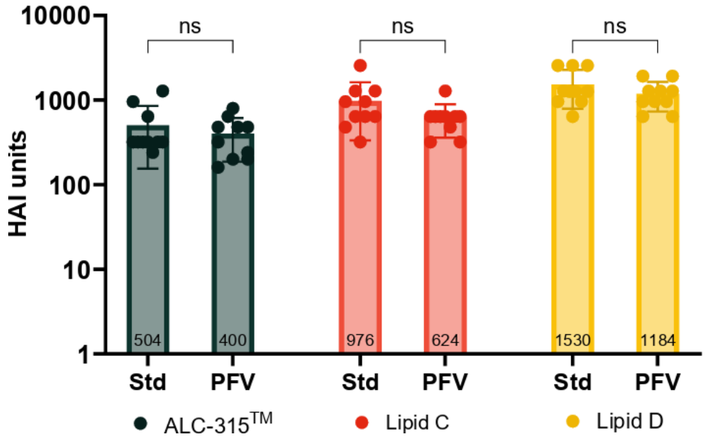


Physical Properties

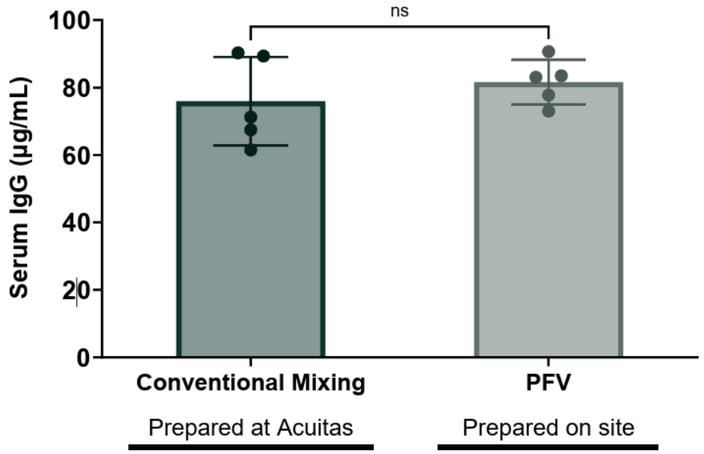
Alternative LNP Manufacturing

PFV: Equivalent Potency to Conventional Manufacturing

IM: Day 28 HAI Titres (0.2 μg)



IV: Day 1 Plasma IgG (0.5 mg/kg)

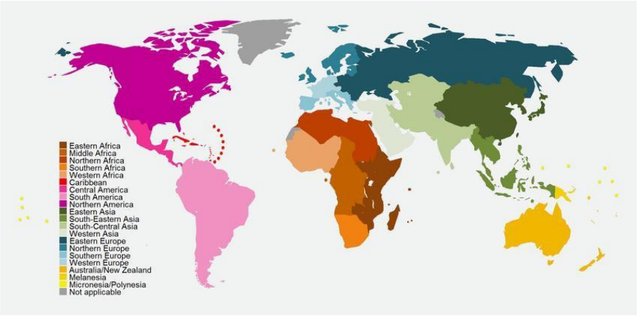


- Conventional and PFV methods exhibit equivalent *in vivo* vaccine performance across lead LNP formulations
- PFV formulation prepared at point of use has IV potency equivalent to conventional formulation of IgG mRNA

Alternative LNP Manufacturing

Opportunities for Point-of-Care Payload Encapsulation

- **Infectious Disease Vaccines**
  - Ambient (RT) or refrigerated (2-8°C) storage & distribution
  - Regional-specific vaccines (tailored to regional viral strains)
  - Delayed selection of prevalent viral strains (e.g. Flu vaccines)



- **Rare Genetic Disease Therapeutics**
  - Need to address diverse patient populations exhibiting similar functional/phenotypic indications
  - Small-scale individualized kit format
  - Patient-specific gene editing protein and/or guide RNA
  - Cost-effective product manufacture

Vial Format

(e.g. of components provided in a kit)

Summary of Recent Innovations

- 33 clinical trials of partnered products with Acuitas LNP initiated in the last 3 years
- Enhanced biodegradable (eBD) lipid candidates identified in mice and monkeys, with equal or greater potency and improved liver tolerability vs clinical benchmark
- Extensive binding/uptake and mRNA expression in T-lymphocytes achieved using targeted, extrahepatic LNP (ehLNP) containing a CD8 DARPin
- mRNA-LNP formulation candidates identified for aerosolization and delivery through mucous barrier and to lung epithelial cells
- Pre-formed vesicles (PFVs) - simple, highly flexible, point-of-care mRNA-LNP manufacturing option that maintains particle characteristics, stability and potency vs conventional methods
  - Significant benefits for cost, storage, distribution and formulating small batches for personalized medicines