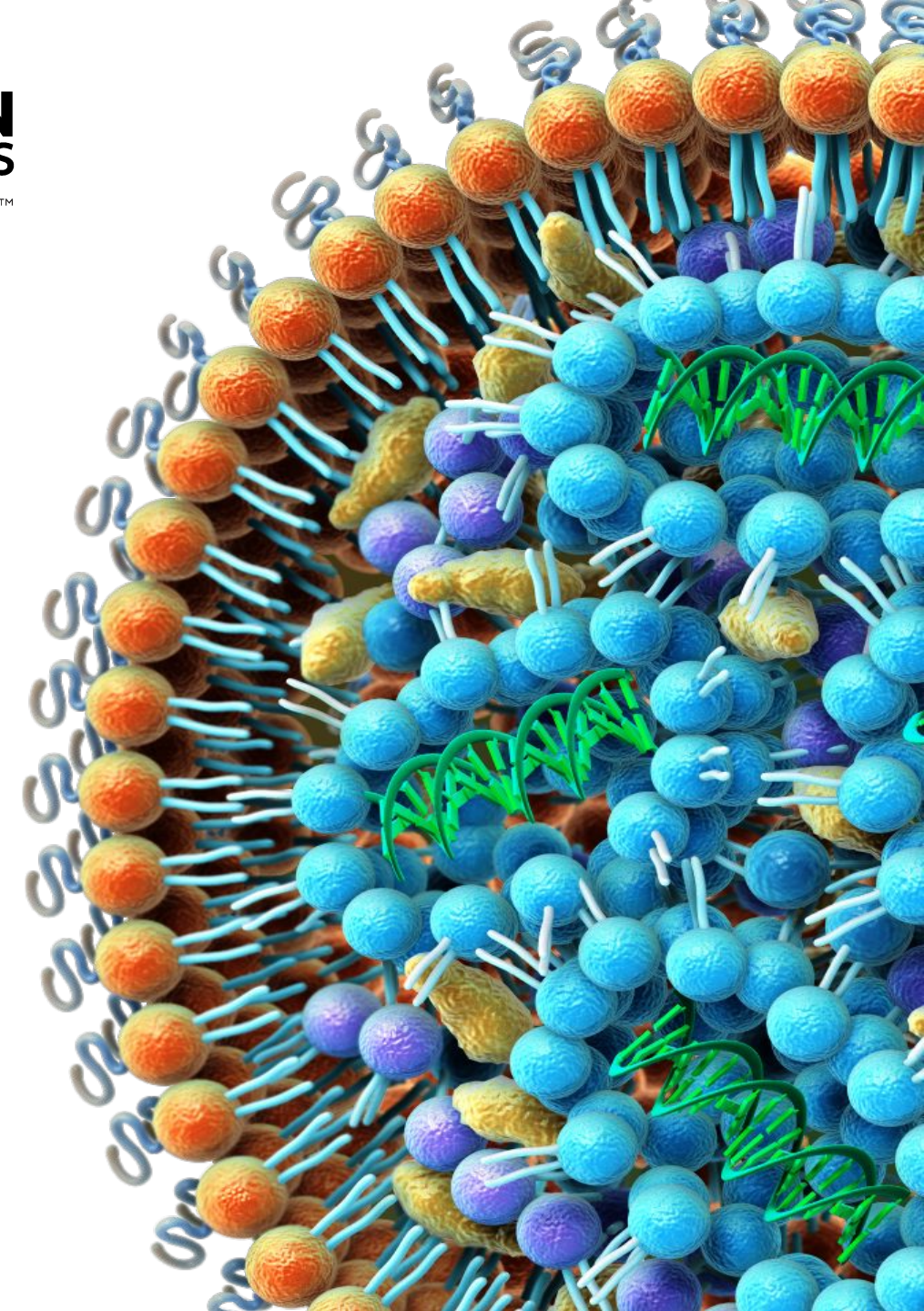




# How Ionizable Lipids RNA-LNP Optimize Delivery Strategies

*Dr. Ian Villamagna*

25 July 2023

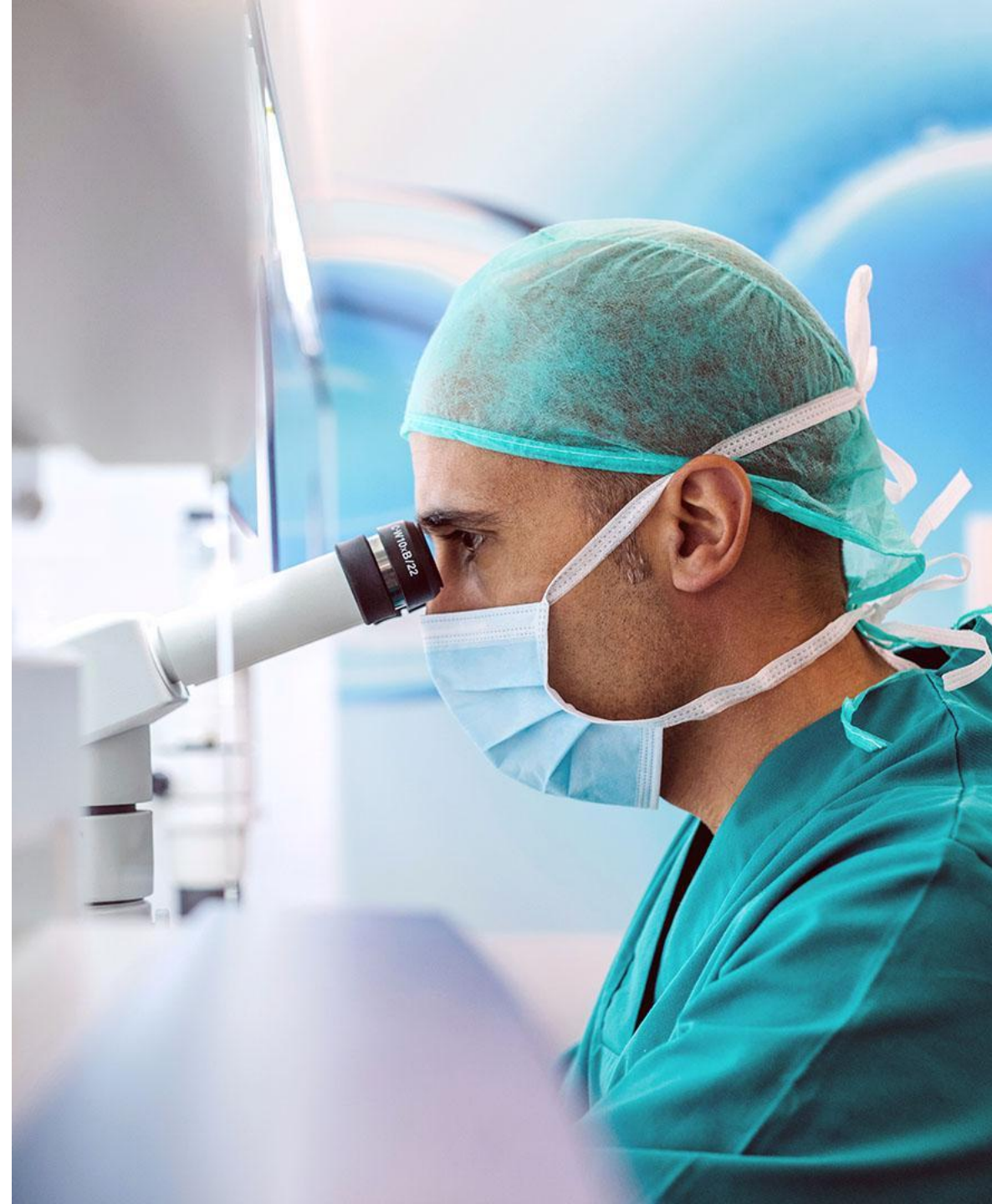




# Mission

**At Precision NanoSystems, our mission is to accelerate the creation of transformative medicine that significantly impacts human well being**

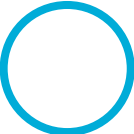



**At Cytiva, it's our mission to advance and accelerate therapeutics.**





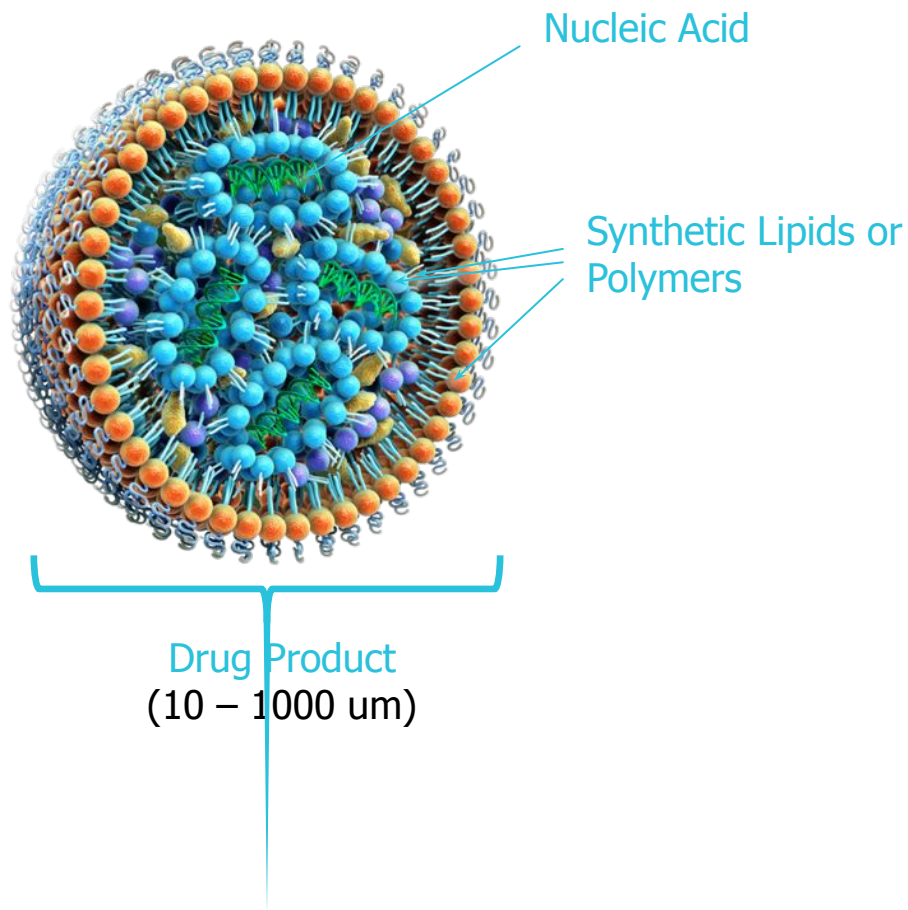
# Within Genomic Medicine Development Delivery is the Biggest Unknown

## What are the Challenges?

- **Nucleic Acid Delivery**  
Nucleic acids are unstable and do not readily enter cells
- **Knowledge Gap**  
Genomic medicines are an emerging technology and few teams have the full compliment of experience in developing this drug product
- **Drug Product Development, Manufacture & Scale-up**  
Genomic medicines are complex drug products and are traditionally difficult to develop and challenging to scale-up
- **Different Needs for Different Programs**  
Genomic medicines are being developed as personalized medicines and for mass administration, and by teams with a wide range of capabilities

# Genomic Medicines are Complex and Require Specialized Reagents, Instruments, and Services

The development of genomic medicines is inherently complex, with multiple different areas that require optimization:



## LNP Composition

- Stability
- Lipid composition
- Excipients

## RNA Quality

- Quantity
- Integrity
- Structure

## Payload Design

- Sequence
- Production
- Biology

## Encapsulation

- %EE
- Location of RNA

## Scale Up

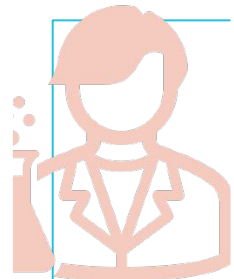
- Larger batches
- Process Development
- Downstream Process

## Biological Testing

- Dosing
- Efficacy
- Safety
- Administration



# Meeting our Customers' Needs Earlier to Accelerate Therapeutic Development



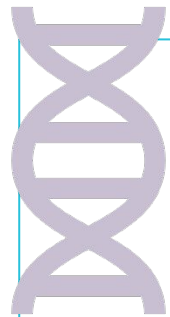
## Every Scientist can be a Genomic Medicine Developer

- Lowering the Barrier to Entry
- Genomic Medicines Designed not Discovered
- Niche to Mainstream



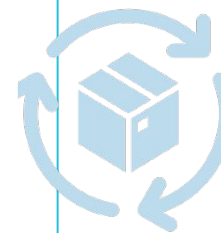
## Partner for the Entire Drug Development Journey

- Ideation to Approved Drug
- Continuity through Drug Development
- **Show-Not-Tell** Therapeutically Relevant Proof of Concept Data



## Be the Platform for Genomic Medicine

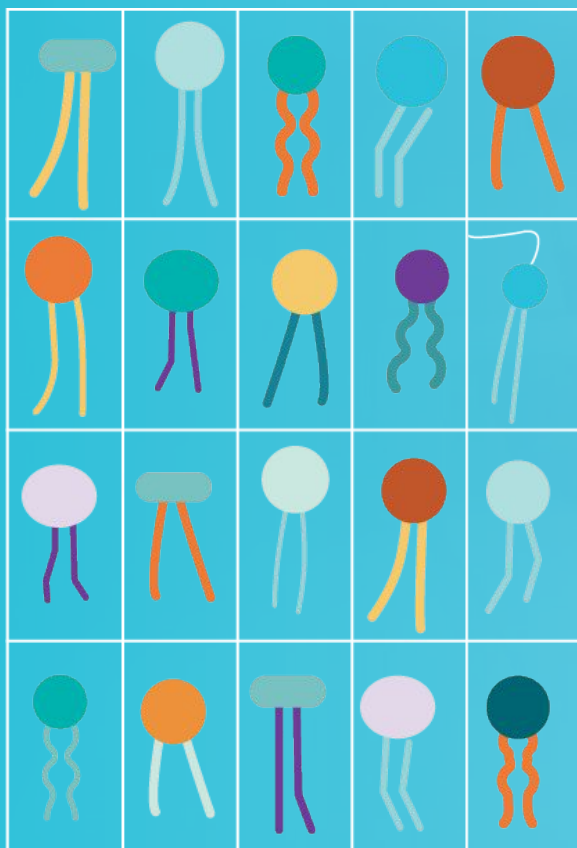
- Optimized Products, Services, Technologies, and Workflows
- Full Stack of Genomic Medicine Development Technologies
- End-to-End Solutions



## The Product is the Process

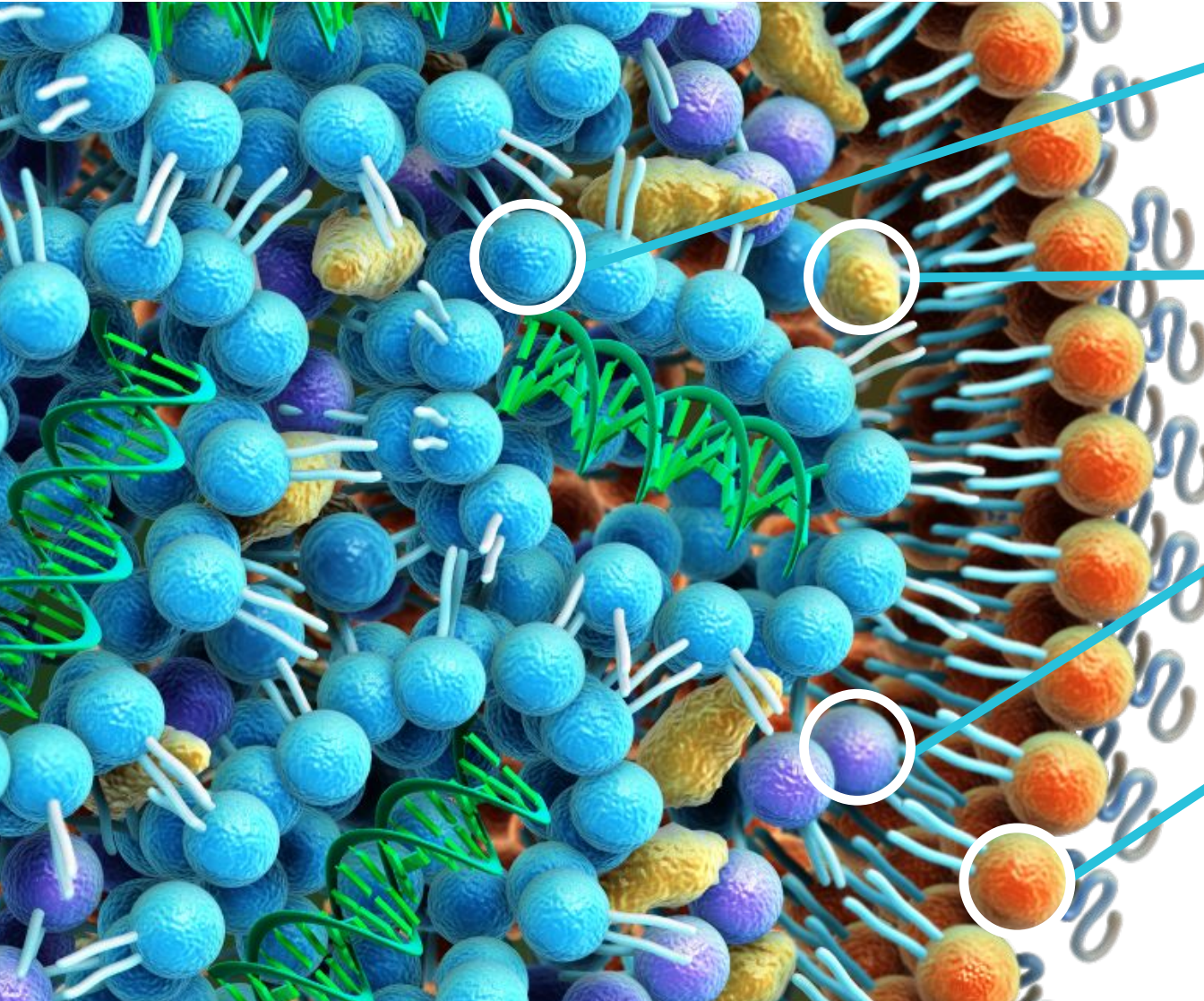
- Therapeutic and Process Development are Linked
- Manufacturing Efficiencies are Critical
- NxGen™ Delivers Precise, Reproducible Nanoparticles across Scales

## Ionizable Lipid Portfolio





# LNP components



## **Ionizable Cationic Lipid**

- Neutral at pH 7, Cationic at pH ~4.5
- Binds & protects RNA
- Facilitates Endosomal Escape

## **Cholesterol**

- Binds ApoE
- Mediates endocytosis via LDL receptor

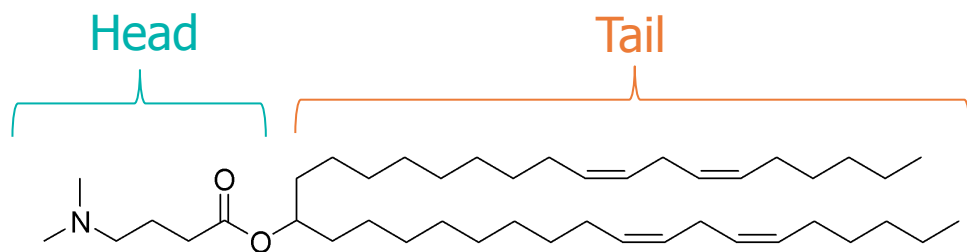
## **Helper Lipid (e.g. DSPC, DOPC)**

- Structural support, fills voids
- Been found to affect release

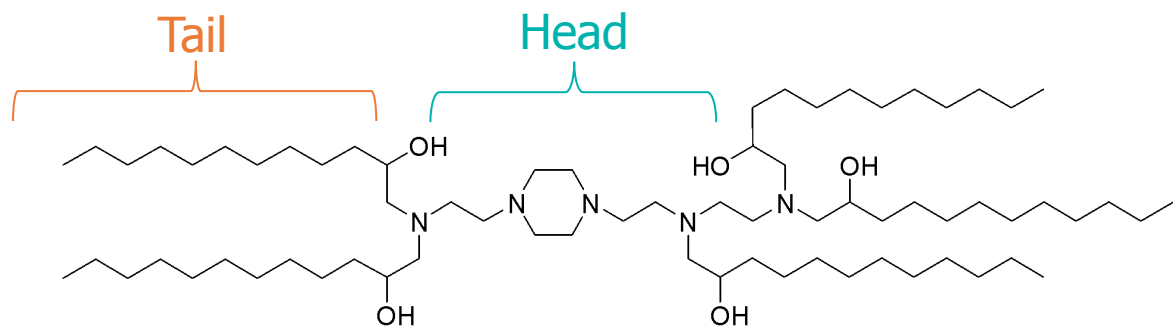
## **PEG-Lipid**

- Stabilizes particle during formation
- Protect from opsonization
- Shed from particle over time following systemic injection

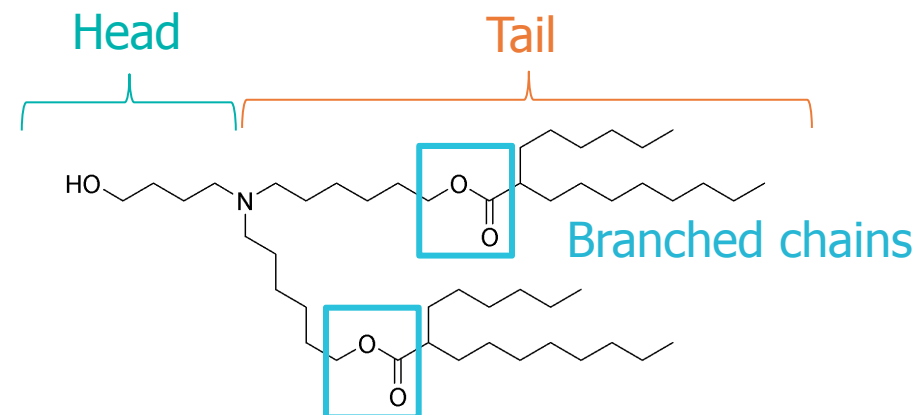
# Common Ionizable Lipids



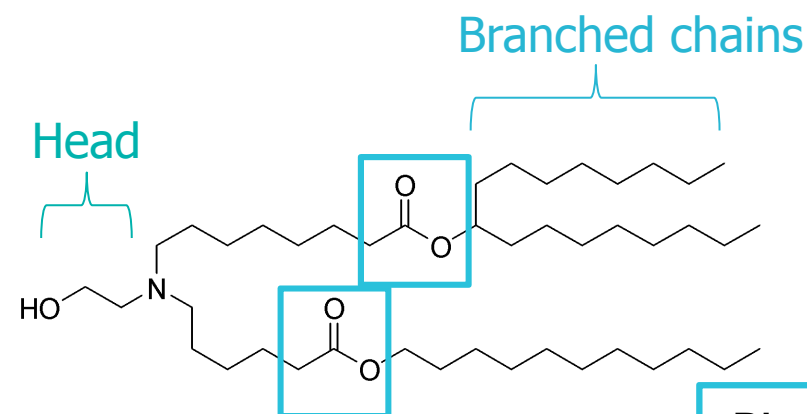
DLin-MC3-DMA



C12-200



ALC-0315



SM-102

Biodegradable  
ester groups



# Strategies to Streamline Ionizable Lipid Selection



**Diverse Library of Novel Ionizable Lipids**

**Lead Candidate Selection**

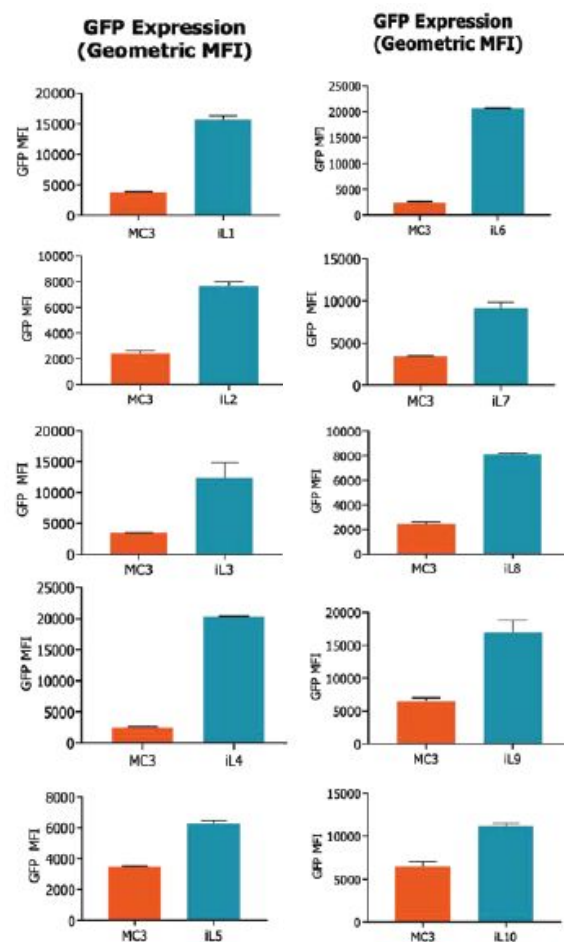
**Novel Compositions Designed for Specific Applications**

Diverse  
Portfolio

*Well-characterized ionizable lipid portfolio streamlines screening across applications*

# Lipid Nanoparticle Portfolio Screening

**Our ionizable lipid portfolio contains multiple ionizable cationic lipids more potent than MC3**



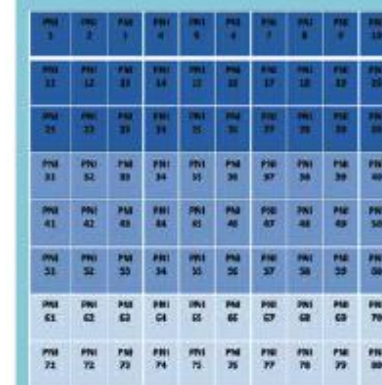
Library - Cell therapy use  
(Ancillary materials)



Hits for Cell Therapy



Main Library



Biodegradable, semi-biodegradable  
and non-biodegradable lipids



## Applications Demonstrated

- Vaccines
- Protein Replacement
- Cell Therapy

Library - In vivo use  
(Drug Product)

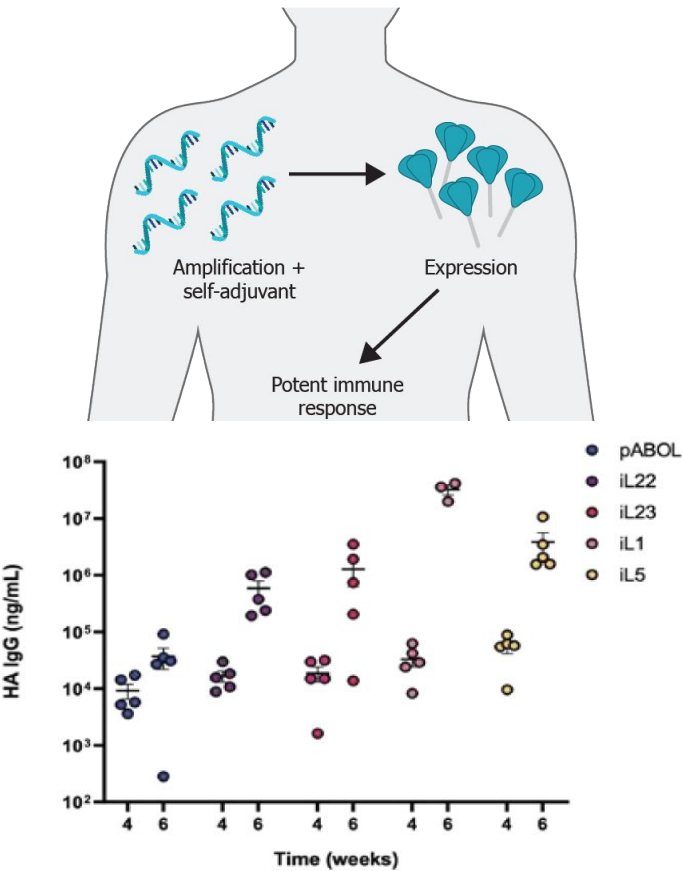


Hits for In-vivo application



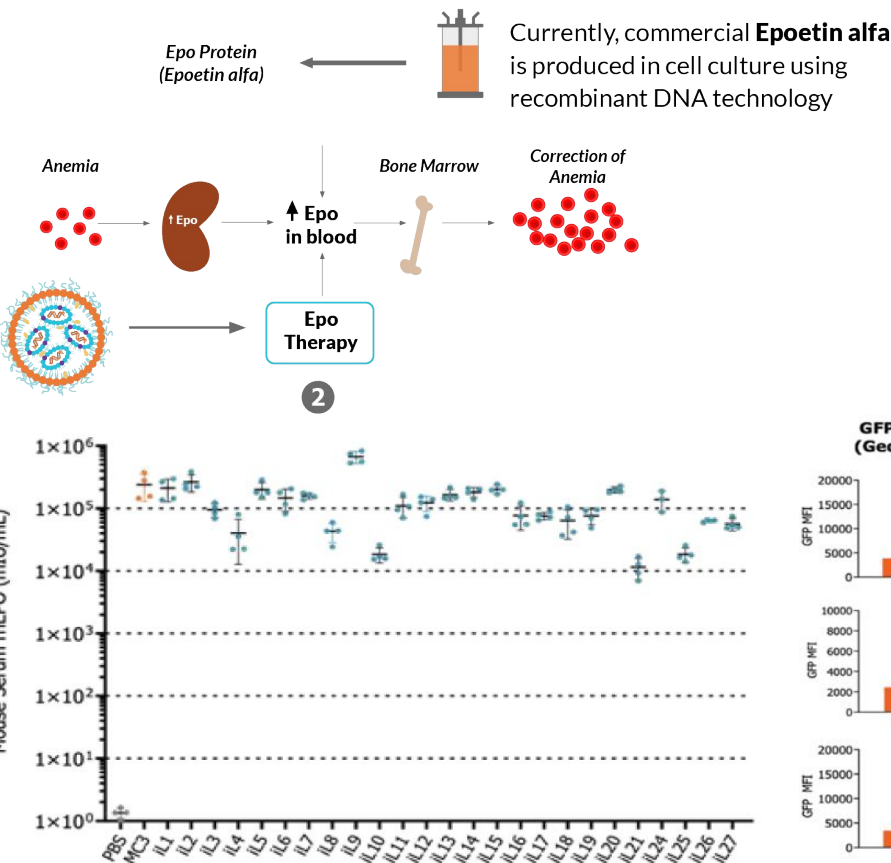
# GenVoy™ LNPs Enable Delivery for Key Applications in Genomic Medicine

## Vaccines



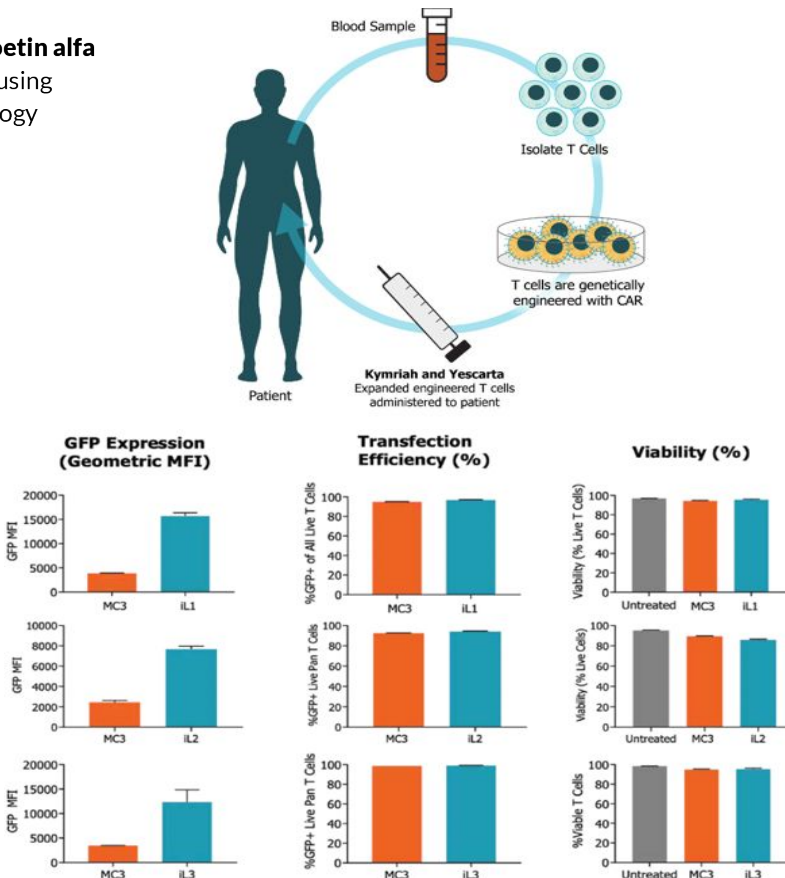
HA IgG Generation in Mice

## Gene Therapy



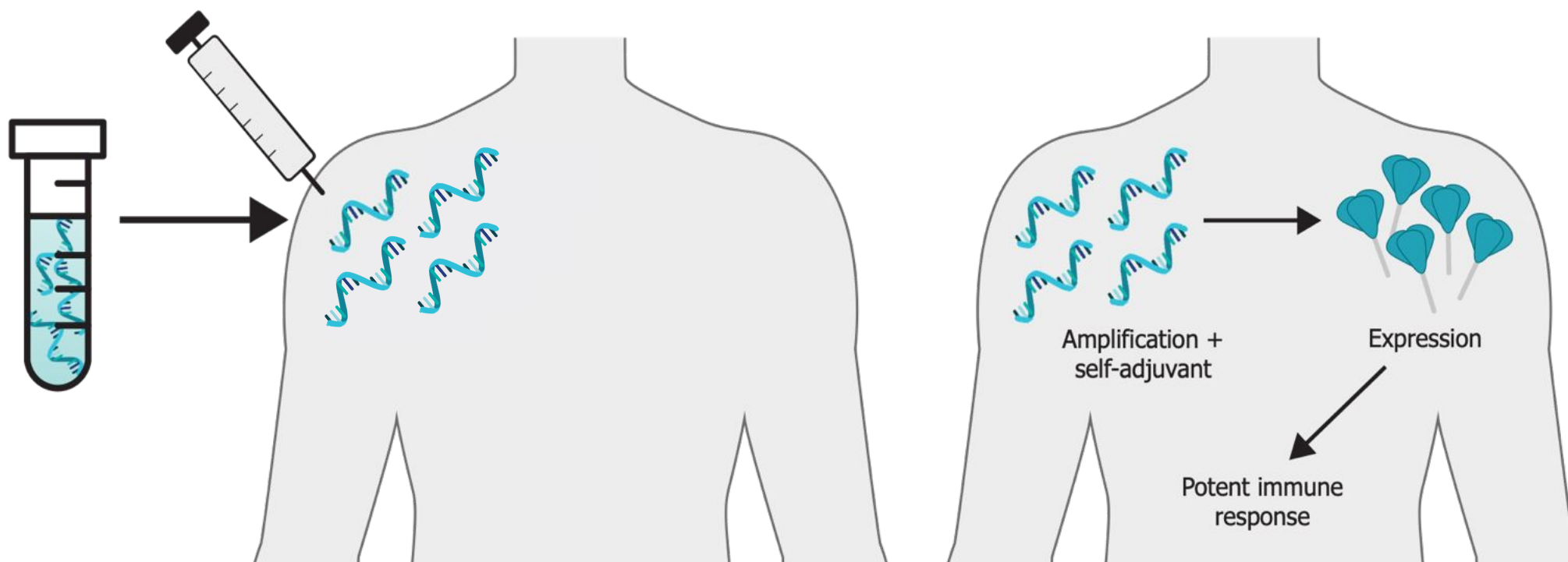
Epo Production in Mice + Gene Editing

## Cell Therapy (ex vivo)



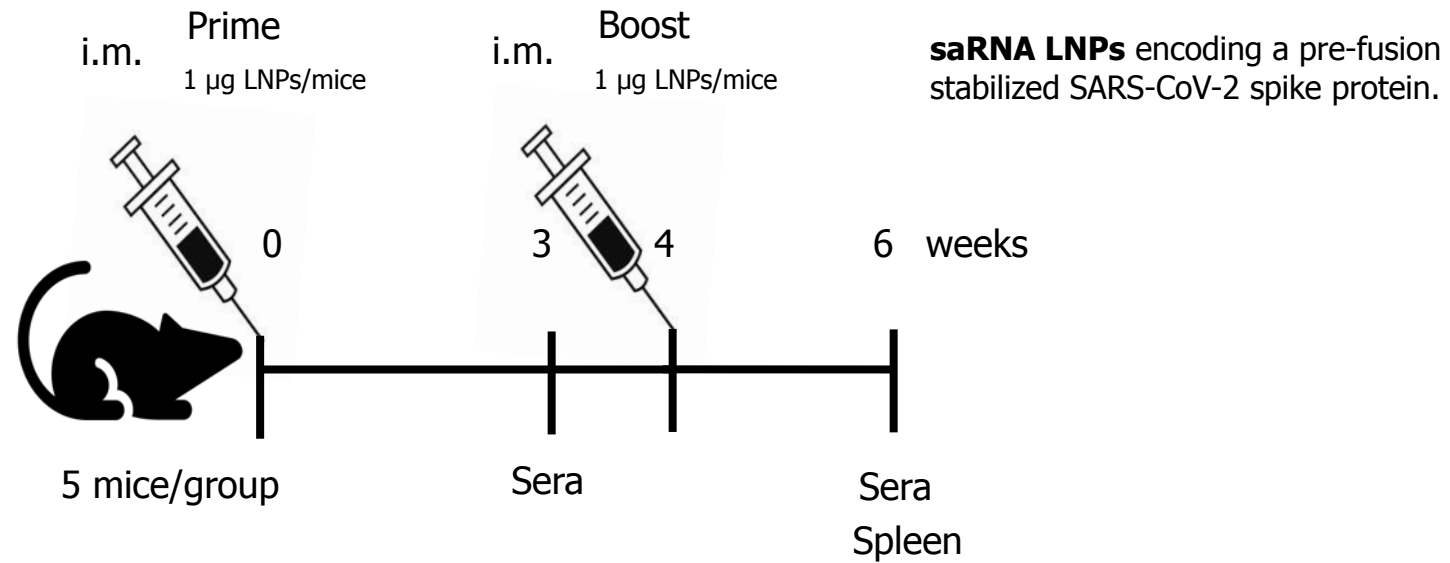
GFP Expression in Primary Human T Cells

# Vaccines





# In Vivo Testing of saRNA LNPs



Sera



- SARS CoV2 specific IgG ELISA
- Cytokine measurements/Neutralization assays
- Isolation of splenocytes

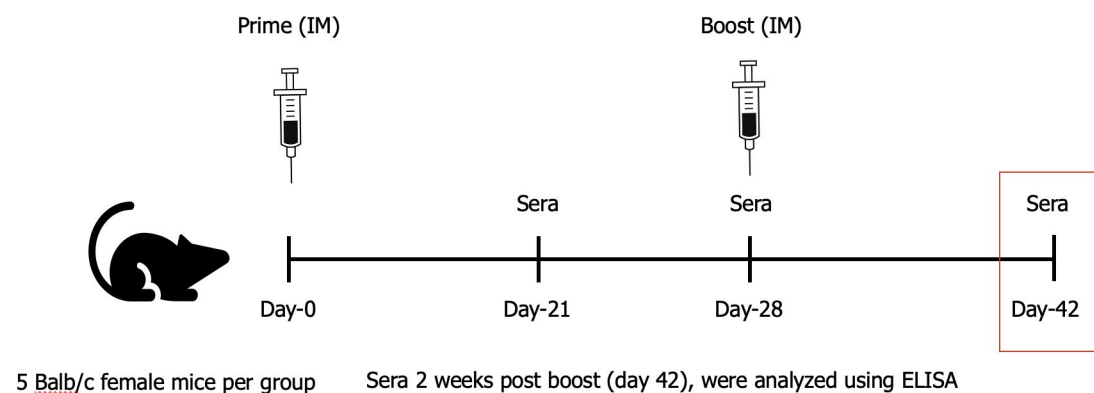
Spleen



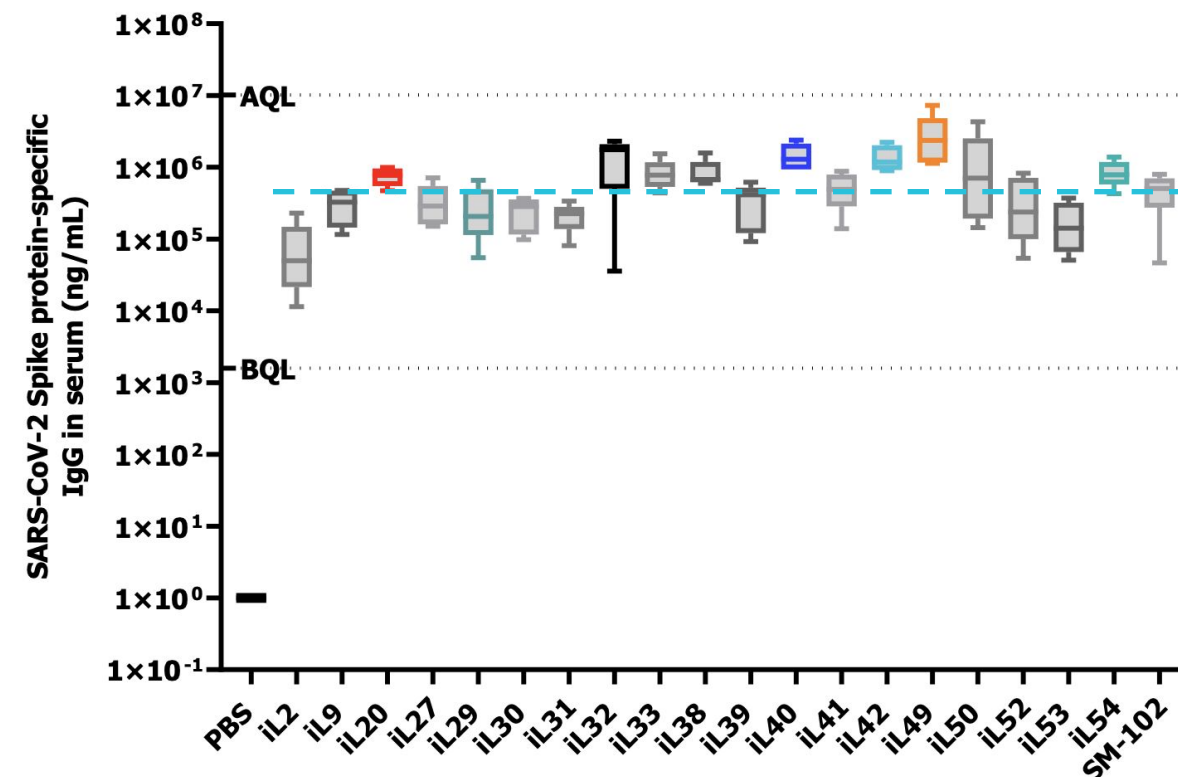
- *Ex vivo* restimulation with SARS-CoV-2 peptides
- Intracellular cytokine staining/Cytokine measurements

# SARS-COV-2 Spike Encoded saRNA

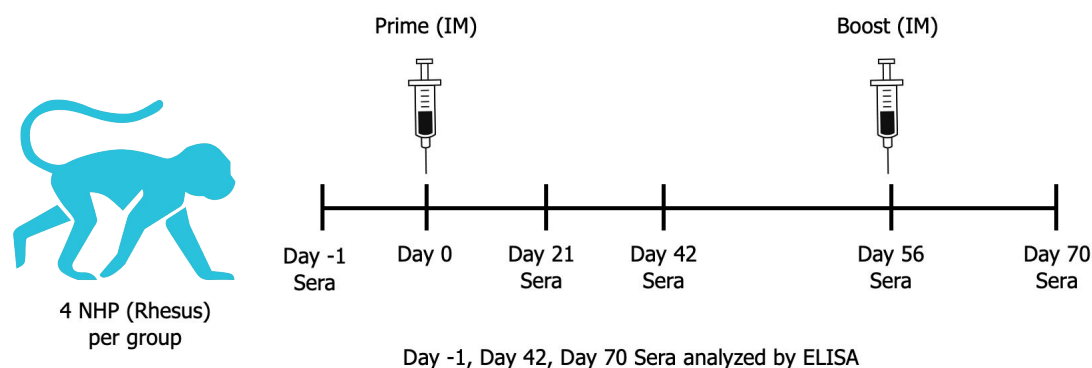
## ELISA Data for 2 weeks sera post boost (Day 42)



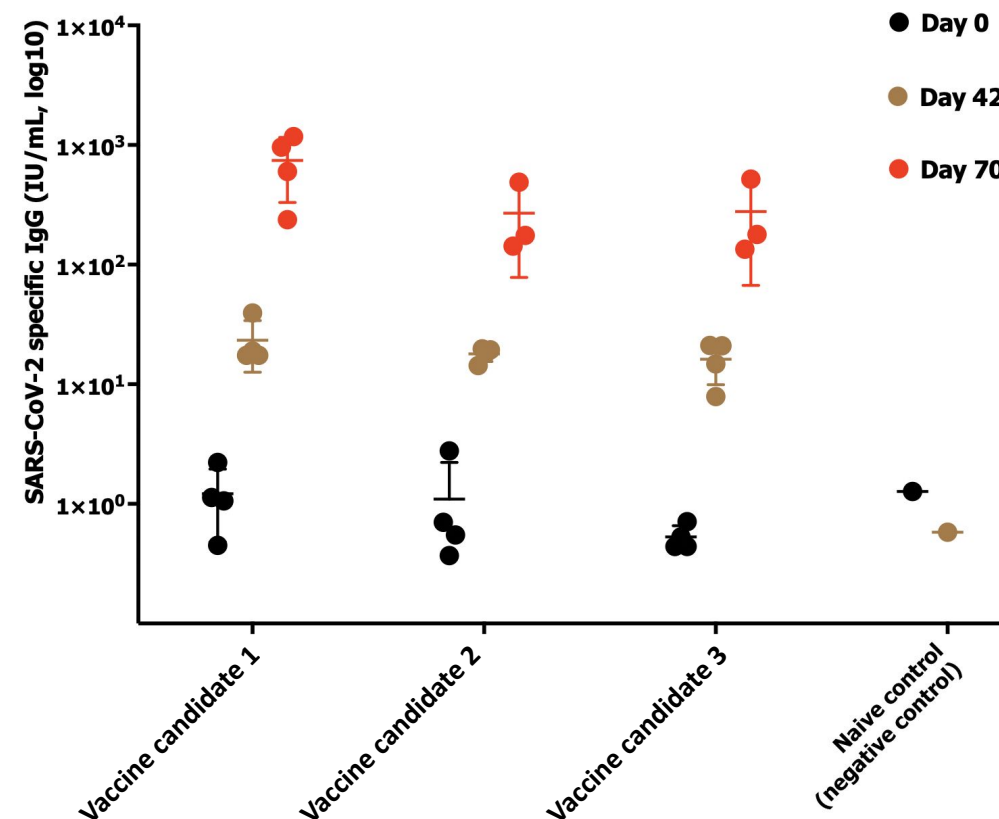
Data indicates that a no. of PNI lipids are extremely effective for vaccine applications



# Vaccine Candidates Showed High IgG Levels in NHPs



- Significant difference in IgG levels at Day 42 compared to pre-bleeds.
- High response was observed on Day 42 for all the candidates following Prime
- The response on Day 70 is much higher than other time points, a response typical of the boost.

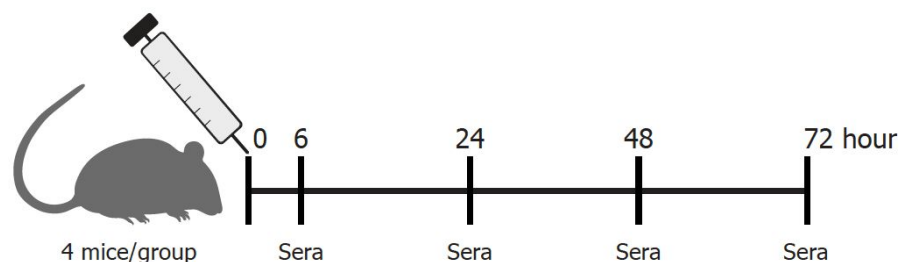


WHO SARS-CoV-2 human IgG standard was used to establish a quantitative curve for relative quantification anti-spike IgG in the serum specimens

Calibration Control with known IgG concentrations and serum naïve Rhesus Monkeys (NHP02SRMUN, BIOIVT) were used as positive and negative controls



# Tolerability of Lipids following IV administration



72h study  
CD-1 male mice  
6wk old  
n=4/LNP

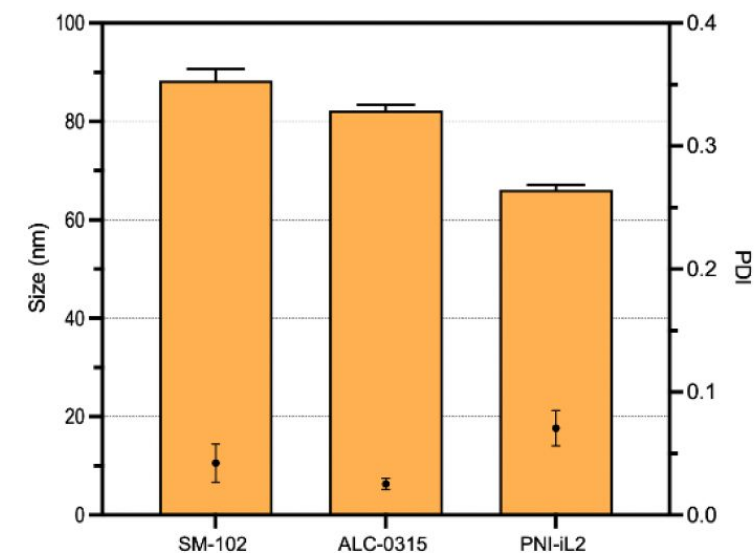
LNP	0-72 h post dose		72 h post dose			
	Clinical Observations		Necropsy		Liver Histology	
	Moderate Dose	High Dose	Moderate Dose	High Dose	Moderate Dose	High Dose
Control PBS	Normal		Normal		Normal	
SM-102	Mild signs recover w/in 6h	Moderate signs lasted full 72h	Normal	¾ Normal ¼ Euthanized @ 1h	Normal	Slight apoptosis & inflammation
ALC-0315	Mild signs recover w/in 72h	Mild to moderate for 6-72h	Normal	¾ Normal ¼ Enlarged Spleen	Mild apoptosis & inflammation	Mild apoptosis & inflammation
PNI iL2	Normal	Mild signs recover w/in 6h	Normal	Normal	Slight apoptosis & inflammation	Slight apoptosis & inflammation
	Normal	Slight	Mild	Moderate	Severe	Severe



**Clinical observations**  
were relatively higher, lasted longer, and occurred with greater frequency in the LNPs with SM-102 and ALC-0315, than compared to a matched mg/kg dose of PNI LNP.

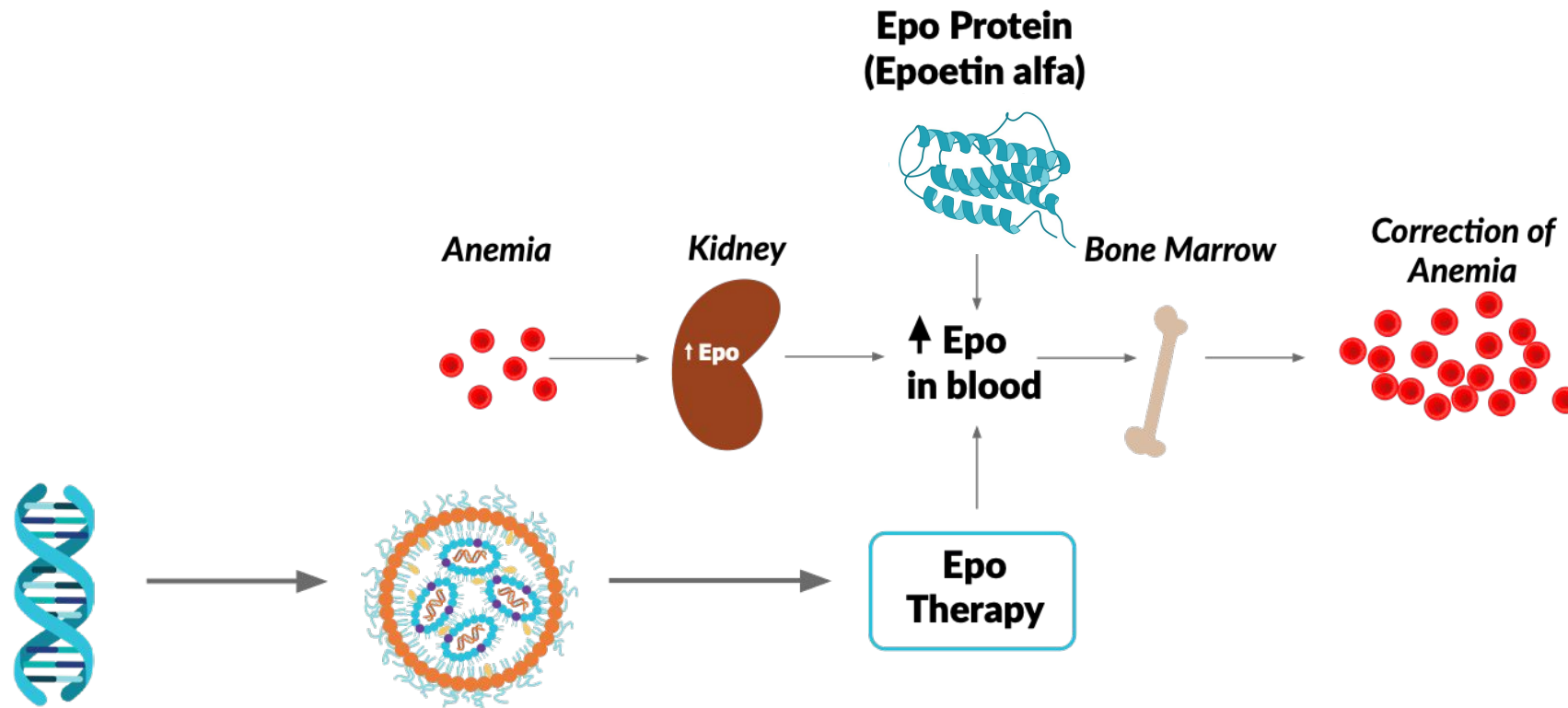
**Necropsy**  
were normal for most groups.

**Liver histology**  
showed relatively higher apoptosis and inflammation in the LNP containing ALC-0315 compared to all other groups at matched doses.



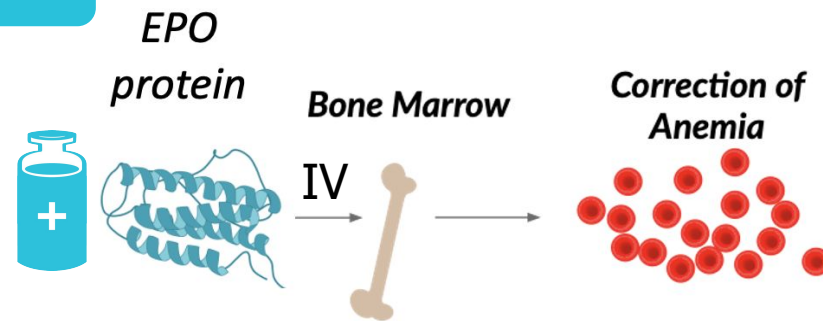
PNI Ionizable Lipid showcased good tolerability in a blinded study.

# Gene Therapy

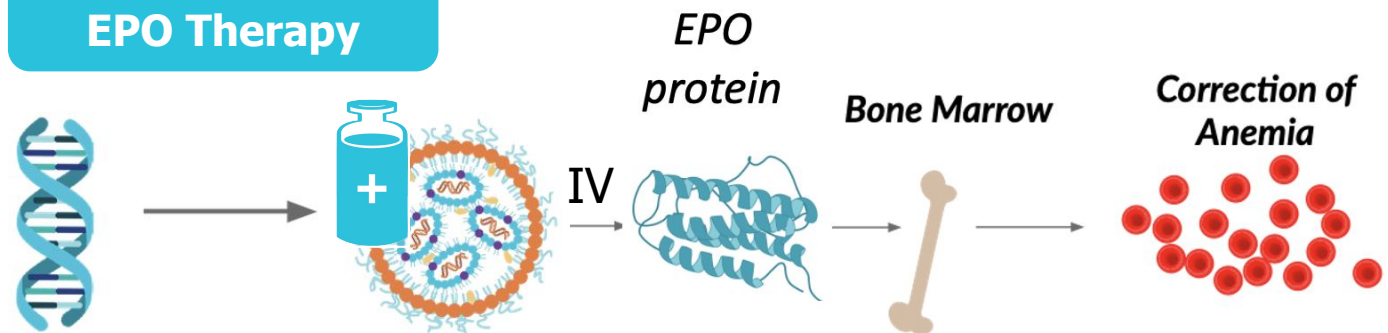


# Gene Therapy

## Current Treatment – EPO Therapy

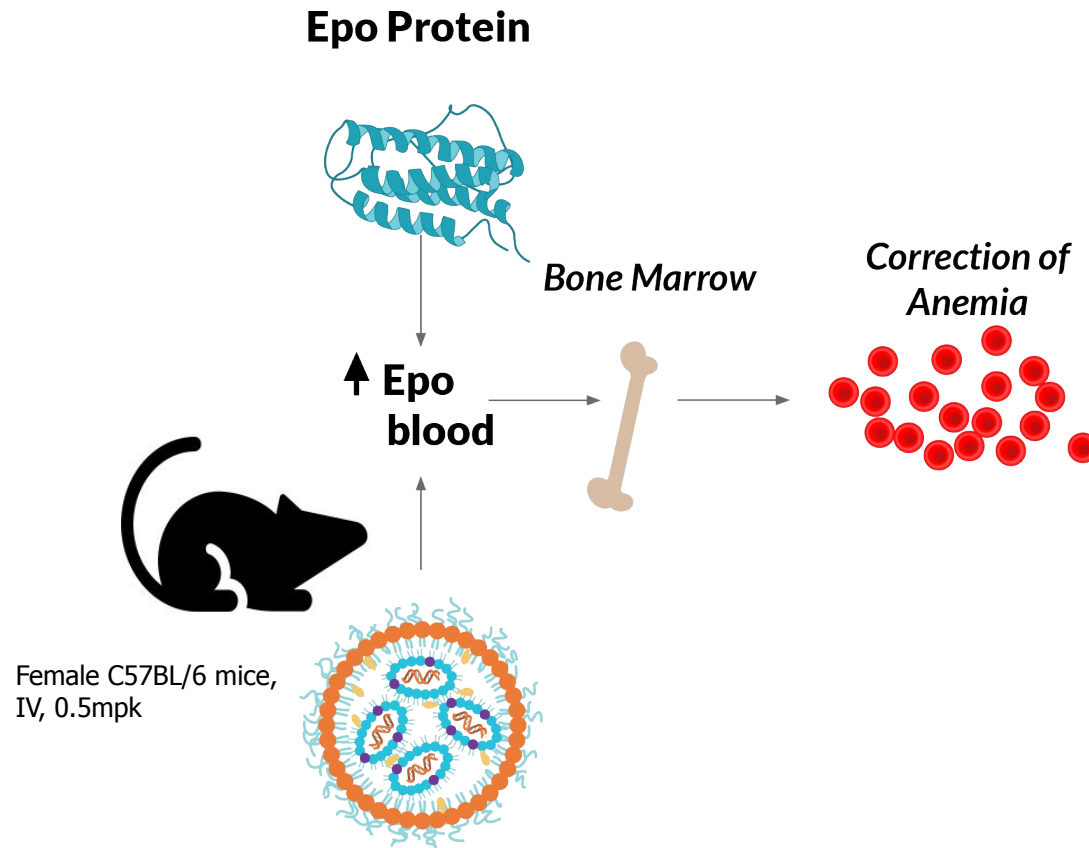


## Genetic Medicine EPO Therapy



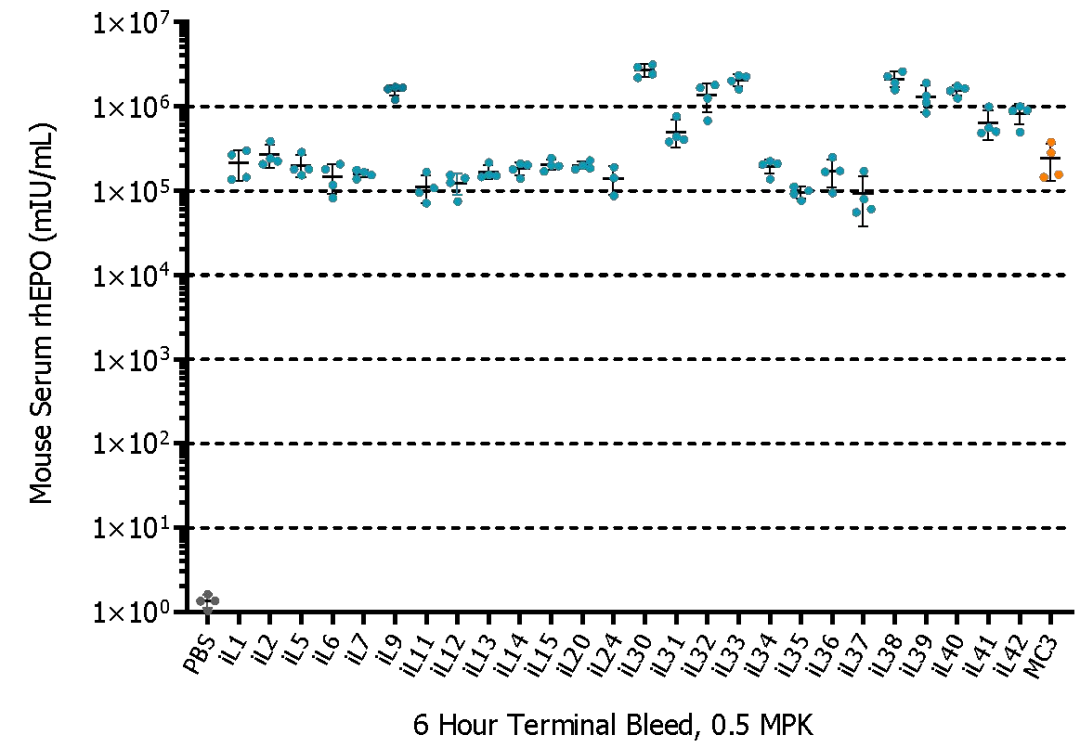


# Erythropoietin (EPO) Expression – An Example for Protein Replacement Therapy



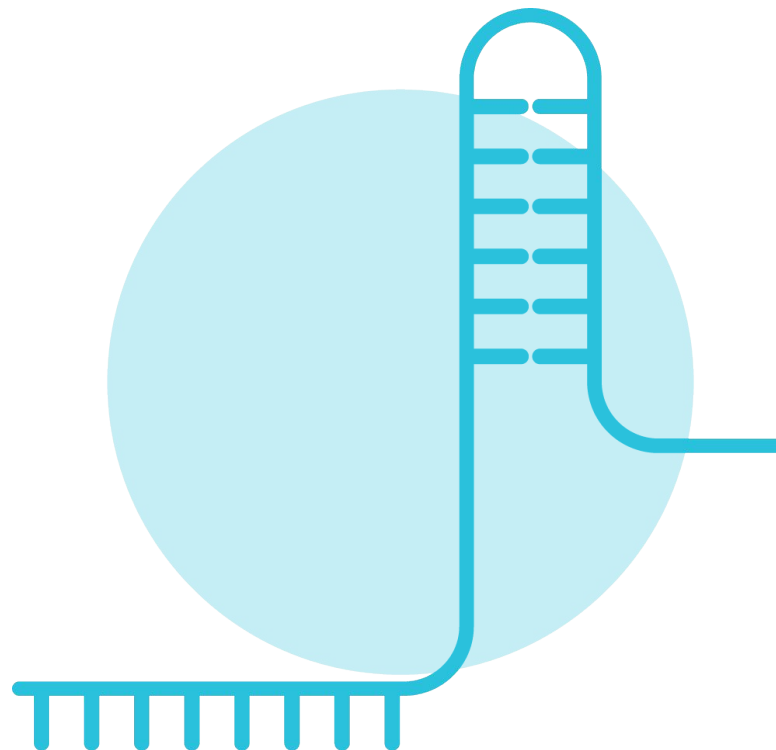
Data indicates that Precision NanoSystems' ionizable lipids demonstrate its ability to deliver mRNAs encoded for therapeutically relevant proteins

**rhEPO Expression in Mice**



# Gene Editing

## CRISPR & Base Editing



# CRISPR to Reduce Transthyretin Amyloidosis - Intellia's NTLA-2001

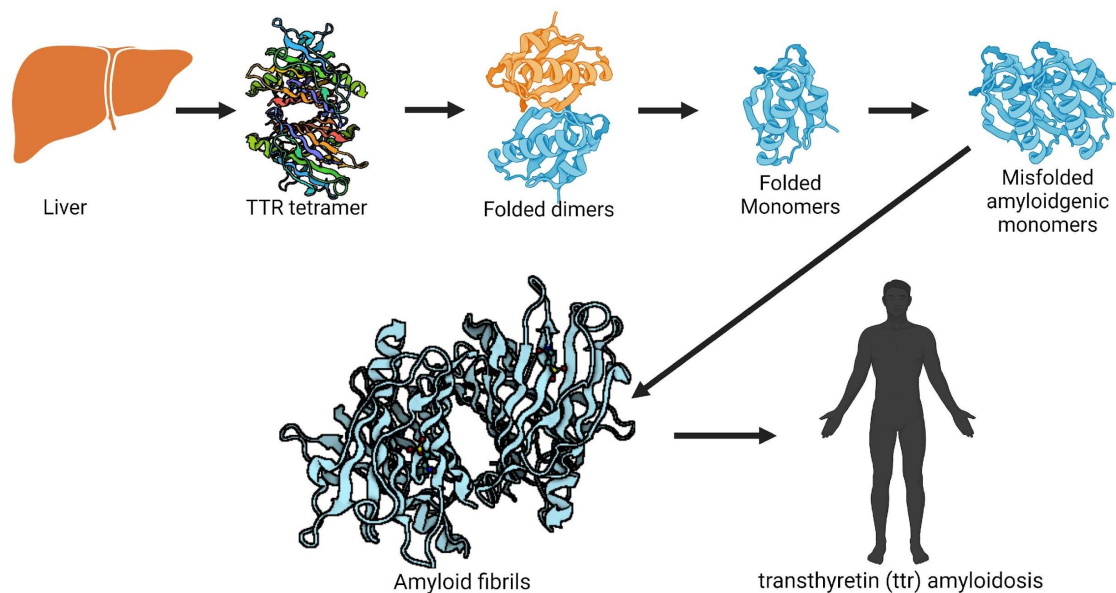


**Intellia's NTLA-2001 Phase -1  
clinical trial data shows high  
promise for Transthyretin  
amyloidosis as single dose  
treatment**

NCT04601051



# Transthyretin Amyloidosis (ATTR)



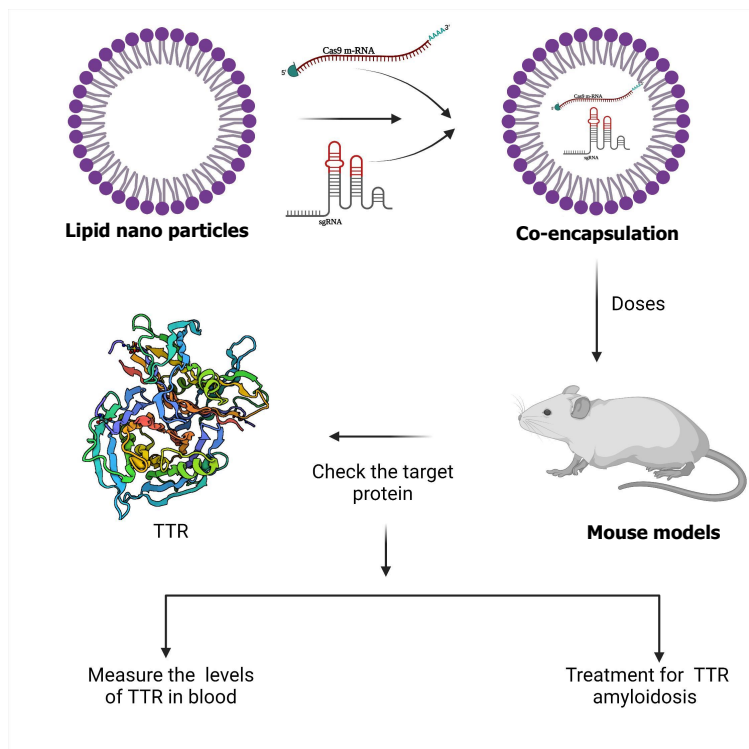
- A life-threatening genetic disease, caused by progressive accumulation of misfolded transthyretin (TTR) protein in tissues of nerves and heart
- Polyneuropathy & Myopathy can result
- Leads to cardiovascular disorders

## ○ Current treatment options

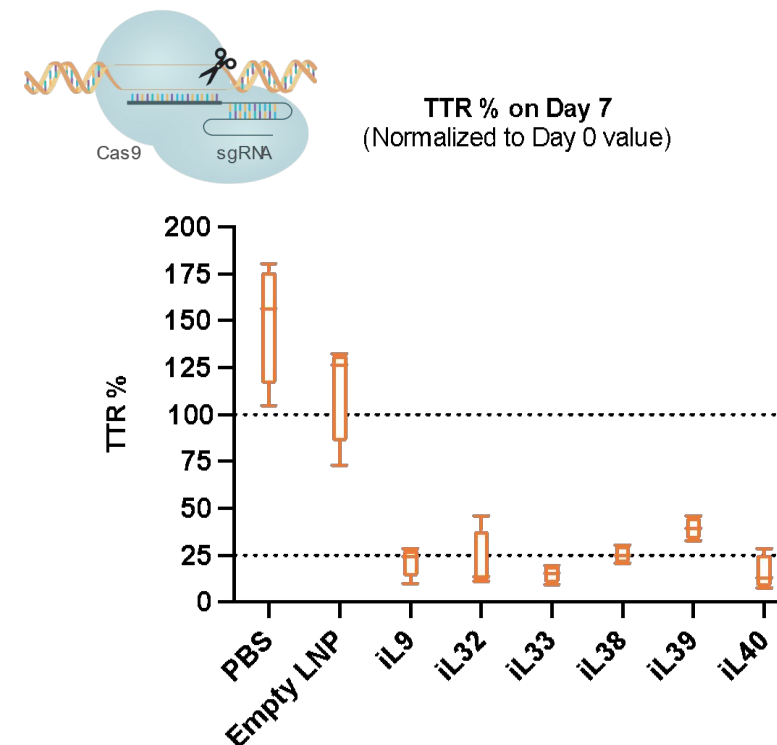
- Patisiran® (Onpattro™)
- Inotersen (Tegsedi™)

Image credit - BioRender

# TTR CRISPR Gene Editing – Preliminary Data with Precision NanoSystems Ionizable Lipids as a Delivery System



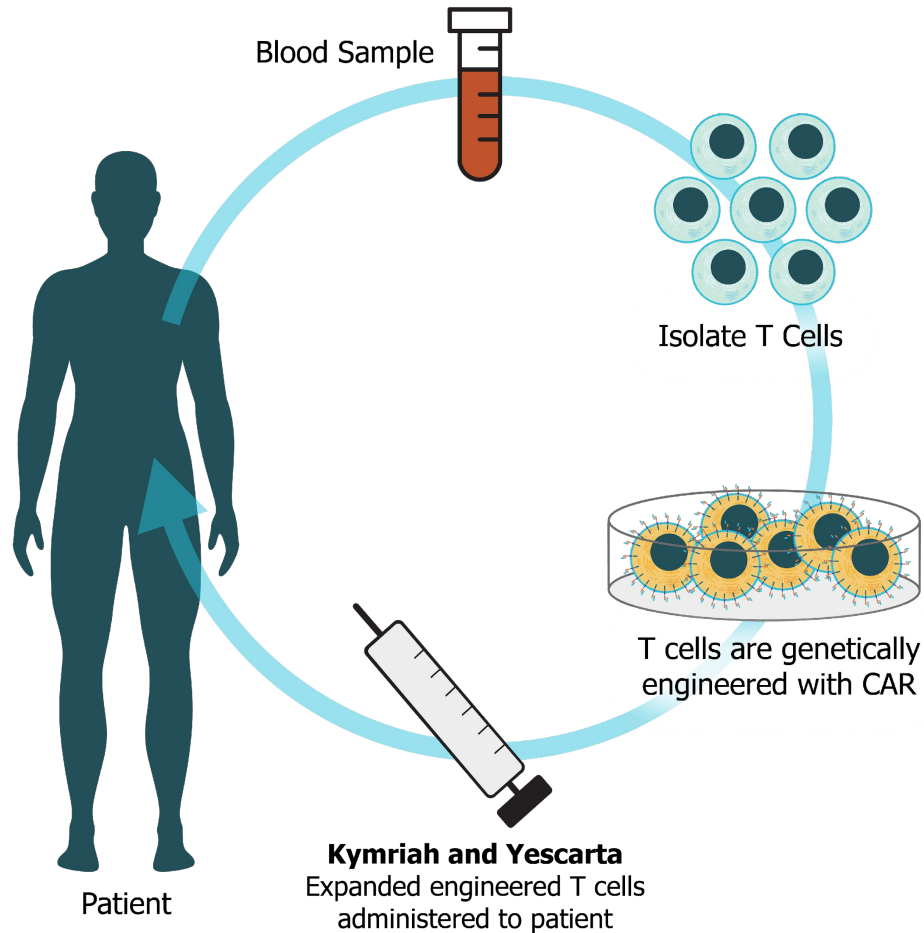
A Single Administration of CRISPR/Cas9 Lipid Nanoparticles Achieves Robust and Persistent In Vivo Genome Editing.)



TTRguide-Cas9 mRNA LNP: Co-encapsulation of guide and Cas9 mRNA at 1:1 wt ratio

Preliminary results show more than 75% reduction in TTR protein levels with majority of tested lipids with one injection (3 mg/Kg)

# Cell Therapy



5 years from FDA approval. Novartis



Image credit: Novartis



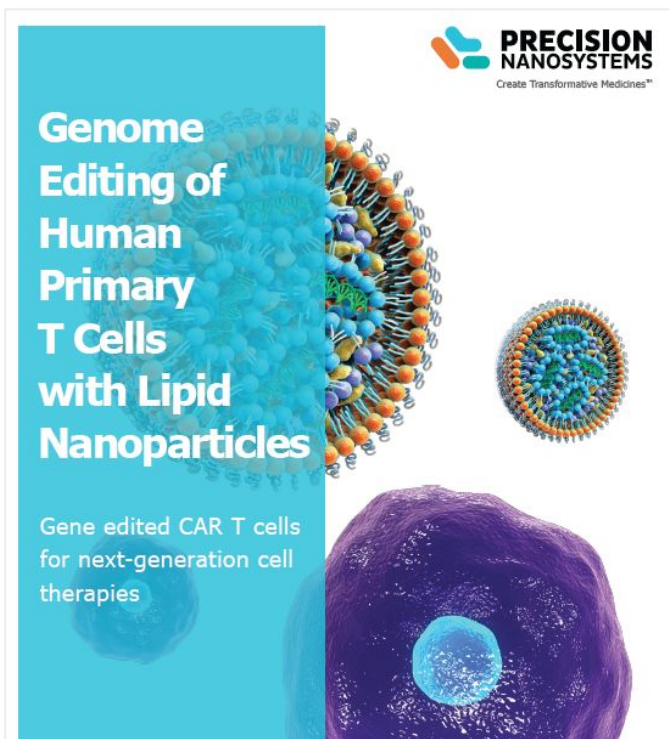
Image source:  
<https://emilywhiteheadfoundation.org/news/celebrating-10-years-cancer-free/>

LNP mediated delivery of nucleic acid therapeutics have the potential to become similar **life saving genomic medicines**



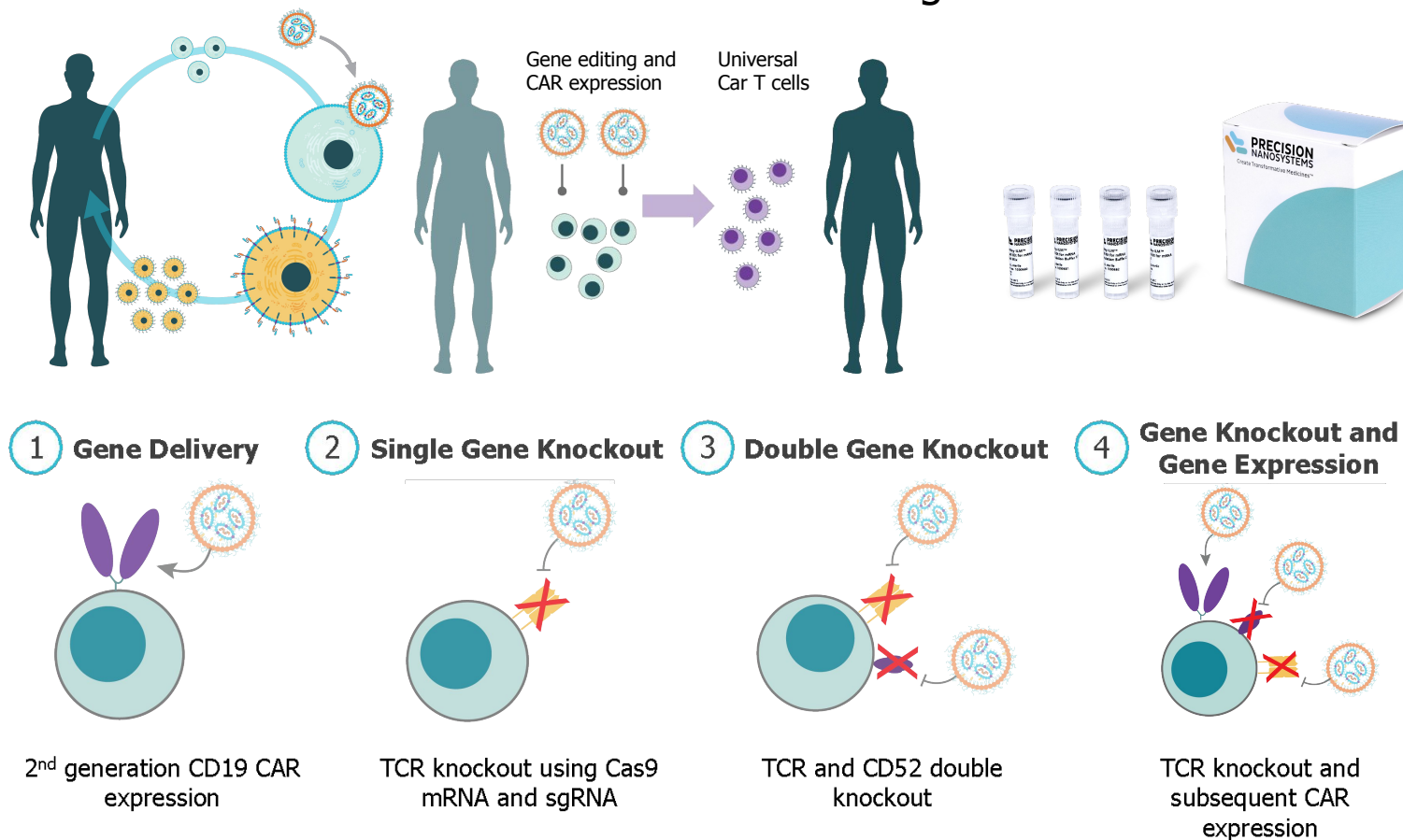
# Off-the-shelf T Cell LNP Reagent for Accelerating T Cell Therapies

Immense possibilities with **Precision NanoSystems' Ionizable Lipids in T cell product kit.**

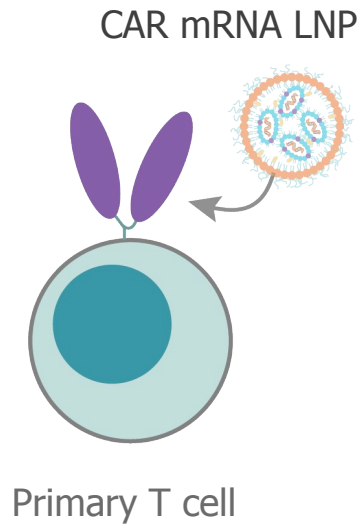


Application Note

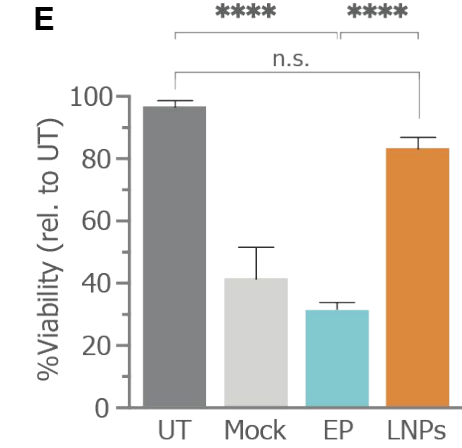
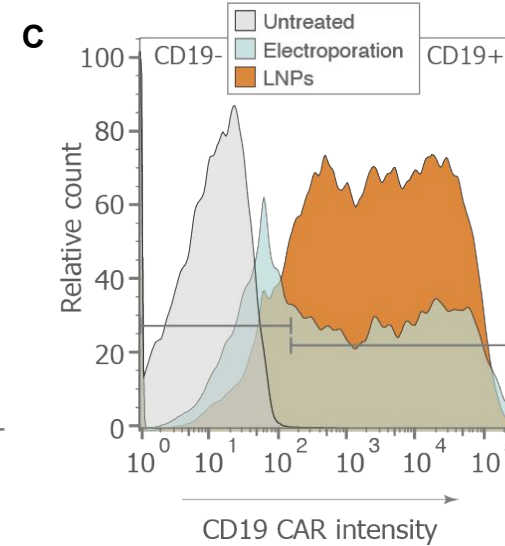
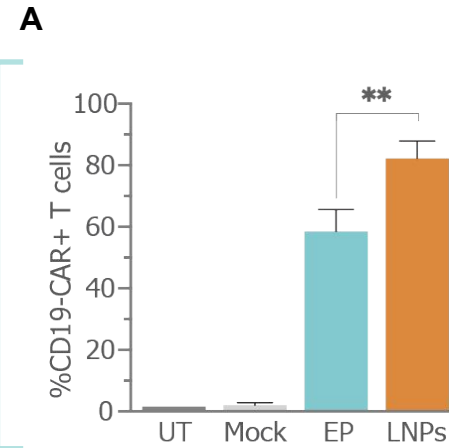
First-to-market: An off-the-shelf T cell LNP reagent



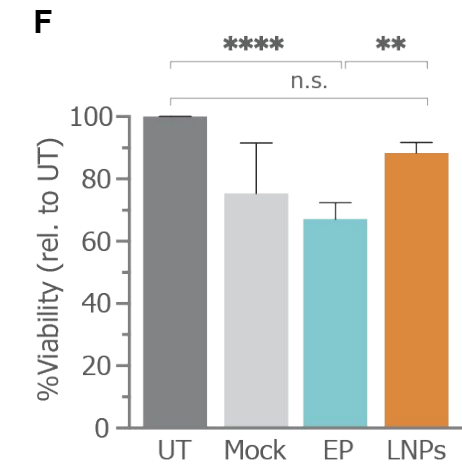
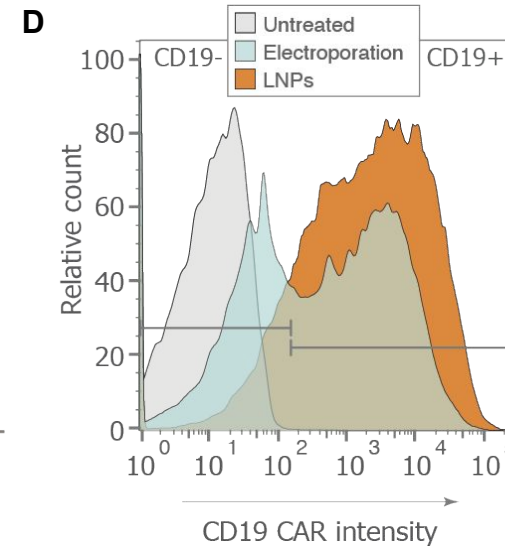
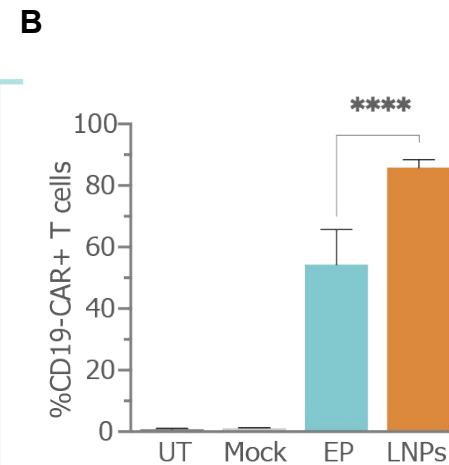
# 1 mRNA Delivery with High Efficiency and Cell Viability



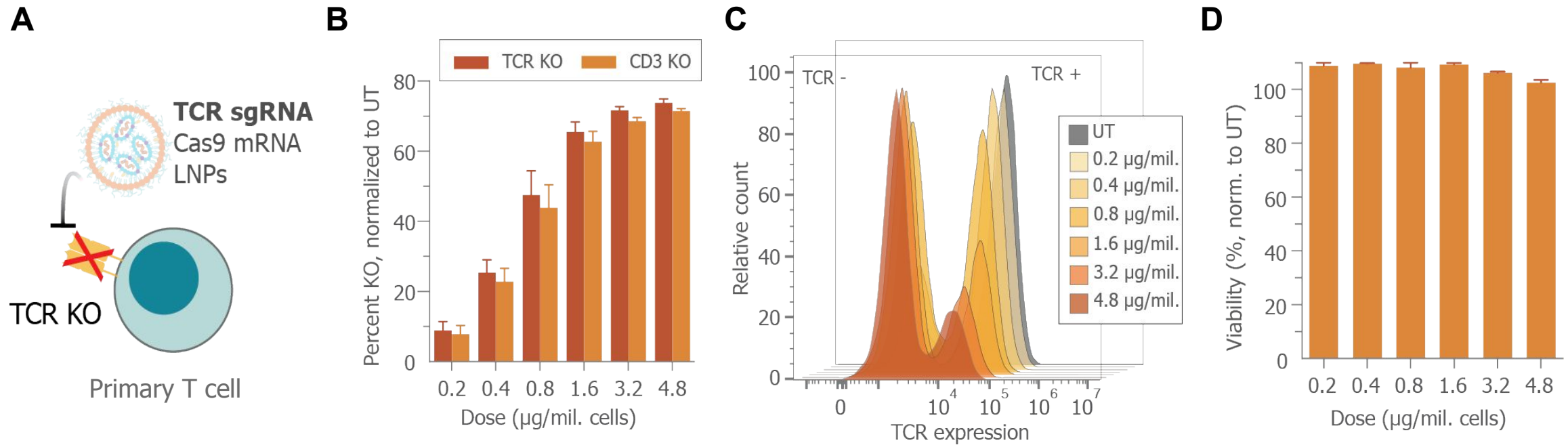
24 h  
detection



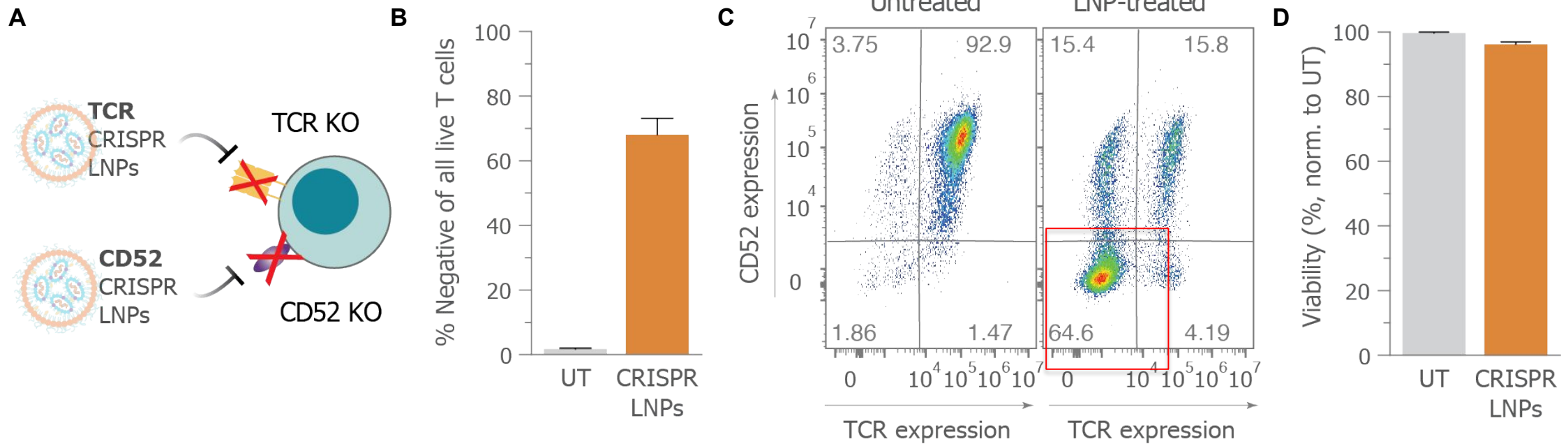
48 h  
detection



## 2 Single Target Knockout with High Efficiency and Cell Viability

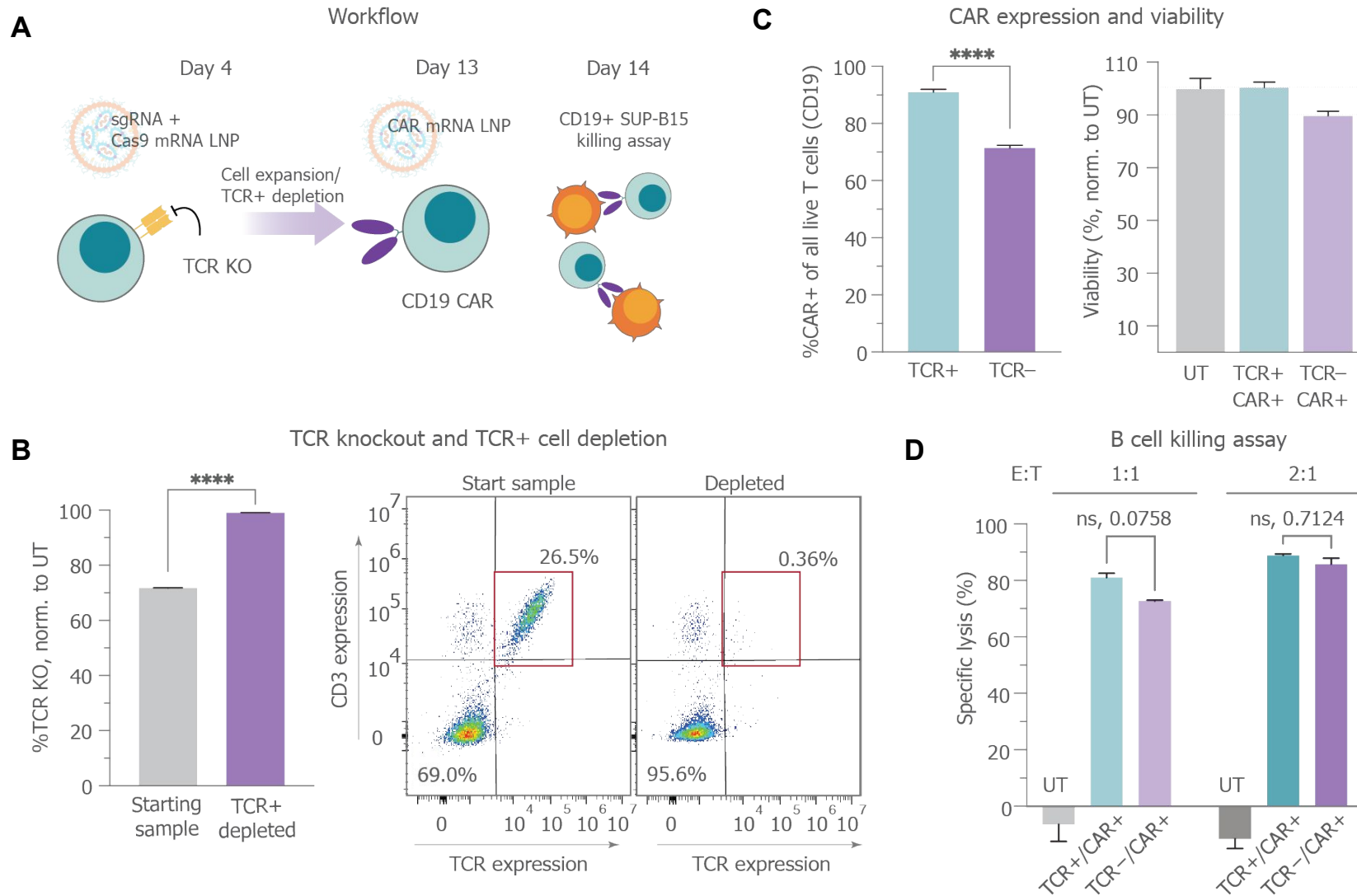


### 3 Double Target Knockout to Model Allogeneic Cell Therapy

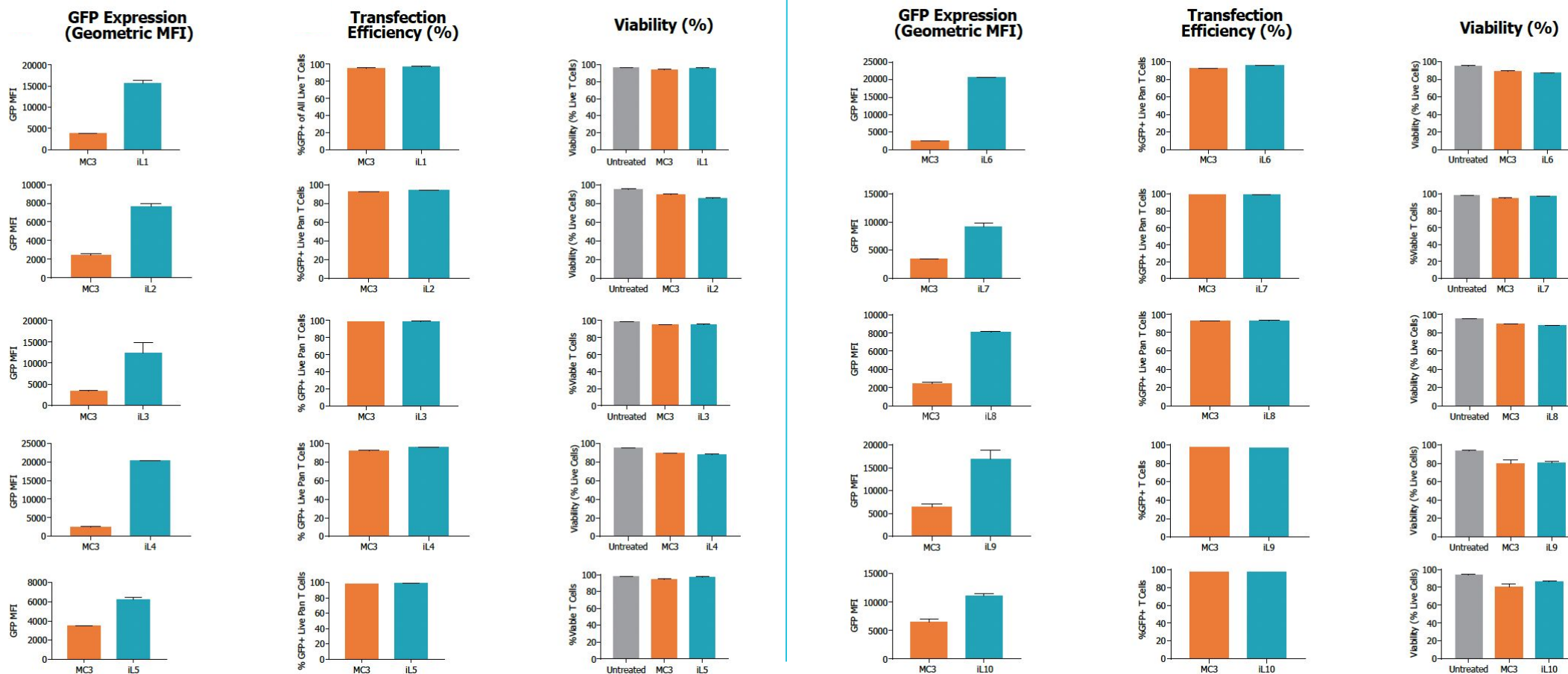




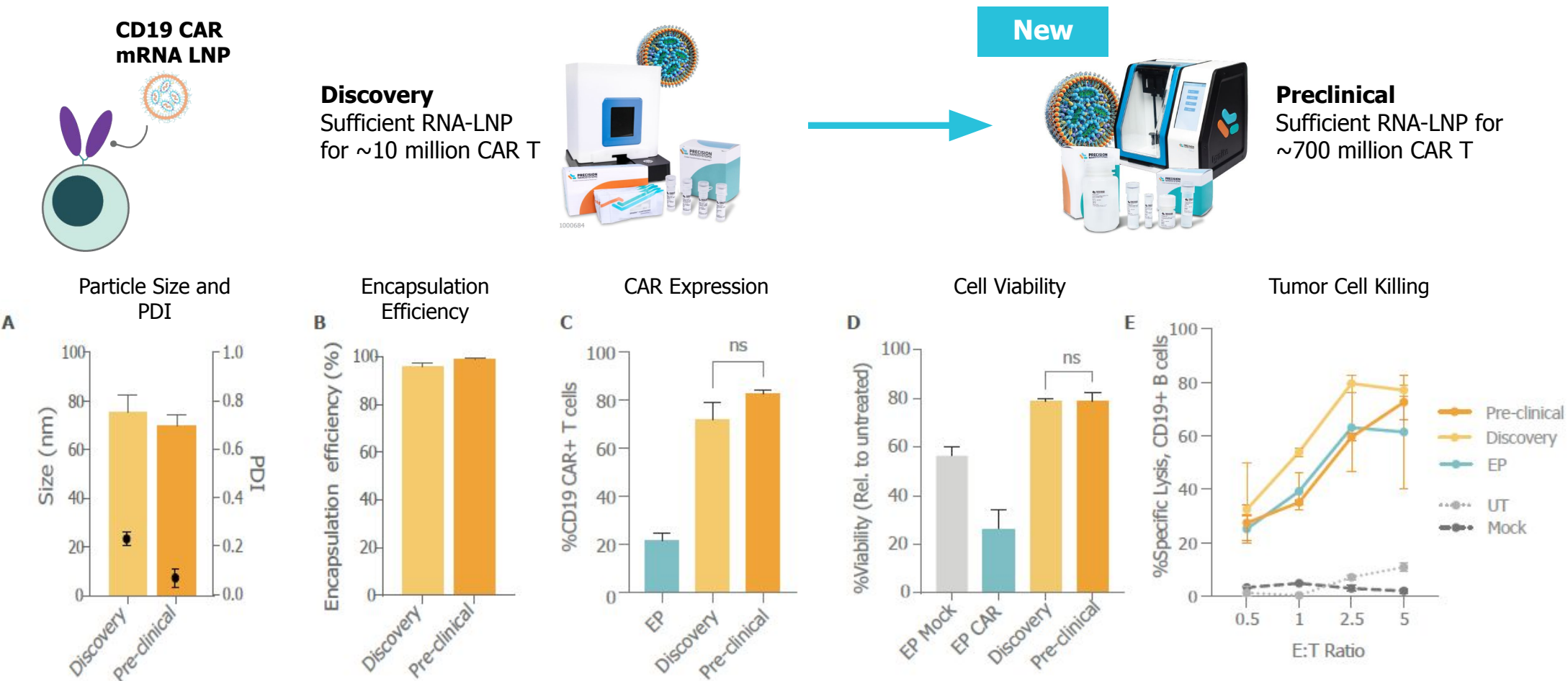
# 4 Multi-step Cell Engineering of Knockout Followed by Delivery



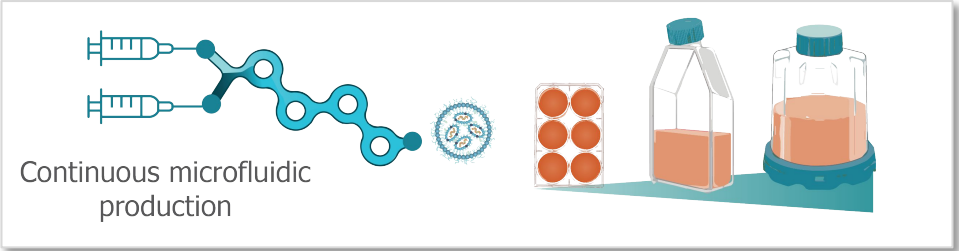
# GFP expression in primary human T cells using PNI proprietary Lipid & LNP composition



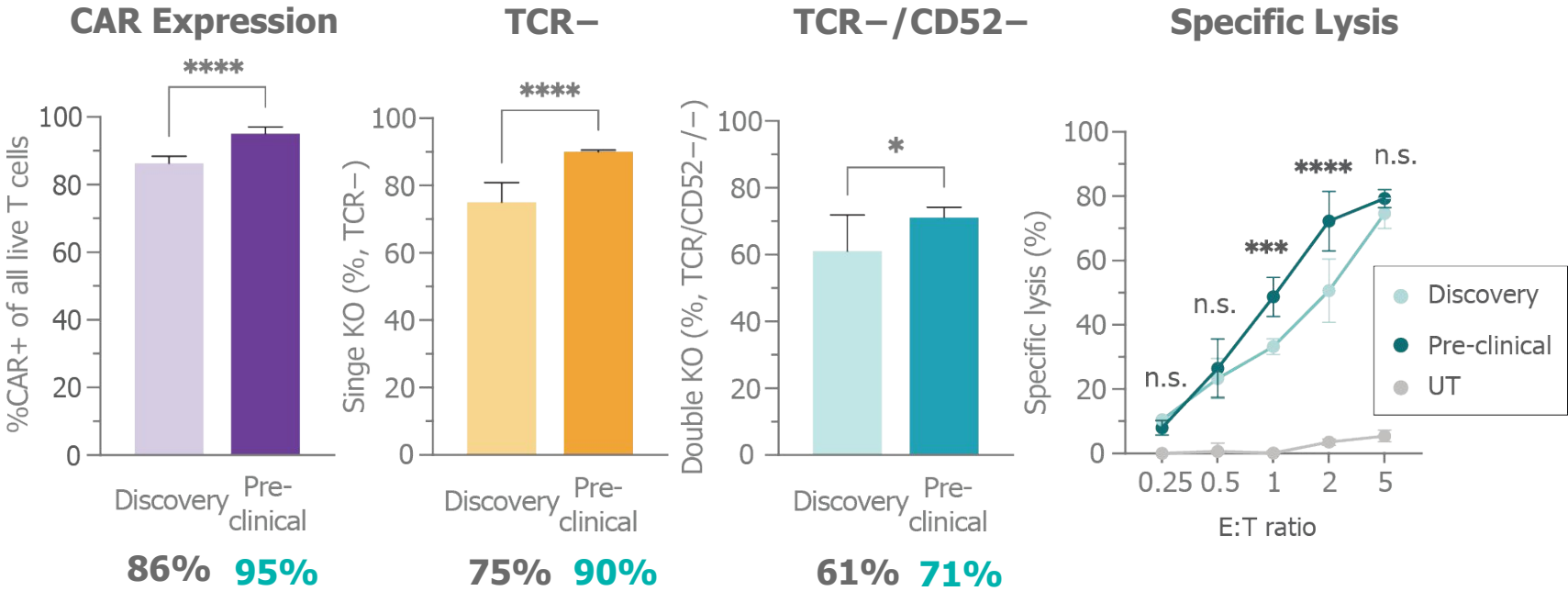
# Enable Gene Editing and Delivery in Human Primary T Cells using Lipid Nanoparticles: Seamlessly Scalable from Discovery to Preclinical



# Enable Gene Editing and Delivery in Human Primary T Cells using Lipid Nanoparticles: Seamlessly Scalable from Discovery to Preclinical



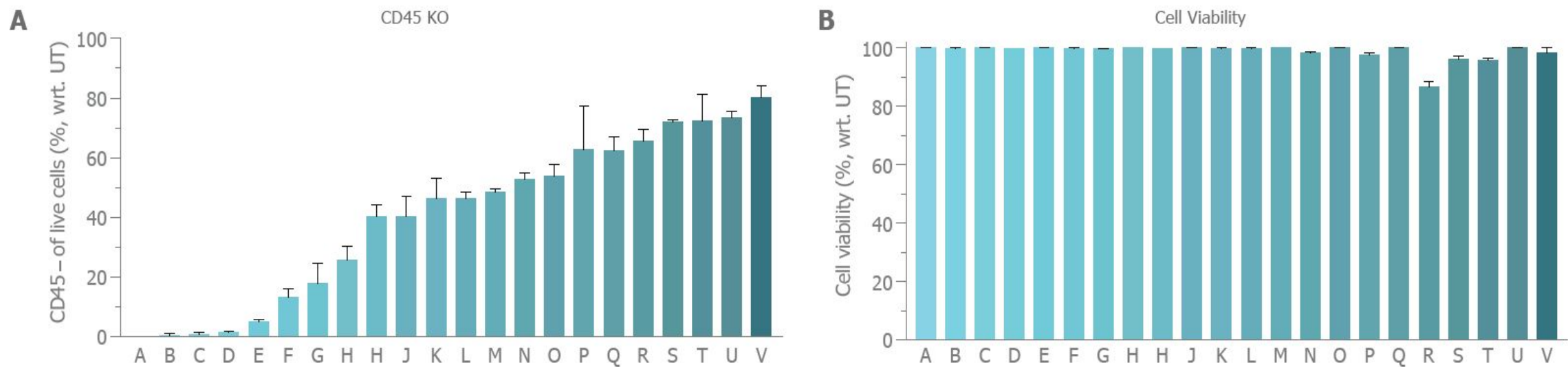
	Discovery Scale	Pre-clinical Scale
RNA wt.	10 µg	4 mg
LNP vol.	0.5 mL	18 mL
CAR T numbers	20 million	1 billion





# Expertise Drives Optimal LNP Formulations for New Applications

## LNP composition significantly affects CD34+ HSPC editing efficiency



**Figure 2.** Various LNP compositions were designed from a proprietary lipid library and screened to identify lead candidates. **A)** CD45 knockout efficiency and **B)** cell viability as analyzed by flow cytometry.

Optimized  
Pharmacokinetic  
Profiles

Leverage Precision NanoSystems expertise optimize delivery efficiency, potency, and biodistribution for novel genomic medicines

# Access to our Ionizable Lipids

# The "Lipid Menu"

## Ionizable lipid portfolio

PNI 1	PNI 2	PNI 3	PNI 4	PNI 5	PNI 6	PNI 7	PNI 8	PNI 9	PNI 10
PNI 11	PNI 12	PNI 13	PNI 14	PNI 15	PNI 16	PNI 17	PNI 18	PNI 19	PNI 20
PNI 21	PNI 22	PNI 23	PNI 24	PNI 25	PNI 26	PNI 27	PNI 28	PNI 29	PNI 30
PNI 31	PNI 32	PNI 33	PNI 34	PNI 35	PNI 36	PNI 37	PNI 38	PNI 39	PNI 40
PNI 41	PNI 42	PNI 43	PNI 44	PNI 45	PNI 46	PNI 47	PNI 48	PNI 49	PNI 50
PNI 51	PNI 52	PNI 53	PNI 54	PNI 55	PNI 56	PNI 57	PNI 58	PNI 59	PNI 60
PNI 61	PNI 62	PNI 63	PNI 64	PNI 65	PNI 66	PNI 67	PNI 68	PNI 69	PNI 70
PNI 71	PNI 72	PNI 73							

Biodegradable, and non-biodegradable lipids with diverse

**Category 3 "Exploratory"**  
Fit-for-purpose lipid formulation

**Category 2 "Ready to test"**  
LNP panels with in-house POC data

**Category 1 "Ready to use"**  
LNP formulation in RUO kits, can be licensed for clinical use

Client tested examples with preliminary positive results

Ocular delivery (intravitreal)  
Myeloid delivery (intravenous)

Vaccine use (intramuscular)  
Liver delivery (intravenous)

T-cell kit for *ex-vivo* use

# RUO Reagent Kits Optimized for NanoAssemblr® Systems

Discovery

Preclinical

Clinical

Commercial

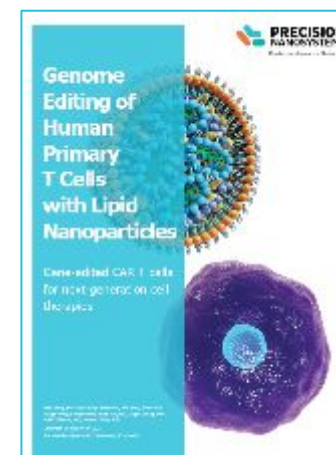
## Research Use Only Lipid Nanoparticle Reagent Kits

### GenVoy-ILM™ for Ignite

- Tool for trying LNP technology for the first time
- Benchmark for developing formulations

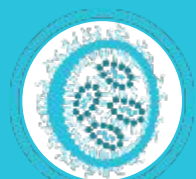
### T Cell Kit for Spark and Ignite

- Validated for CRISPR and multiplex gene editing



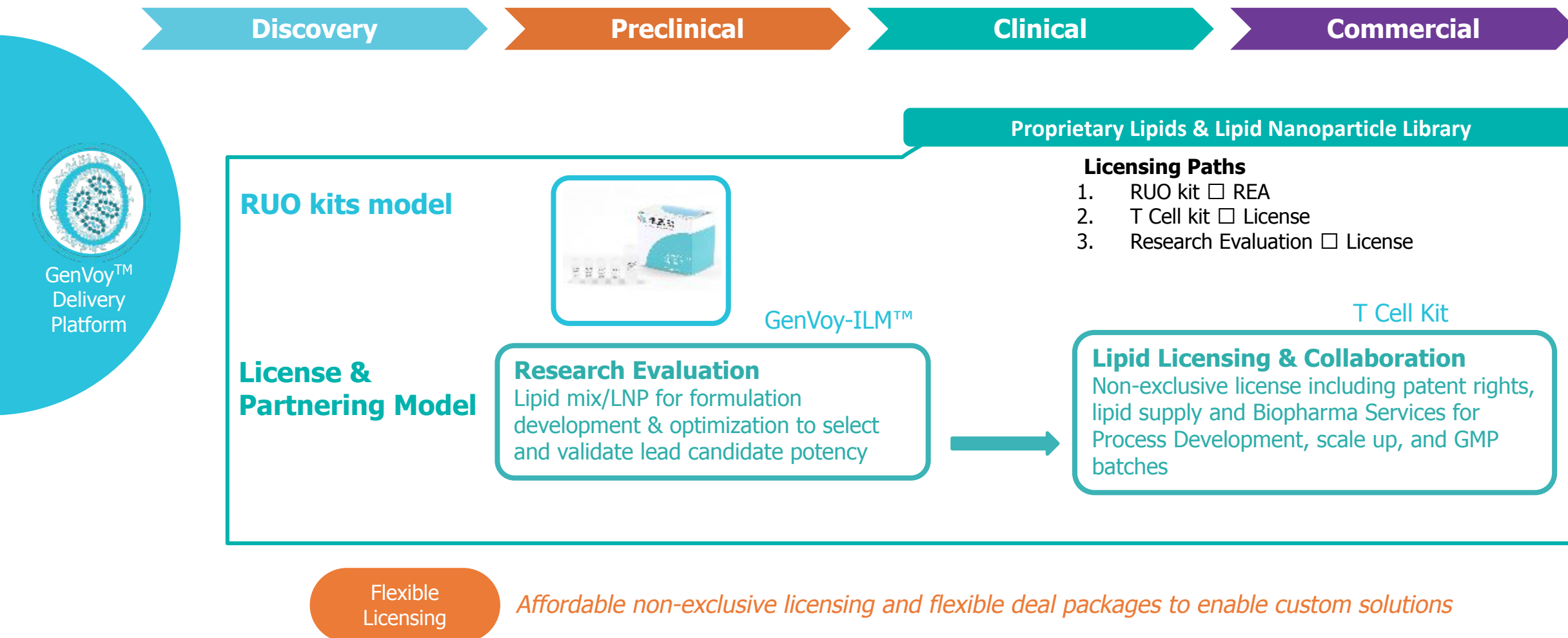
Off-the-shelf  
LNP kits

*Lowering the barrier to entry for developing genomic medicines by offering easy to use LNP kits with a clear path to the clinic*



GenVoy™  
Delivery  
Platform

# Proprietary Clinical Lipids & Lipid Nanoparticle Portfolio





# Selected Lipid Licensee & Collaborators

## Precision NanoSystems and Replicate Bioscience in licensing deal to scale up genomic medicines

NEWS PROVIDED BY  
[Replicate Bioscience](#) →  
Jul 26, 2022, 08:00 ET

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Precision NanoSystems

REPLICATE

(PRNewsfoto/Replicate Bioscience)

- Companies aim to support development of up to 15 new genomic medicine products
- Replicate's commercial licensing of lipid nanoparticle technology from Precision NanoSystems at foundation of deal

<https://www.newswire.ca/news-releases/precision-nanosystems-and-replicate-bioscience-in-licensing-deal-to-scale-up-genomic-medicines-888682943.html>

## Precision NanoSystems and Aurora Vaccines Join Forces to Accelerate the Development of Vaccine Candidate for Hepatitis C Virus

Thursday, July 14, 2022

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Vancouver, BC, July 14, 2022--(T-Net)--**Precision NanoSystems** (PNI), a leader in non-viral delivery of genomic medicines, announced that it has entered into a strategic collaboration agreement with Aurora Vaccines to accelerate the development and manufacture of its Hepatitis C vaccine candidate.

<https://www.bctechology.com/news/2022/7/14/Precision-NanoSystems-and-Aurora-Vaccines-Join-Forces-to-Accelerate-the-Development-of-Vaccine-Candidate-for-Hepatitis-C-Virus.cfm>



## End to End Solutions for Genomic Medicine Development

# Genomic Medicines are Complex and Require Specialized Reagents, Instruments, and Services

The development of genomic medicines is inherently complex



## Biopharma Services

Drug development expertise  
at all stages



## Payload— Genetic API

saRNA Platform



## GenVoy Delivery Platform

Proprietary PNI lipids to  
enable development from  
the bench to the clinic  
and beyond

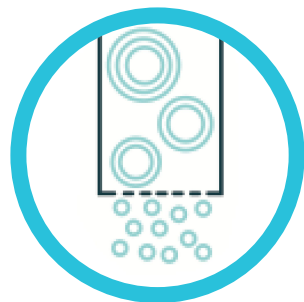


## NanoAssemblr Manufacturing

Scalable production for all  
stages of development

- Process Development
- Downstream Process
- Quality Control
- Safety Administration

# Nanoparticles can be made a number of ways



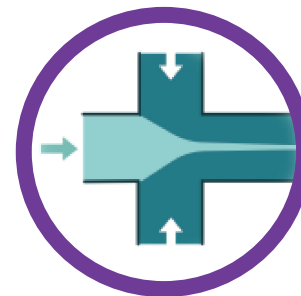
## Sonication/Extrusion

- Limited applications
- Difficult to reproduce
- Harsh process conditions
- Difficult to scale



## T-tube and Impingement Jet Macromixing

- Limited applications
- Difficult to reproduce
- Not suited for rapid development
- + Gentler process conditions
- + Demonstrated scale-up for limited applications



## Other Microfluidic Approaches

- Challenges scaling up
- Not designed for specific nanoparticle manufacturing
- + Expanded applications
- + Reproducible
- + Non-turbulent process conditions
- + Suited to small volume formulations



## NxGen Microfluidics

- + Easy to scale
- + Broad range of applications
- + Potential multi-mixer integration opens possibilities
- + Reproducible
- + Non-turbulent process conditions
- + **PNI's Second Generation Technology** compatible with series mixing and other complex architectures

**PNI/Pall NxGen technology comes with freedom of usage (IP protected)**

# Scalable Solutions from Research Through to Commercial



*Existing preclinical systems to accelerate drug development through de-risking MFG runs at bench scale*



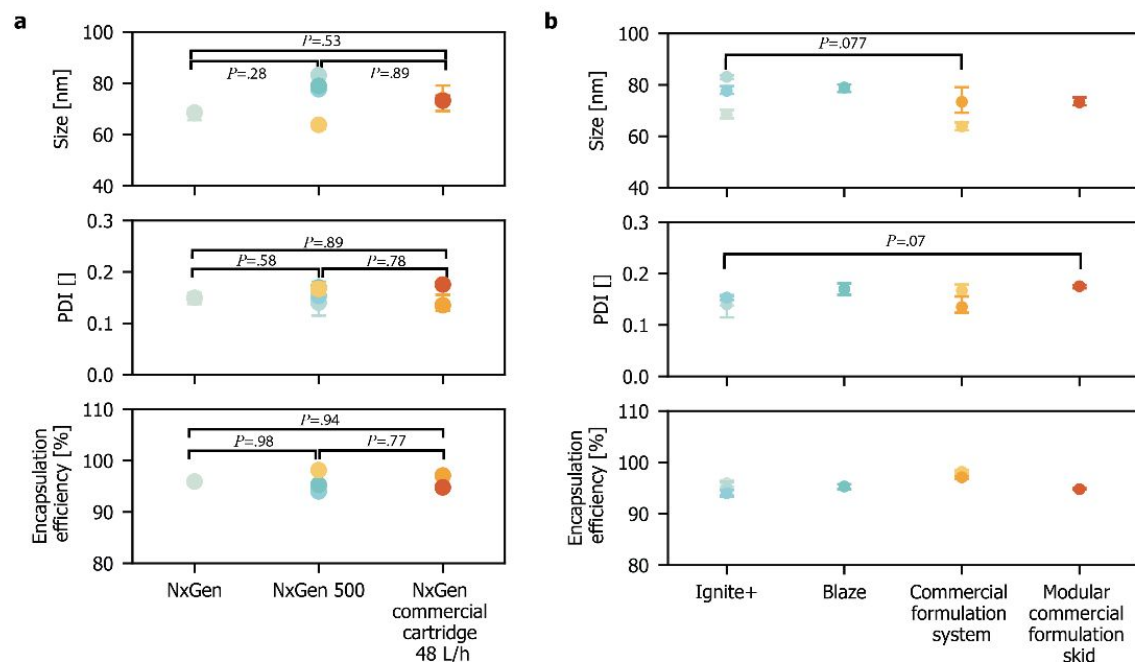
<b>NxGen™ Mixer</b>	NxGen™	NxGen™ NxGen™ 500	NxGen™ 400 NxGen™ 500	NxGen™ commercial cartridge 12 L/h* NxGen™ commercial cartridge 48 L/h
<b>Volume per Run^</b>	25 µL – 250 µL	1 mL – 60 mL	20 mL – 10 L	1L – 100 L (8.3hr) 1L – 400 L (8.3hr)
<b>RNA per Run#</b>		Up to 4.5mg Up to 13.5mg	Up to 2.3g	Up to 22.3g Up to 90g

**NxGen™ technology enables consistent mixing parameters across all scales**

\*NxGen™ commercial cartridge 12 L/h is equivalent to NxGen 500  
^Undiluted mRNA-LNP formulation  
#3:1 FRR, 0.3mg/ml RNA



# Consistent CQAs Following Downstream Processing

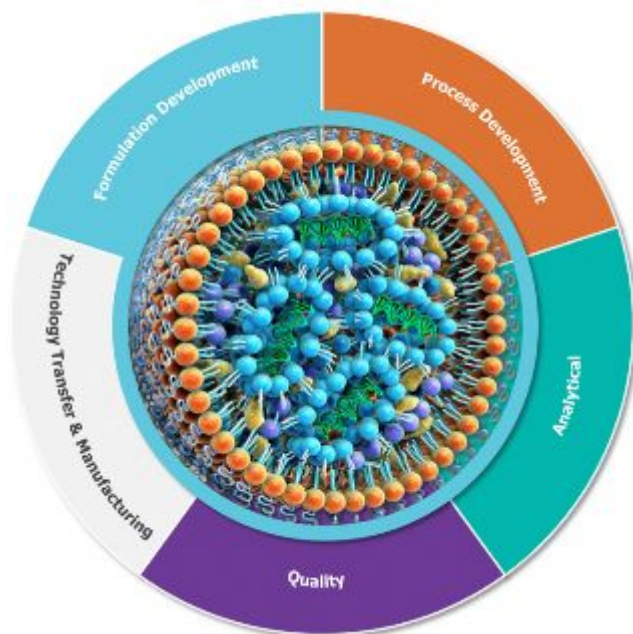


Condition	NanoAssemblr(r) system	NxGen mixer cartridge	Total flow rate [L/h]	Batch volume [mL]	RNA Encapsulated [mg]
1	Ignite+	NxGen	0.72	30	1.1
2	Ignite+	NxGen 500	6.9	30	1.1
3	Ignite+	NxGen 500	12	30	1.1
4	Blaze	NxGen 500	6.9	30	1.1
5	commercial formulation system	NxGen commercial cartridge 12 L/h [NxGen 500]	12	100	3.3
6	commercial formulation system	NxGen commercial cartridge 48 L/h	48	100	3.3
7	modular commercial formulation skid	NxGen commercial cartridge 48 L/h	48	150	5.0

- LNP size, PDI, and EE% post-formulation, dilution, TFF and sterile filtration are consistent across a range of flow rates on Ignite+, Blaze, GMP System and commercial systems

# Expertise & Capabilities for Developing Nucleic Acid Therapeutics

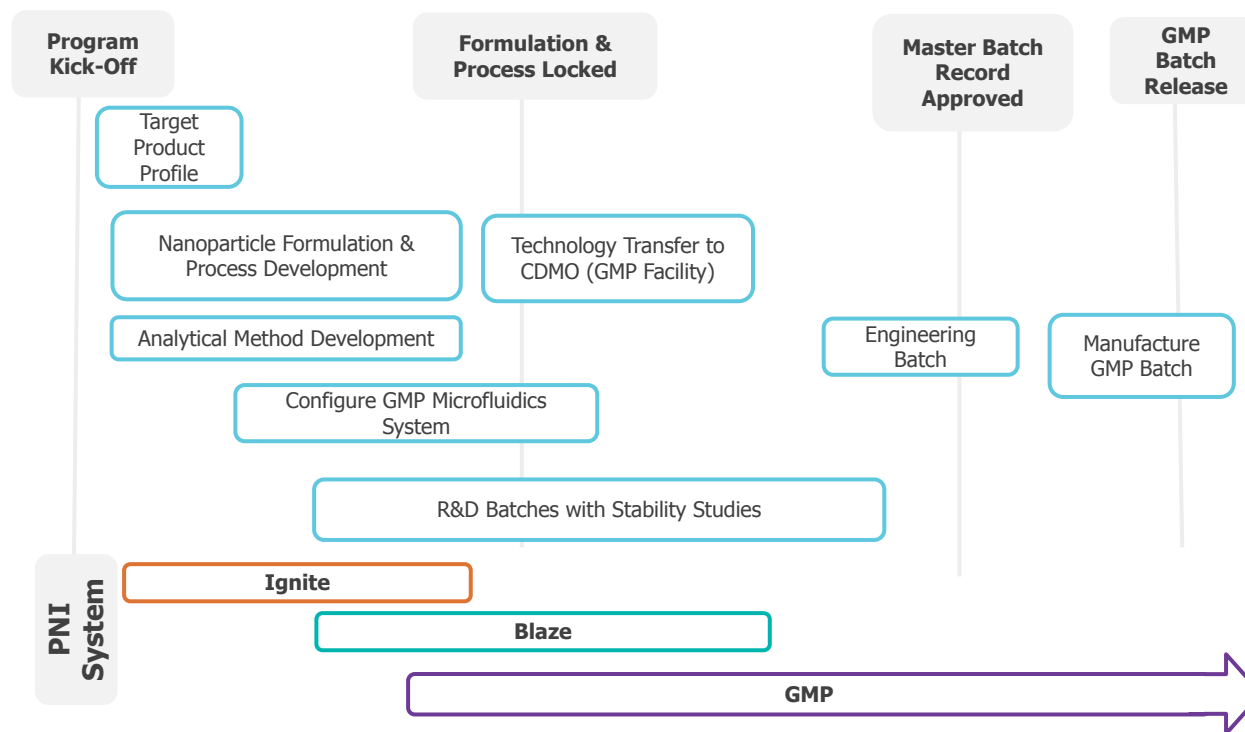
## Core Capabilities for mRNA-LNP Vaccine Development



Drug  
Development  
Expertise

Trusted Expertise

## Expertise Across the Entire Path to the Clinic



*Experience across a broad range of drug payloads and carriers. Cumulative experience on over 150 projects, 26 clinical stage programs, production of >44 GMP batches producing 2 approved drugs.*

# Unparalleled Formulation and Drug Development Expertise



Expertise

Value-Added Services and Expertise

## Formulation Development

- Formulation development to optimize product's physico-chemical properties and biological activity
- Identify critical quality attributes and draft drug product specifications
- Projects encompass a broad spectrum of payloads and delivery technologies

## Process Development

- Process transfer from Ignite™ and scale-up with Blaze™ and GMP
- Example projects include scale-up process development, nanoparticle purification, terminal sterilization processes, and design of experiment (DoE) studies to optimize manufacturing parameters

## Analytical

- Comprehensive analytical capabilities to support drug development, including genomic medicines
- Example methods include: UPLC (QQQ, CAD, PDA, ELSD) for lipids/drug substance, bioanalyzer for DNA, RNA, protein quantification & in vitro RNA potency assays

## Technology Transfer & Manufacturing

- Build custom GMP platform per client specifications and prepare demonstration batch at scale on GMP manufacturing system
- Prepare GLP-Tox batch & perform release and stability testing
- Provide relevant documentation & on site training

## Quality

- Support IQ/OQ of GMP manufacturing systems
- Assist with CMC regulatory submissions
- Programs supported by relevant Quality Management Systems (QMS) including Pharmaceutical Quality System for drug products and ISO-9001 (under development) for manufacturing systems

# Precision NanoSystems Biomanufacturing Centre – Coming Soon

1055 Vernon Drive, Vancouver, BC, Canada

## 2024 Biomanufacturing Centre



### New Headquarters

**Approx 75,000**

**sq.ft. 4<sup>th</sup> floor**

**Offices**

**3<sup>rd</sup> floor Labs and**

**Offices 2<sup>nd</sup> floor E**



**BMC – 2<sup>nd</sup> Floor**

**32,000 sq.ft. Launching H1, 2024**  
**Cleanrooms, Labs,**  
**Office, Warehouse**

- An end-to-end, turnkey facility offering genetic payload, lipid delivery, and NanoAssemblr manufacturing platforms
- Includes modular GMP cleanrooms for mRNA drug substance manufacturing and two suites for drug product manufacturing with solution preparation, drug compounding, and fill/finish facilities
- Controlled non-classified GMP space with QC lab, QC microbiology lab, QC stability and raw material storage chambers, process engineering lab, and controlled ambient storage with shipping and receiving area
- First GMP manufacturing line qualification expected in 2023

### Manufacturing capacities\*:

#### Clinical Formulation

- Clinical batches up to 20-50 L pre-dilution
- Formulated drug product with up to 10 g of RNA payload
- Filling capabilities up to 2400, 2-mL vials

#### Commercial Formulation

- Commercial batches up to 50-100 L pre-dilution
- Formulated drug product with up to 50 g of RNA payload



**\*Tentative estimations based on an  
saRNA LNP vaccine product**

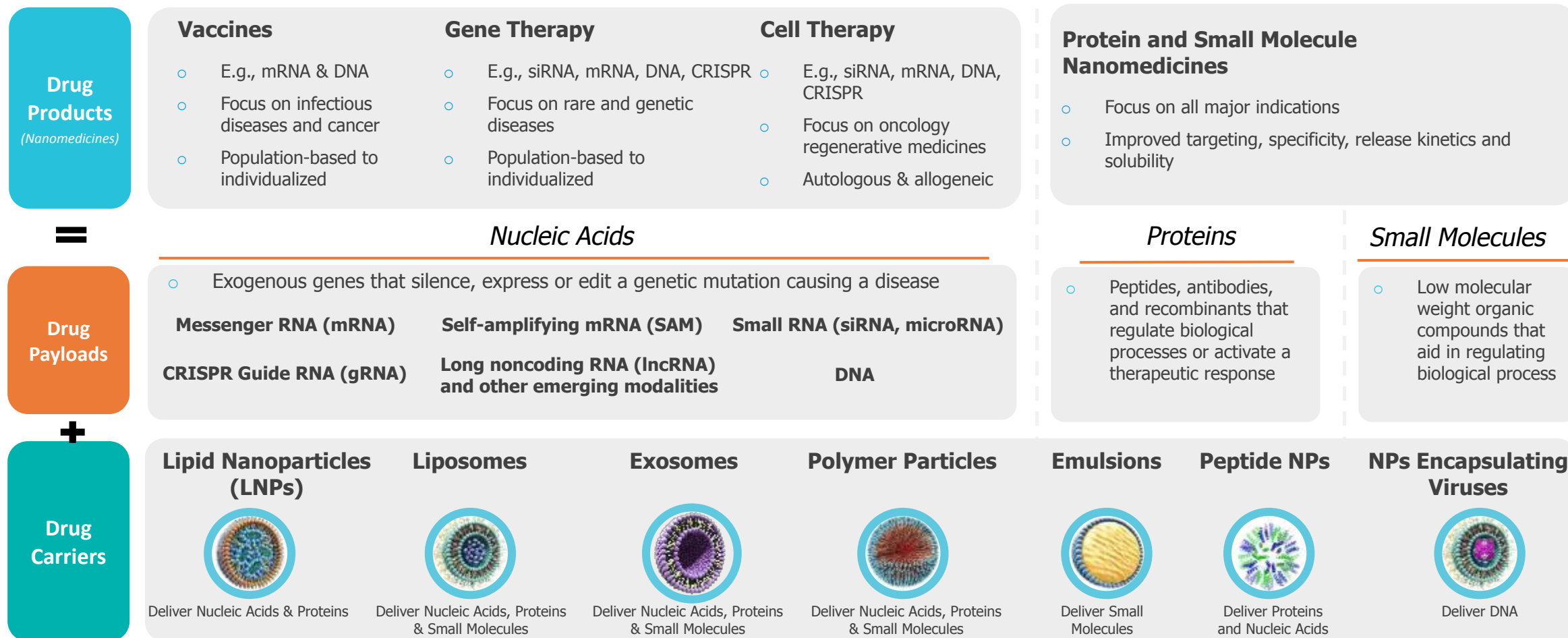




# Summary



# Precision NanoSystems Solutions Are Versatile

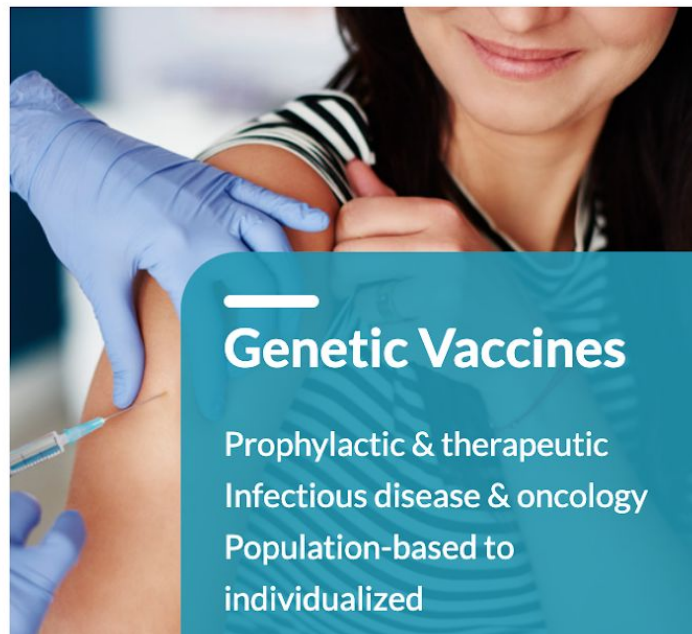


We are part of the Danaher Life Sciences family



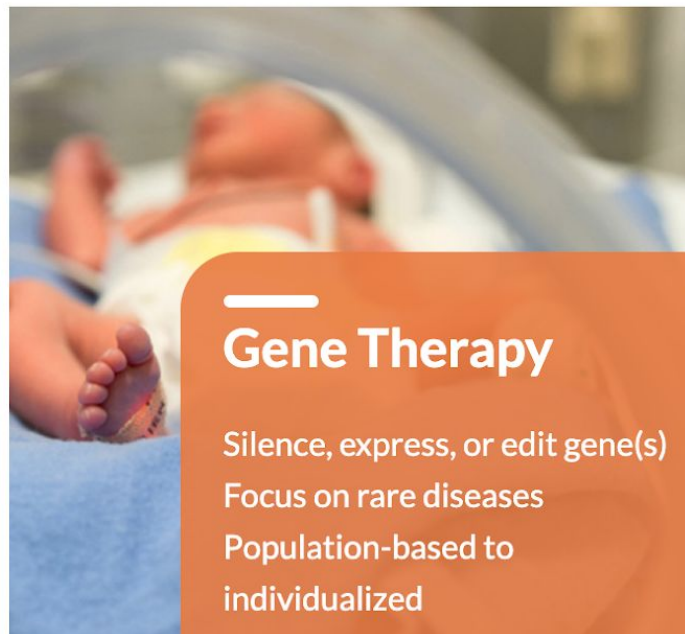
# Accelerating Tomorrow's Genomic Medicines

From idea to approved medicine.



## Genetic Vaccines

Prophylactic & therapeutic  
Infectious disease & oncology  
Population-based to  
individualized



## Gene Therapy

Silence, express, or edit gene(s)  
Focus on rare diseases  
Population-based to  
individualized



## Cell Therapy

Immune cells including T-Cells  
Focus on oncology  
Autologous & allogenic

These therapeutic modalities have broad application in the prevention and treatment of diseases including infectious disease, rare disease and cancer



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# Thank you for listening!

*Questions?*

*Visit us at Booth 310!*

