Tech Session 3: Immuno Delivery

Kunwoo Lee, Ph.D.
CEO and Co-founder
GenEdit

CRS 2022 Annual Meeting & Expo
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Advanced Delivery Science

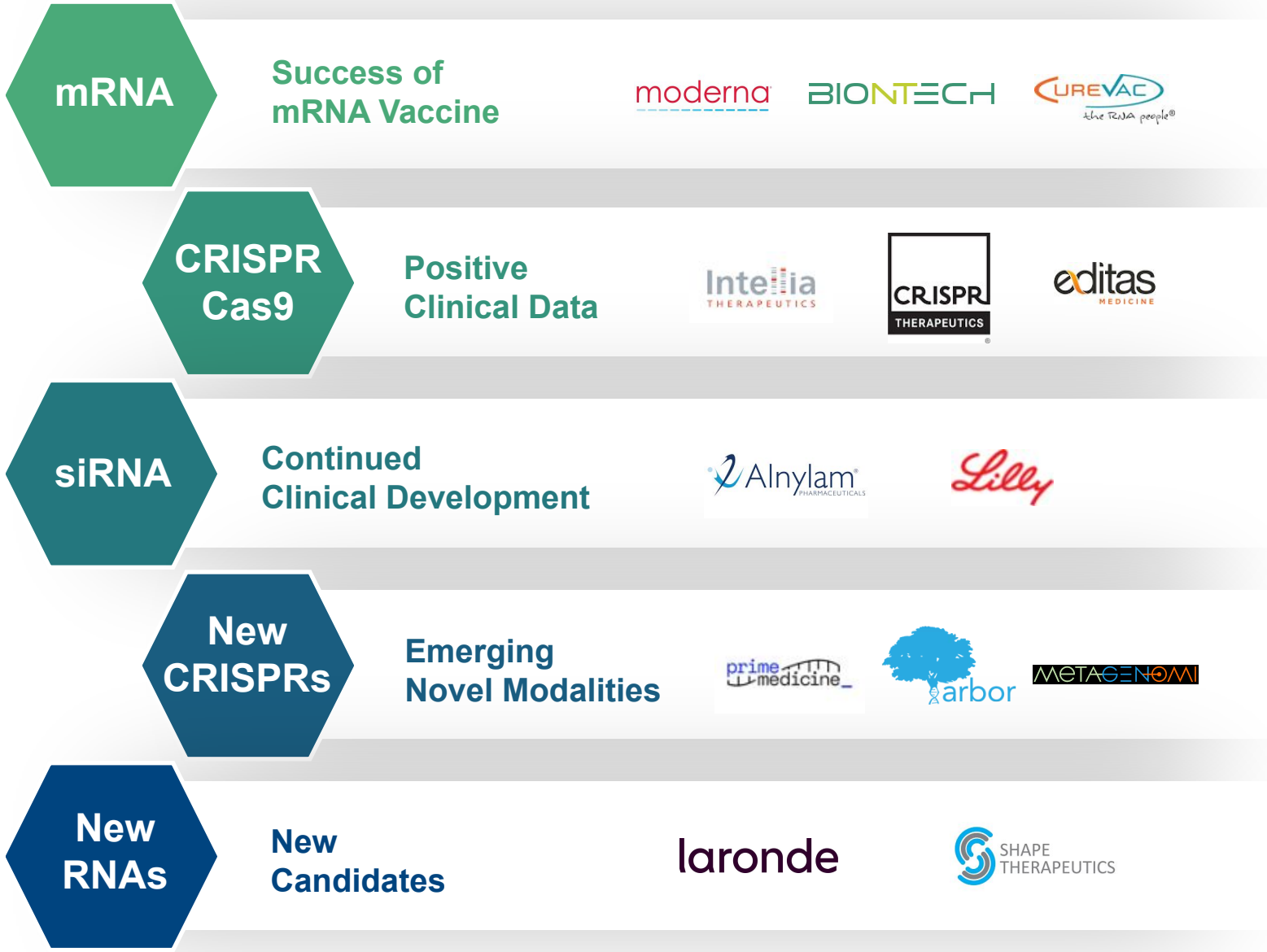
GENEDIT
GENE THERAPY DELIVERED

Delivery of Genetic Medicines with Non-Viral Polymer Nanoparticles

Kunwoo Lee, Ph.D.
CEO and Co-founder

Rise of New Genetic Medicine Modalities

But success is **Limited** to Vaccine and Liver Targeting



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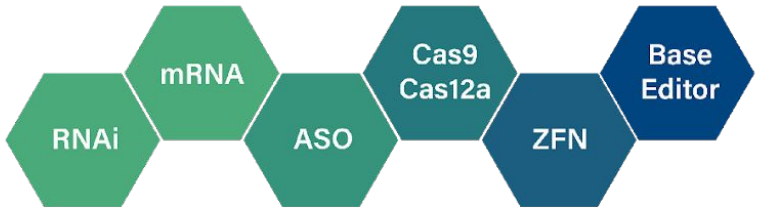
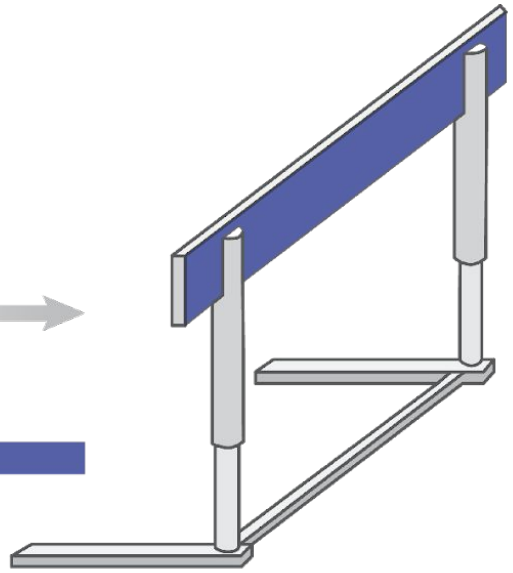
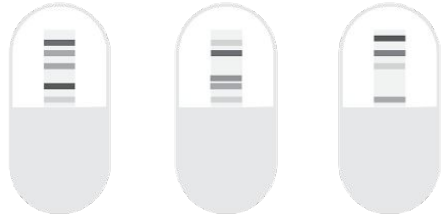
Genetic Medicine Has Potential to Treat Many Diseases

However, Delivery Is THE HURDLE







Payloads | **Delivery Challenge** | **Unrealized Therapy**

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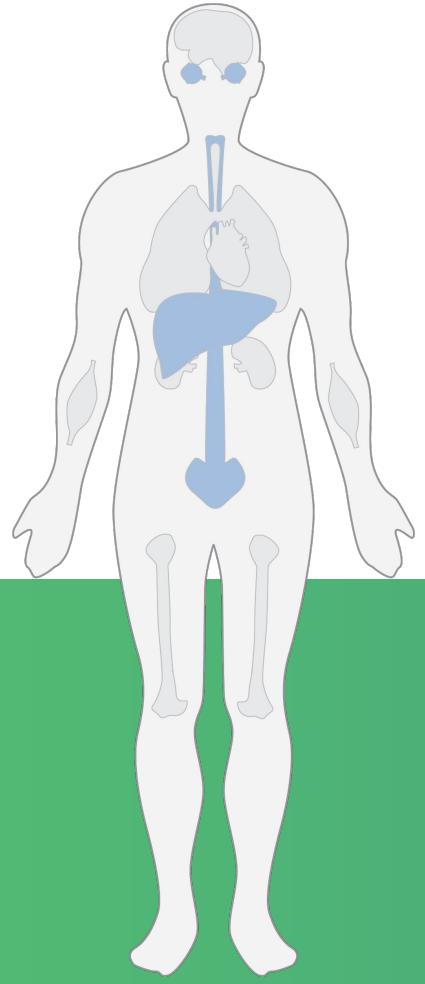
Conventional Delivery Technology Has Limitations

Major Challenges of Delivery Technology



Limited Delivery with Conventional Technology

1. **Tissue Selectivity**
2. **Payload Flexibility**
3. **Ability to Redose**
4. **Ease of Manufacture**

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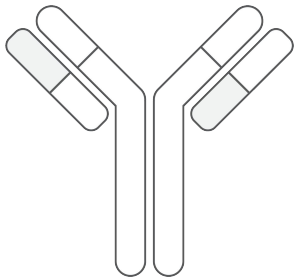
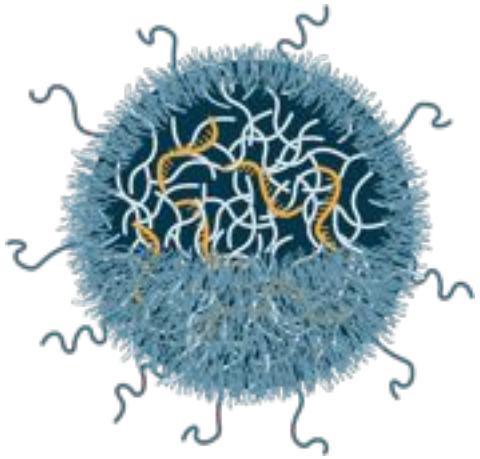
Screening of Hydrophilic Polymers Enables Selective Tissue Targeting

Hydrophilic Domains



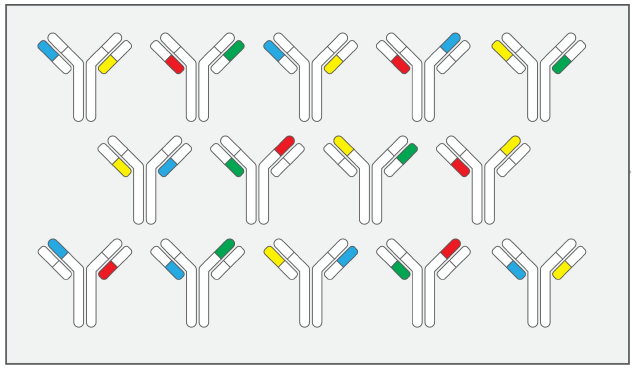
Antibody

Hydrophilic Polymer



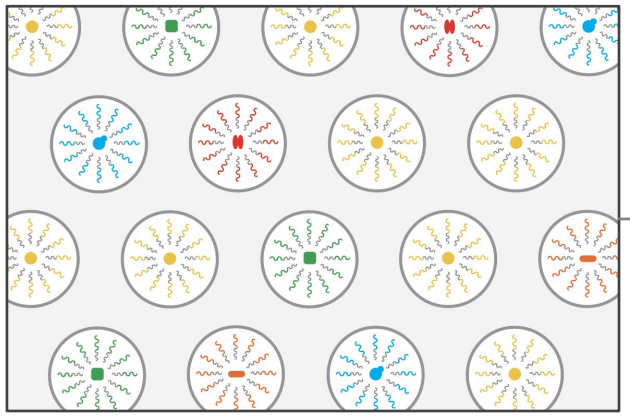
NanoGalaxy™ Polymer

Natural selection from Library

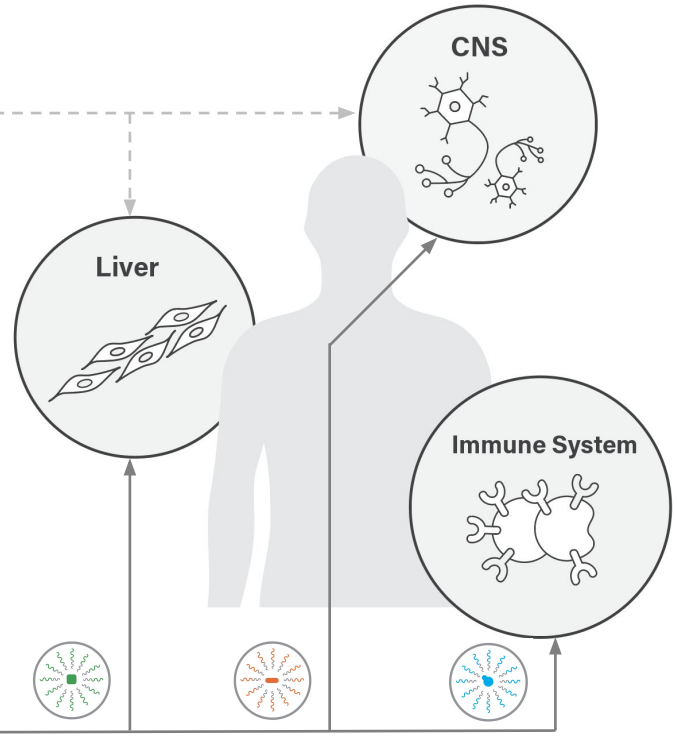


NanoGalaxy™ Polymer Library

with variations of physicochemical properties



Target Organs



Systematic screening and optimization enables selection of hydrophilic polymers for targeted delivery to specific organs

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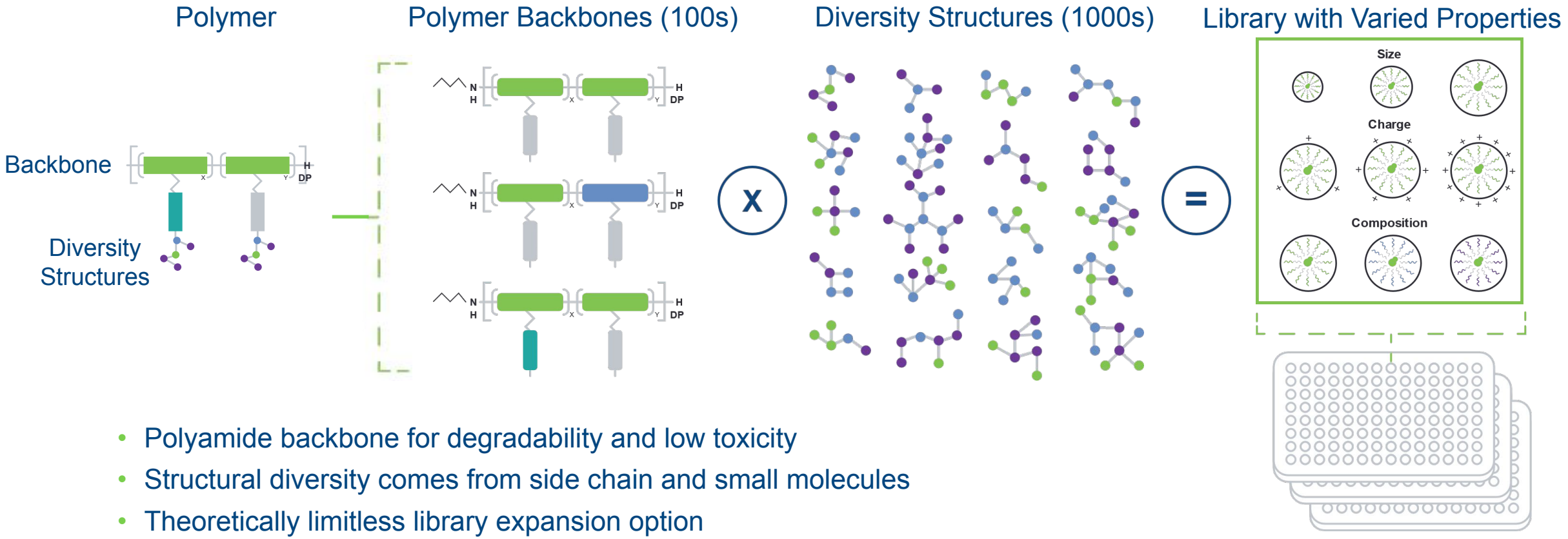
Diversity of Polymer Library Is the Key for Targeted Delivery

Optimized Chemistry Produces Diverse Set of Polymers in One Step



- Polyamide backbone for degradability and low toxicity
- Structural diversity comes from side chain and small molecules
- Theoretically limitless library expansion option

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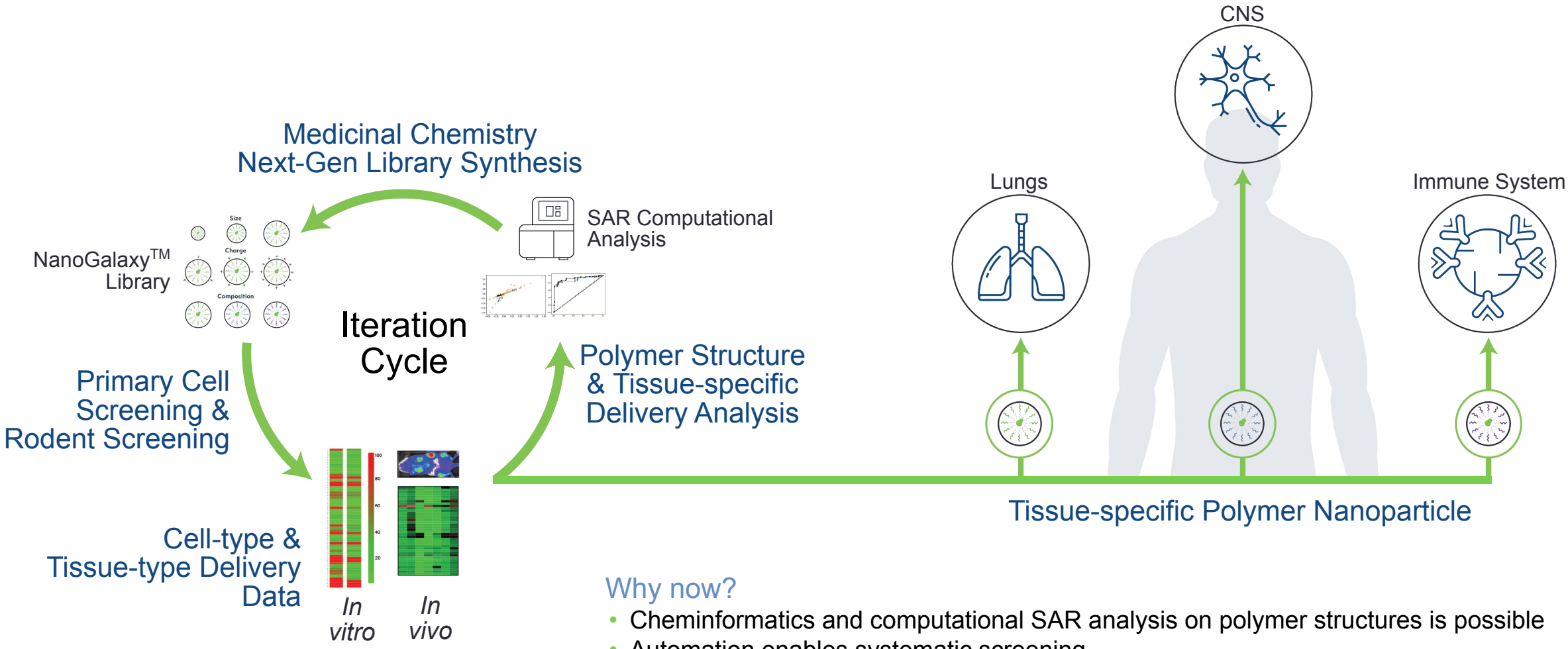
Iterative Screening Process Identifies Tissue-Selective PNPs



Why now?

- Cheminformatics and computational SAR analysis on polymer structures is possible
- Automation enables systematic screening

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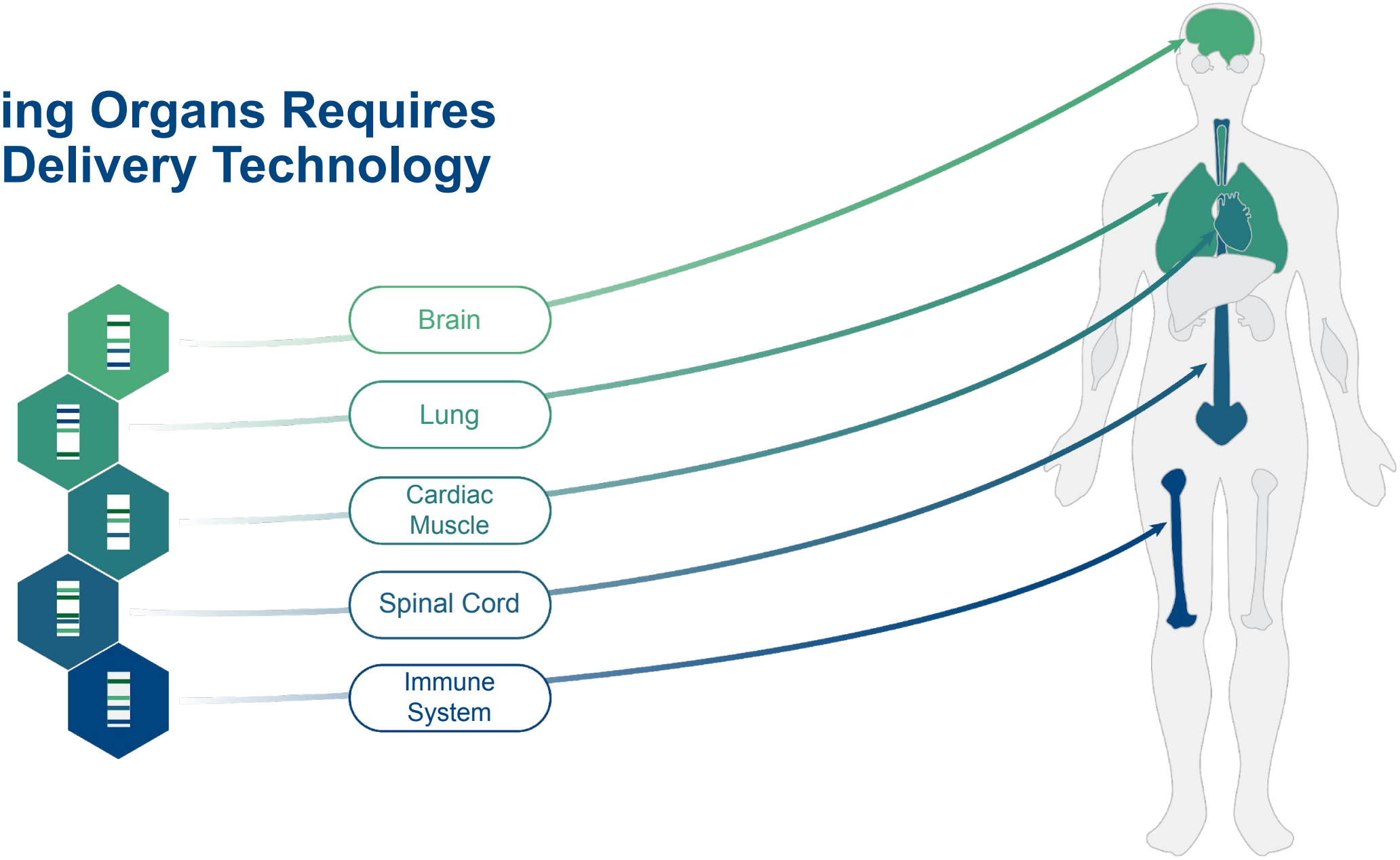
Unmet
Needs

Targeting Organs Requires Novel Delivery Technology



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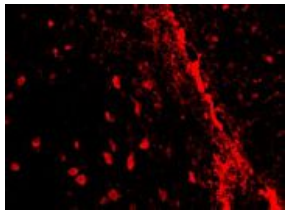
Delivery PoC to CNS, Immune system, and Lung

Tissue Selective Delivery of NanoGalaxy™

IT Administration

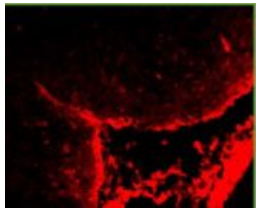
Brain

GEN-153



Hindbrain

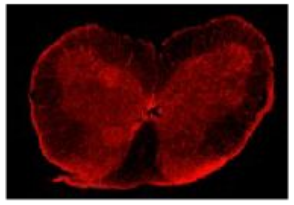
GEN-152



Ependyma

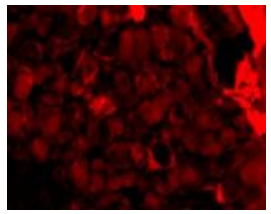
Spinal Cord

GEN-160

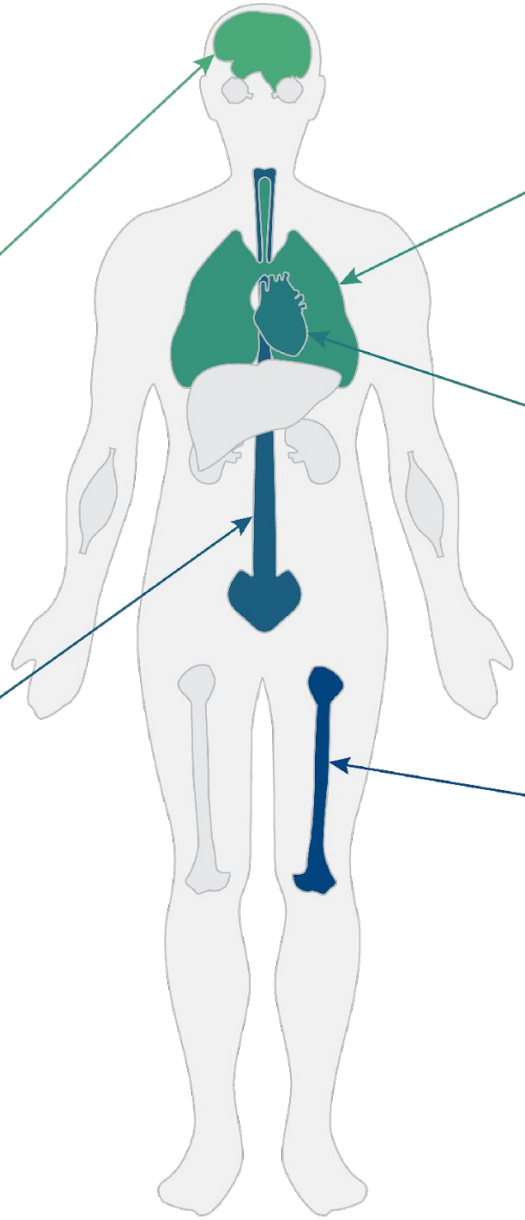


Spinal Cord

GEN-160

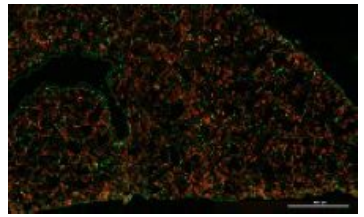


DRG



Lung

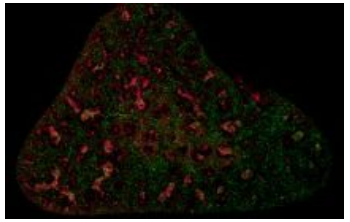
GEN-13, 27



Cardiac Muscle

Immune System

GEN-115



IV Administration

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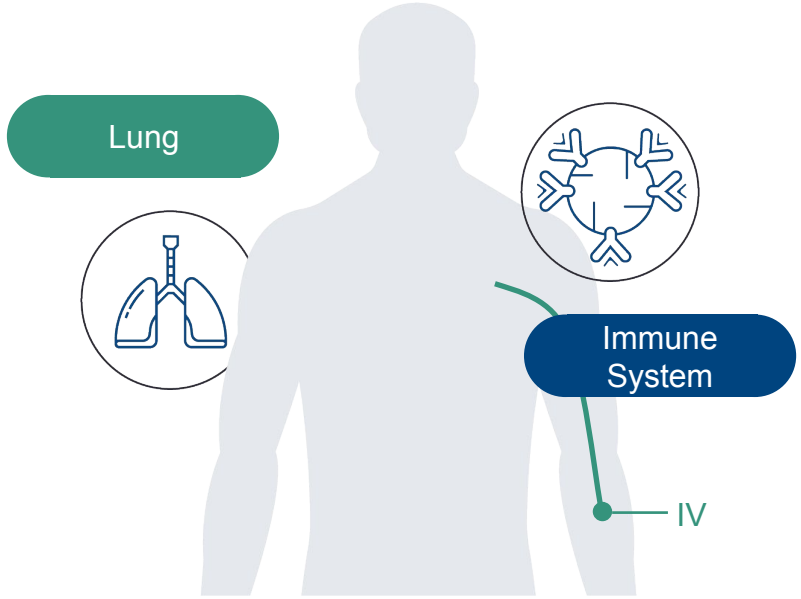
Demonstration of Targeted Delivery via IV Administration

Challenges

- Stability of PNP in serum
- Access the target tissue and de-targeting liver

Our approach

- Serum stable PNP selection
- Iterative screening of serum stable PNPs toward target organs



Results

- Delivery beyond liver and targeting other organs

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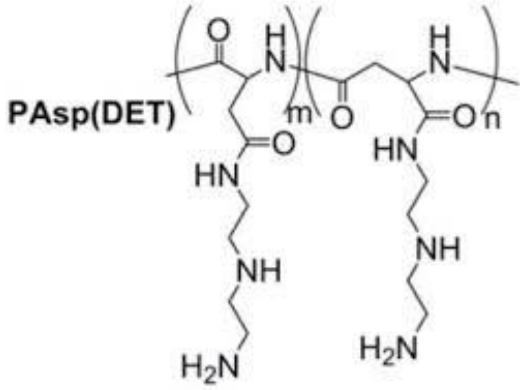
The First Step Is Making PNPs Serum-Stable

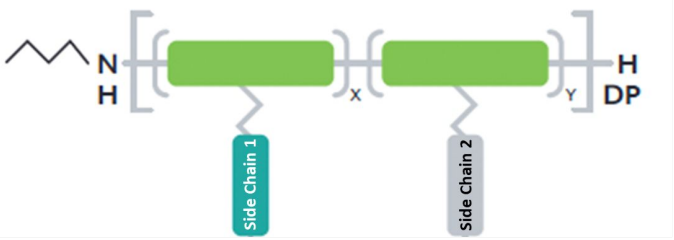
Inspiration



Kazunori Kataoka, Kanjiro Miyata

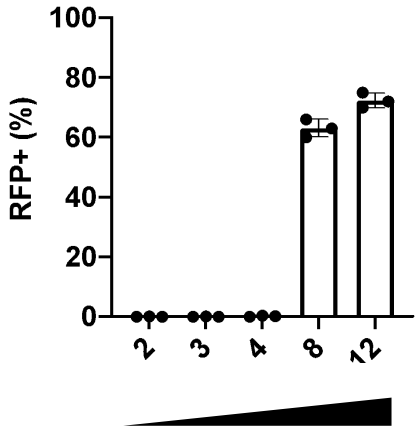
Challenge

- Stability of PNP in blood



Our approach

- Serum-stable PNP selection

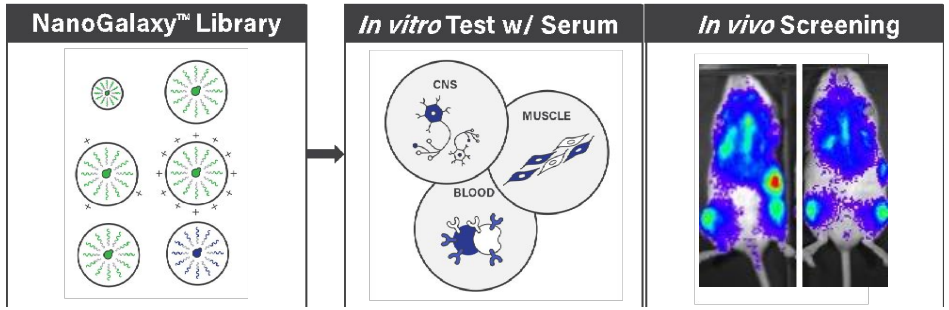


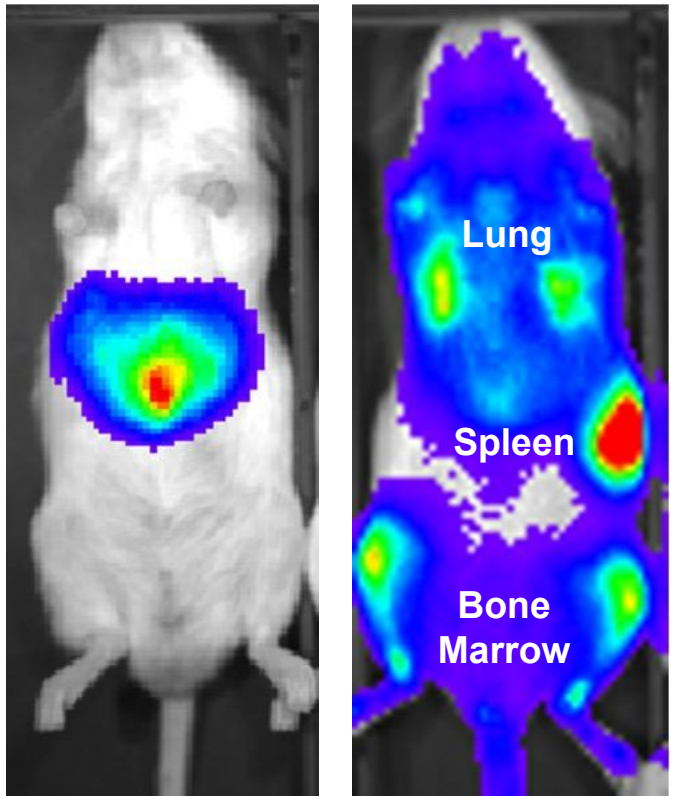
Transfection of RFP mRNA in serum-containing medium

- Screened side chain structures can add serum stability
- Side chain 2 shows serum stability once ≥8 chains are added

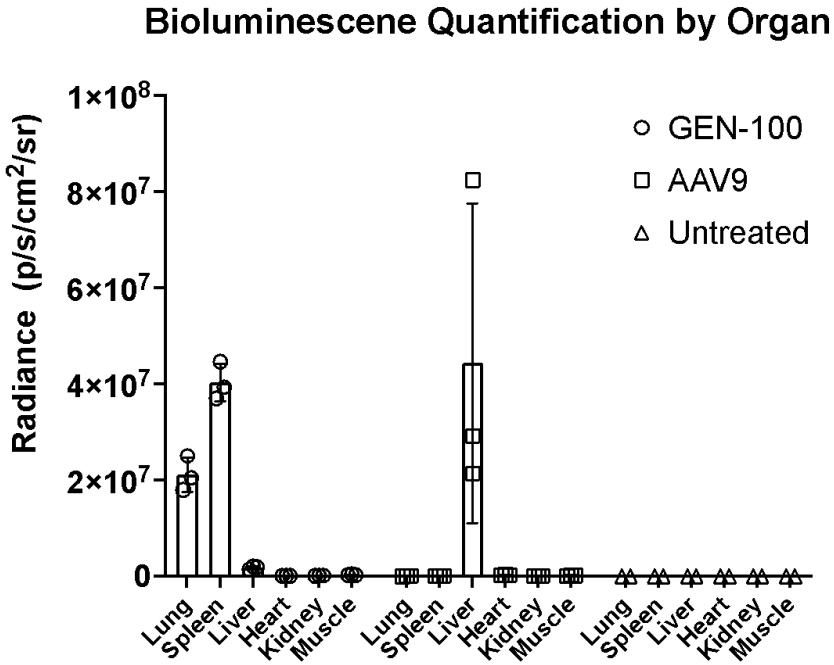
GENEDIT

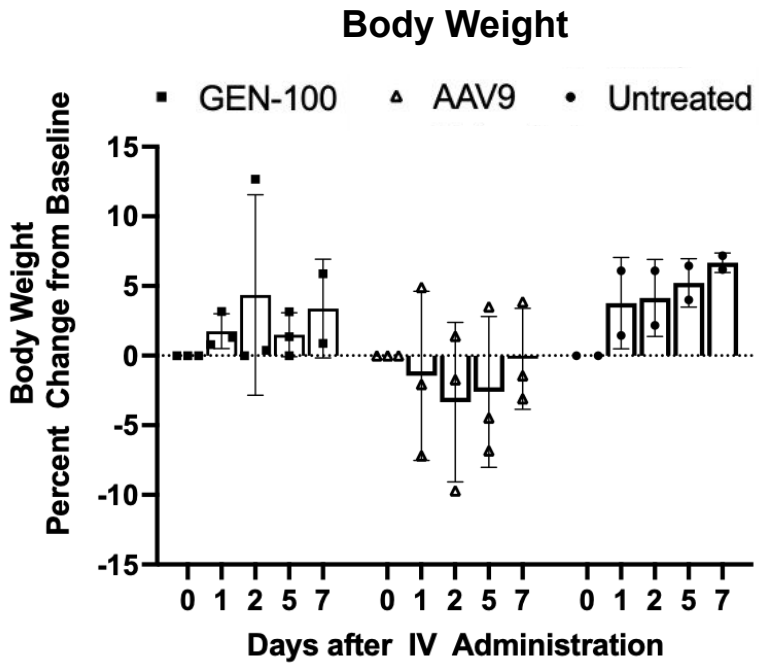
Systematic Screening Identified a PNP Delivering Outside Liver





AAV9 GEN-100

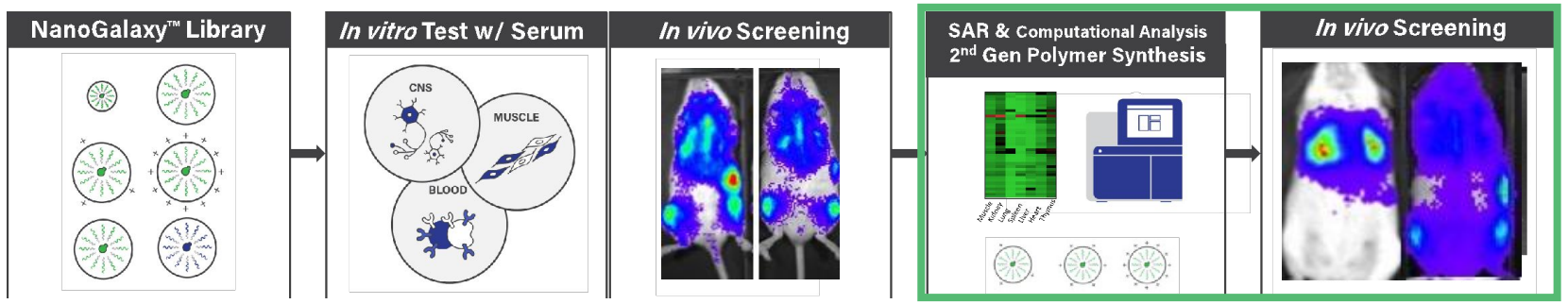


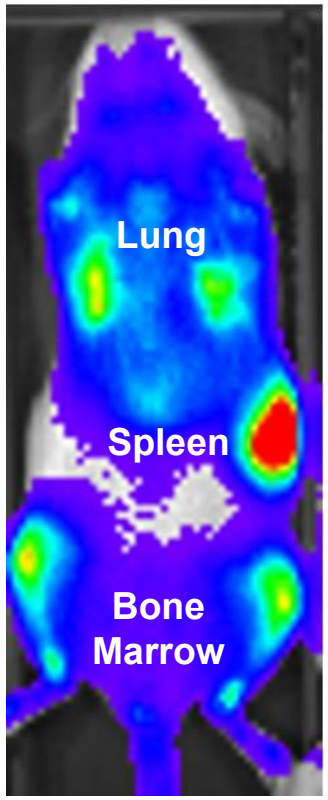


GEN-100: 2 mg/kg Cre mRNA
AAV9: 5e13 vg/kg

GENEDIT

Iterative Screening Tunes Tropism of PNP for Select Tissues

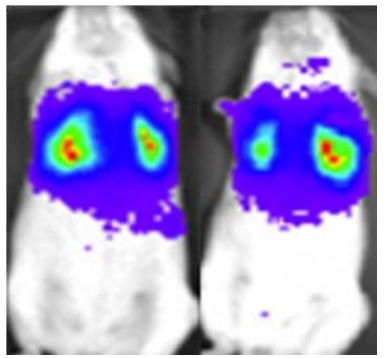




GEN-100

Medicinal Chemistry
2nd Gen PNP Screening

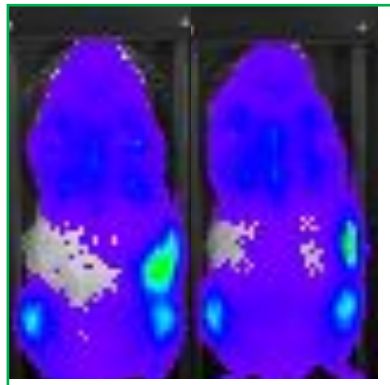
Property A
Focused Library



GEN-13

Reduced
Immune Delivery

Property B
Focused Library



GEN-115

Enhanced
Immune Delivery

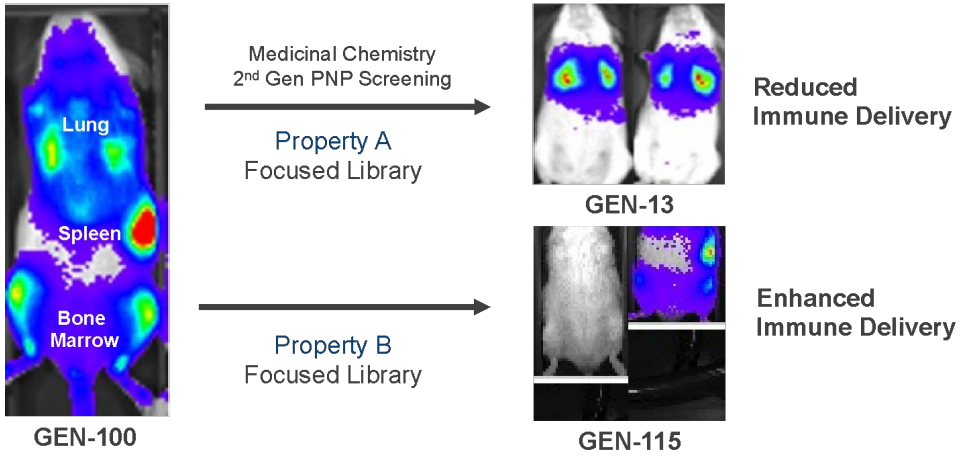
GENEDIT

Delivery in NHP with PNPs identified from Screening in Mouse

- Objective of the study is to test translation of PNP result in rodent to NHP
- Conventional technologies like AAV and LNP showed challenges in NHP delivery previously (rodent result does not guarantee NHP result)

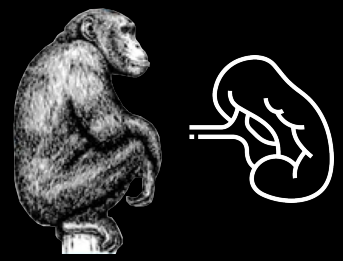
Study Details

- Bolus administration of single dose (2 mg/kg GFP mRNA)
- Tissue distribution histology at 24hr post-injection
- Preliminary assessment of tolerability of a single dose



GENEDIT

GEN-115 Delivers mRNA to Immune System in NHP



GEN-115

GFP
CD3

GFP mRNA (2 mg/kg dose) in Cyno (NHP)
Spleen harvested 24 hr after IV administration, IHC staining

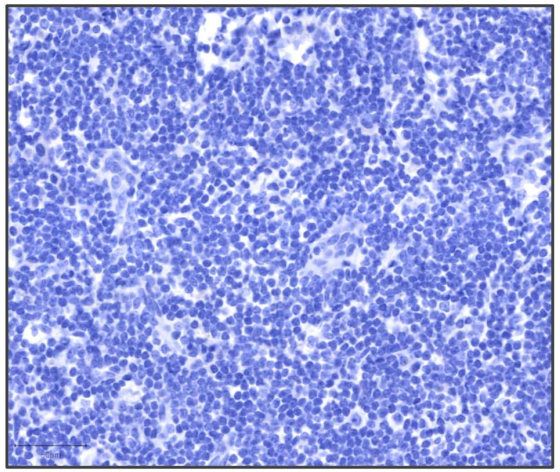
GENEDIT

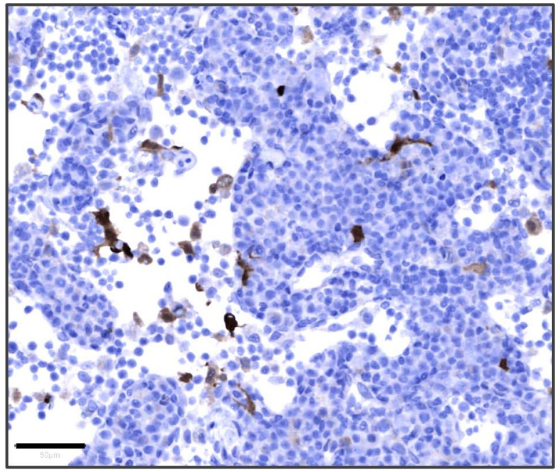
GEN-115 Delivers mRNA to Lymph Node But Minimally to Liver and Lung

Control

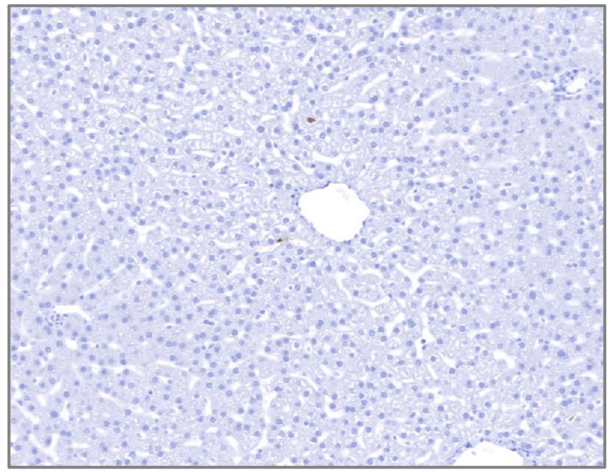
GEN-115

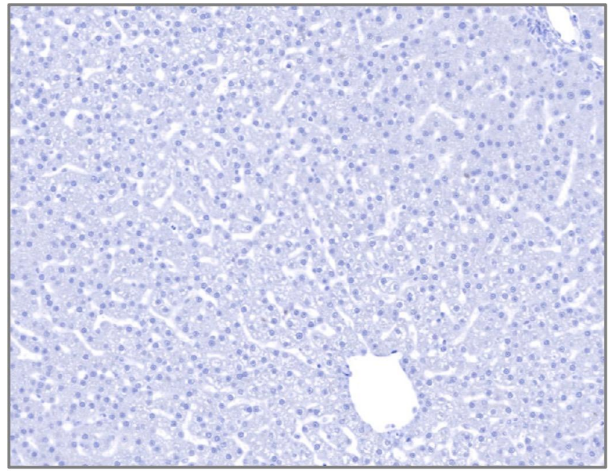
Lymph Node



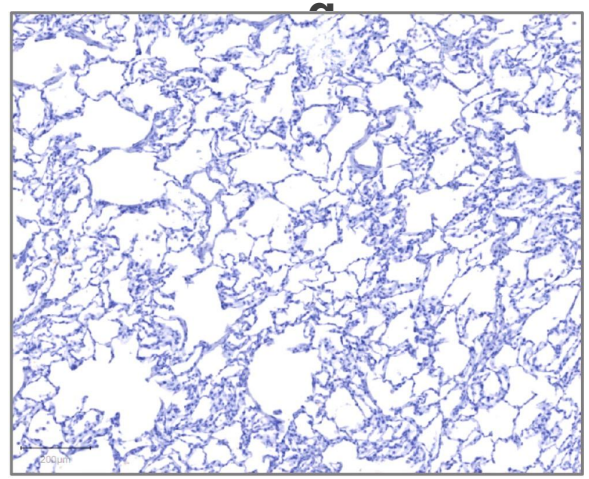


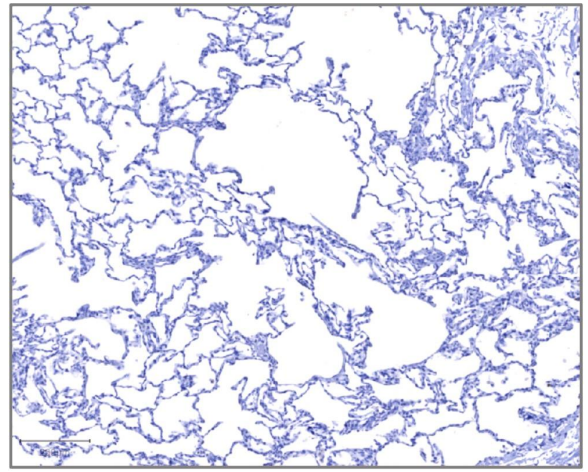
Liver





Lung

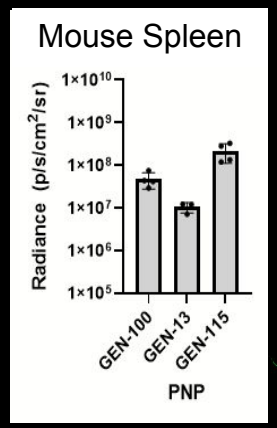




GFP mRNA (2 mg/kg dose) in Cyno (NHP)
Spleen, lymph node harvest 24 hr after IV administration, DAB staining, Scale bar: 50 µm

GENEDIT

Three PNPs Show Similar Delivery Trend in NHP to Mouse



Vehicle Control

GEN-13 Family

Reduce Immune Delivery

Enhance Immune Delivery

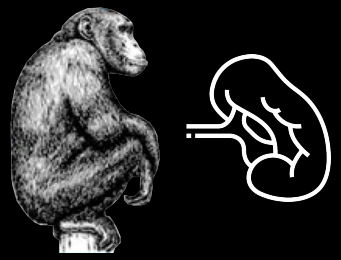
GEN-1 00

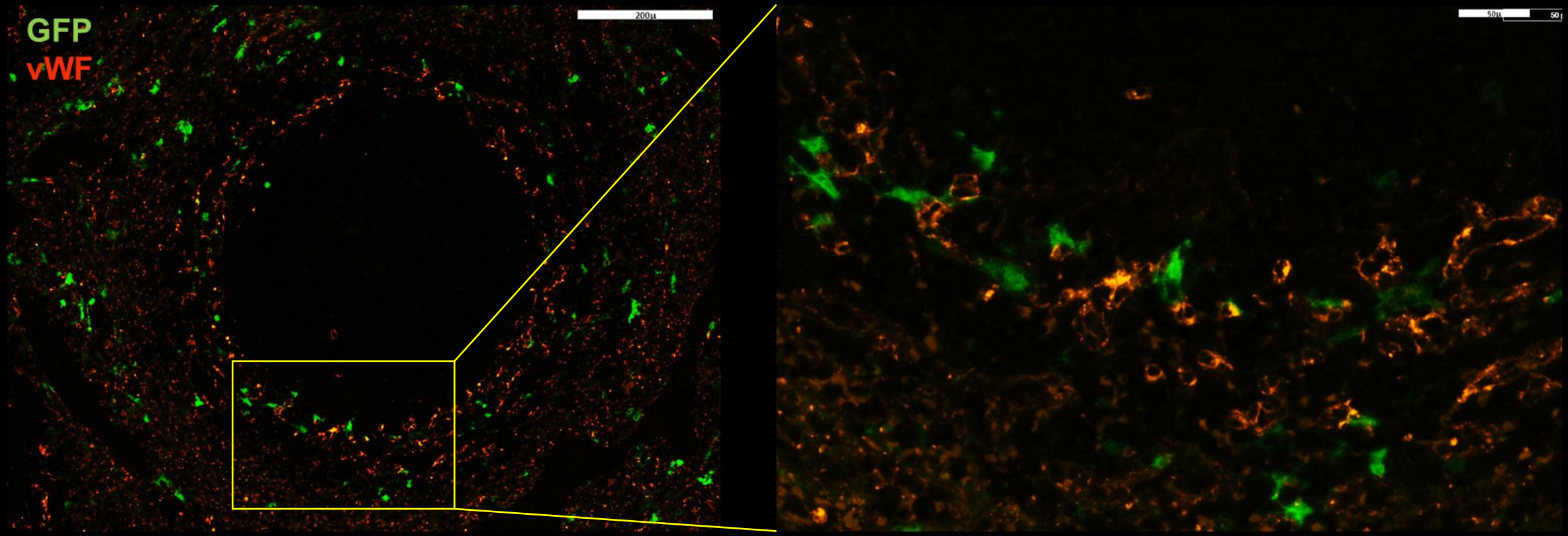
GEN-1 15

GFP mRNA (2 mg/kg dose) in Cyno (NHP)
Spleen harvested 24 hr after IV administration, IHC staining

GENEDIT

No Significant Co-staining of GFP with vWF Endothelial Cell Marker

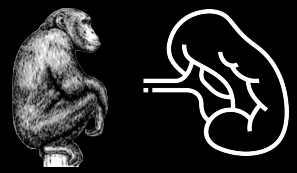


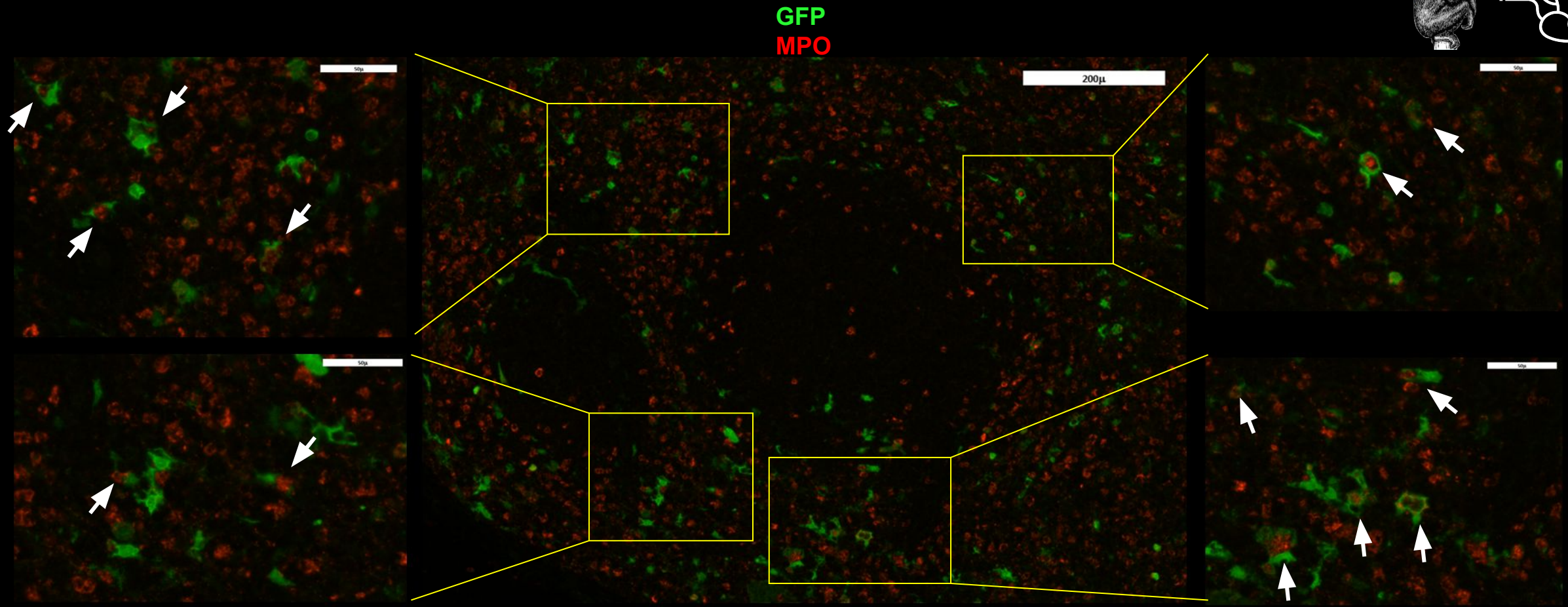


Single injection of GEN-115 transfected some endothelial cells in the marginal zone

GEN-115 Delivers to Splenic Macrophages/Myeloid Cells in NHP

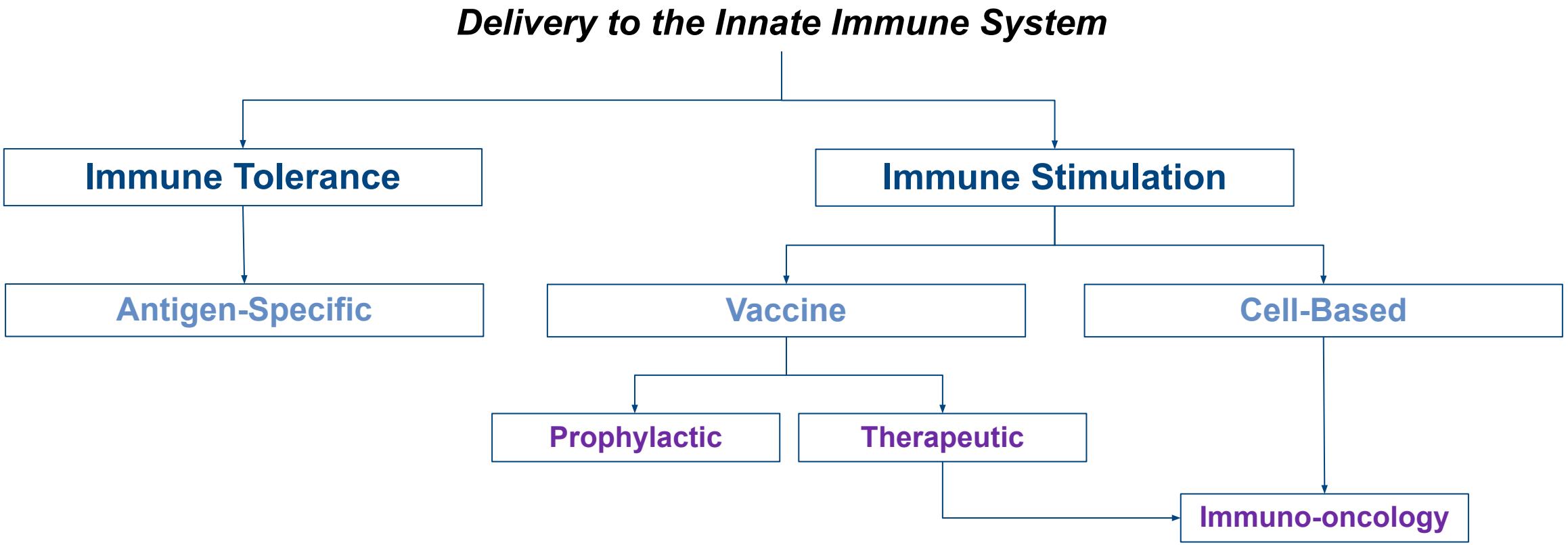
Co-staining of GFP$^+$ cells with MPO observed throughout the spleen





GFP$^+$ MPO$^+$ cells are highlighted (arrows)
MPO protein is found in granules and IHC staining results in a punctate red pattern within the cytoplasm surrounded by GFP (green)

Opportunities for Immune Tolerance and Stimulation

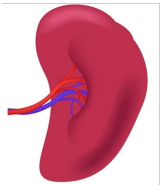


GENEDIT

Further Iteration from GEN-115 Is On-going

New PNPs Show Enhanced Delivery or New Target Tissues

PNP	% T cells ($CD3^+$)	% B cells ($CD19^+$)	% NK cells ($CD335^+$)	% Macrophages ($F4/80^+$)	% DC ($CD11c^+$)	% Endothelial ($CD45^{neg}CD31^+$)
GEN-1016	0.5 ± 0.2	0.5 ± 0.2	2.4 ± 0.8	14.9 ± 2.8	2.3 ± 1.2	2.0 ± 0.8
GEN-1021	0.2 ± 0.1	0.2 ± 0.0	3.2 ± 3.3	12.2 ± 2.0	2.9 ± 0.6	1.1 ± 0.5



Spleen

PNP	% Total Live RFP+ cells	% Live RFP+ HSC
GEN-1016	0.78 ± 0.20	3.07 ± 1.04
GEN-1021	0.30 ± 0.07	1.83 ± 1.59

Bone marrow

- Conducting additional iteration generated new PNPs like GEN-1016 and 1021 that show enhanced delivery to Macrophages, NK cells, and dendritic cells in spleen
- Additionally, they show potential hematopoietic stem cell (HSC) delivery in bone marrow

cre mRNA (2 mg/kg dose) in ai9 mouse
Spleen and bone marrow harvested 10 days after IV administration tissue and flow cytometry to identify target cells

Summary of Delivery with GEN-115 in NHP

- Immune-specific *in vivo* delivery translation from mouse to NHP demonstrated with GEN-115

- GEN-115 shows efficient delivery to hematopoietic tissues with low levels of delivery to other organs

- No signs of significant sustained acute tolerability issues

Various Payload Can Be Loaded to PNP

1

Tissue Selectivity

Ability to diversify polymers and assess SAR enables identification of targeted polymers

2

Payload Flexibility

Hydrophilic polymers are compatible with all nucleic acid and ribonucleoprotein payloads

3

Ability to Redose

Low potential immunogenicity and degradable backbone

4

Ease of Manufacture

Fully chemically synthesizable, scalable formulation, water soluble and lyophilizable

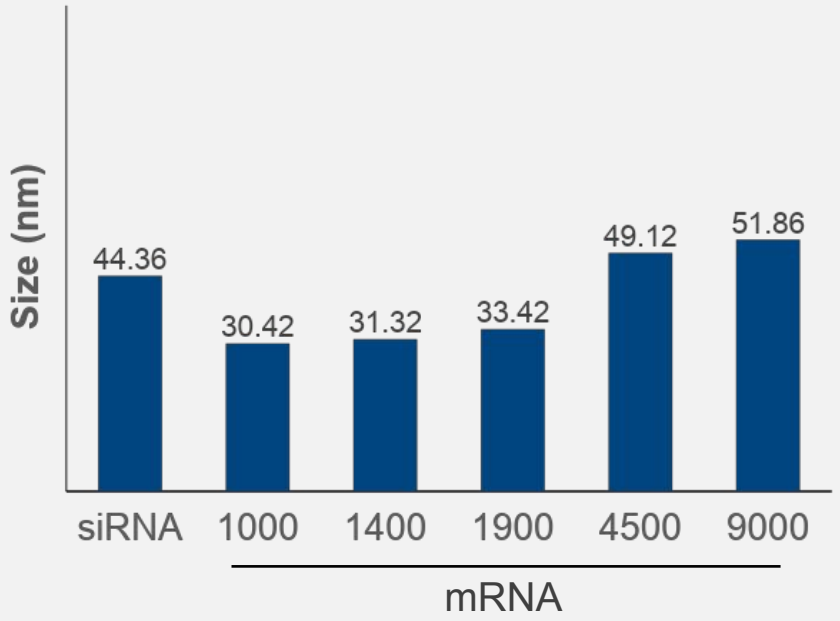
GEN-115 Can Encapsulate A Large Range of RNA Sizes

Optimized Formulation

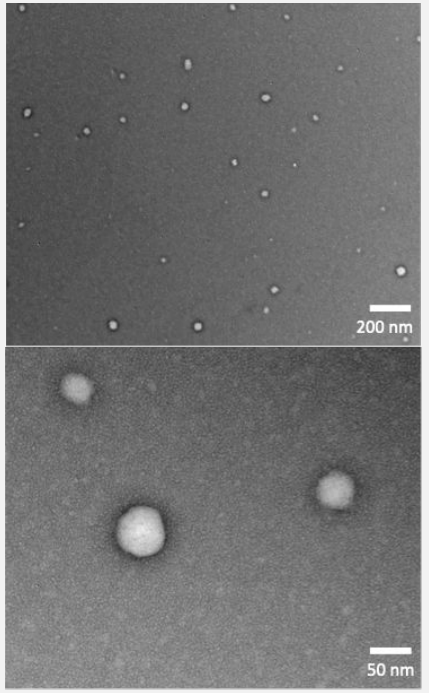
- Optimized formulation condition for all nucleic acid cargos from siRNA to 10 kb DNA/RNAs
- Encapsulation yield was >95% for all DNA/RNAs
- Established scale up formulation method

Various Cargo Encapsulation

Dynamic light scattering (DLS) of GEN-115 with various Cargoes



TEM image of GEN-115



~50 nm diameter

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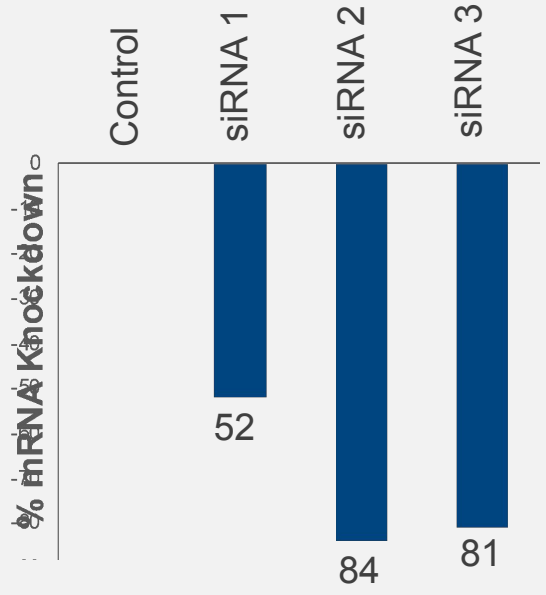
PNP Can Deliver Various Payloads

In vitro PoC with GEN-115

24 bp

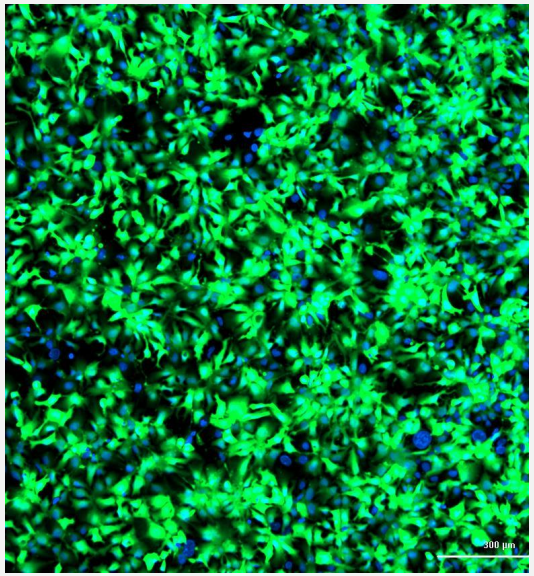
siRNA



~84% knock down

1000 nt

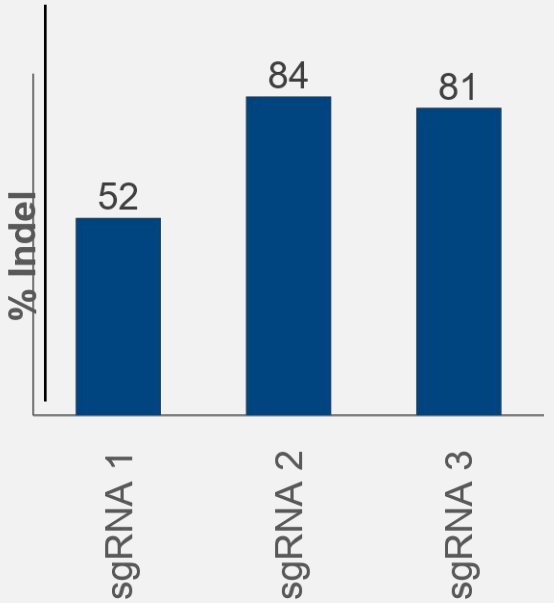
GFP mRNA



~98% transfection efficiency

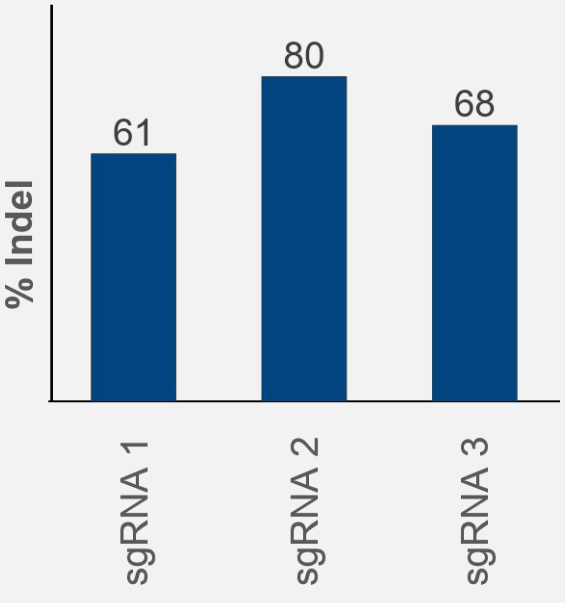
4500 nt

Cas9 mRNA + sgRNA



~84% indel

Cas9 Protein + sgRNA



~80% indel

GEN-115 PNP encapsulating either GFP mRNA, siRNA, Cas9 mRNA, or Cas9 RNP treated in Hep3B cells
GFP expression imaging 24 hr after treatment, mRNA expression: 72 hr after treatment, DNA sequencing: 72 hr after treatment

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Summary

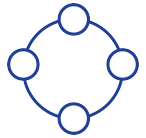
NANOGALAXY™ PLATFORM

Non-viral, non-lipid delivery technology for genetic medicine

TARGETED DELIVERY

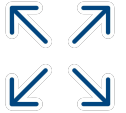
PoC delivery data show that iterative screening achieves targeted *in vivo* delivery in NHP

KEY ADVANTAGES



Payload flexibility, Repeat dosing, Ease of manufacture

APPLICABILITY



Rapidly expandable platform with optimized proprietary discovery engine to develop various genetic medicines

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GenEdit R&D

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Sam Wong
Yvonne Angell
Santanu Maity
Gopi Tiruchinapally
Meng Zhuang
Justin Tran
Carlos Medina
Tin Mao
Seyeol Yoon
Beobsoo Kim

Hiring

We are actively hiring 10 positions across all R&D teams
Visit **Genedit.com**

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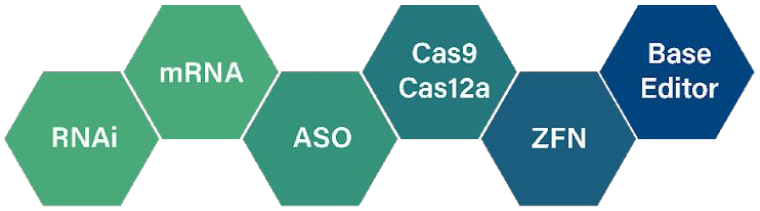
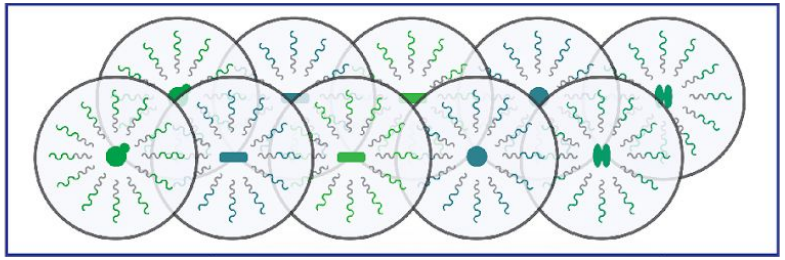
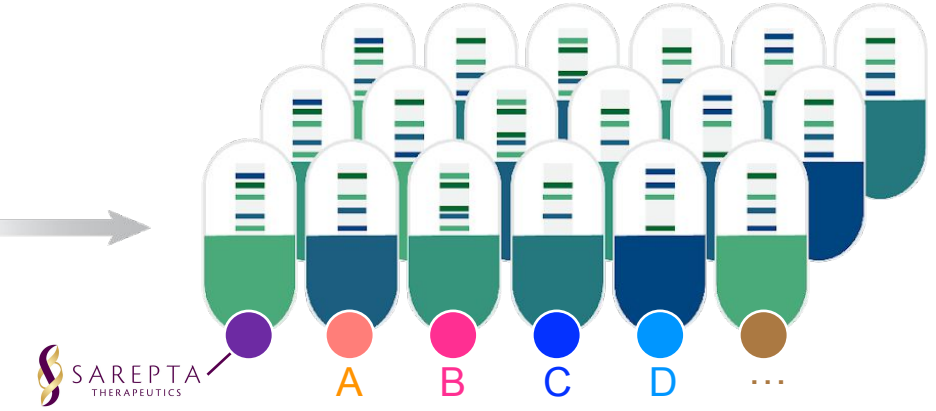
Various Partnership





NanoGalaxy™

Partnership



Payloads | Delivery Platform

Genetic Medicine

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Thank You to Our Session Sponsor

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Comparison of Key Delivery Technologies

GenEdit PNPs address limitations of existing technologies

		Limitations of existing technologies		The Solution
Criteria	**Attributes**	**AAV** (adeno associated viruses)	**LNP** (lipid nanoparticles)	**GenEdit PNP** (polymer nanoparticles)
Tissue Selectivity	Target Tissues	Broad distribution; high off-target expression	Limited to liver	Targeted tissues
Payload Flexibility	Potential Payloads	DNA only (<5kb)	DNA/RNA only	DNA/RNA (any size) Protein
Safety & Immunogenicity	Pre-Existing Immunity	High	Low	Low
	Ability to Re-Dose	No	Yes	Yes
	Genome Integration	Yes	No	No
Manufacturing	Cost & Complexity	Costly cell-based production	Low cost, complex mixture, requires cold chain	Low cost, chemical synthesis in aqueous solution
	Lyophilization	No	No	Yes

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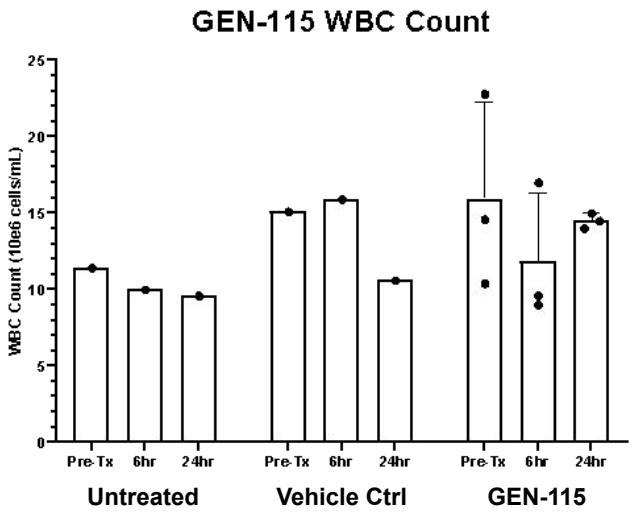
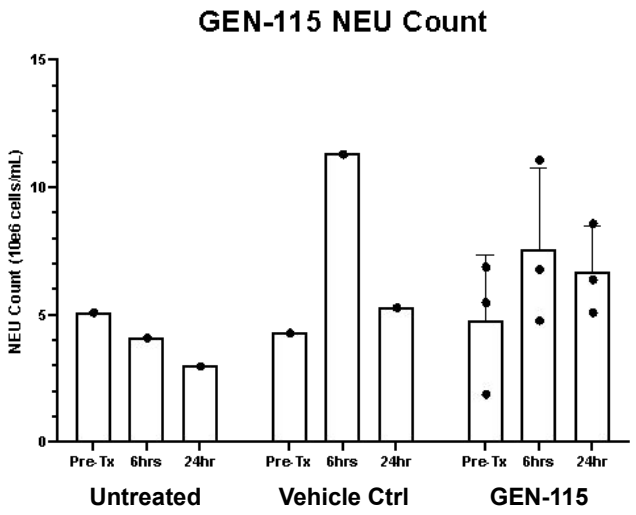
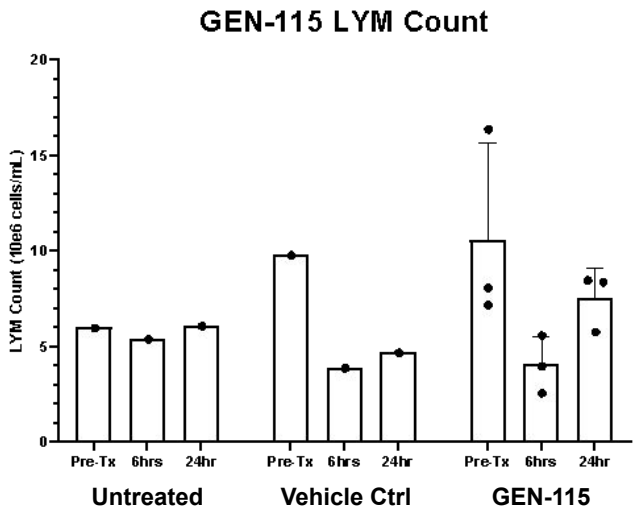
GEN-115 Dosing Did Not Induced Significant Changes in NEU and LYM Counts





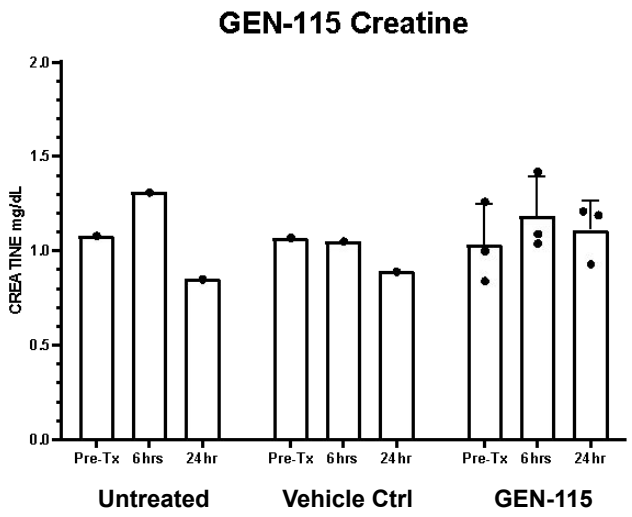


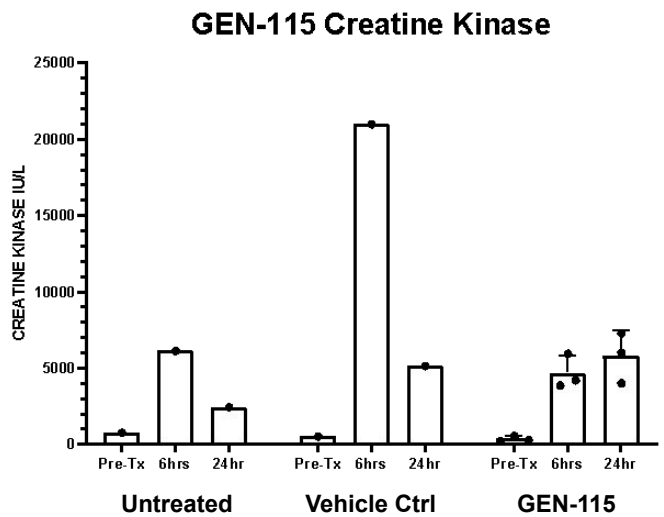
- A slight increase in neutrophil counts and slight decrease in lymphocyte counts was observed at 6 hours post-injection but levels returned to pre-injections by 24 hours

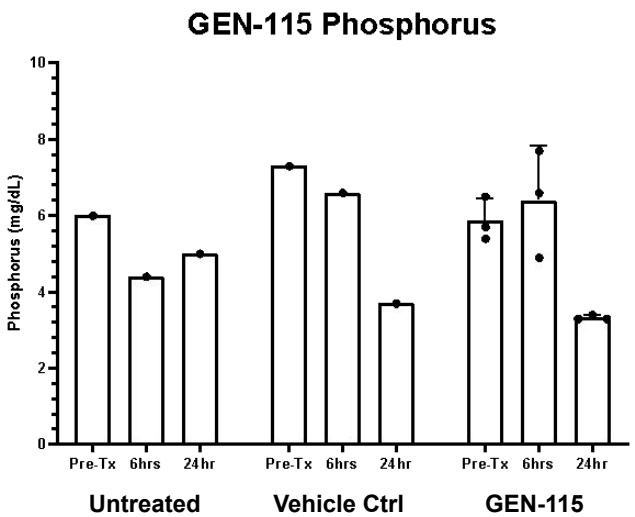
Vehicle control: buffer only IV administration
GFP mRNA (2 mg/kg dose) in Cyno (NHP)
Blood collection either 18hr or 24 hr after IV administration

GENEDIT

GEN-115 Did Not Elevate Creatine, Creatine Kinase, Phosphorus Levels





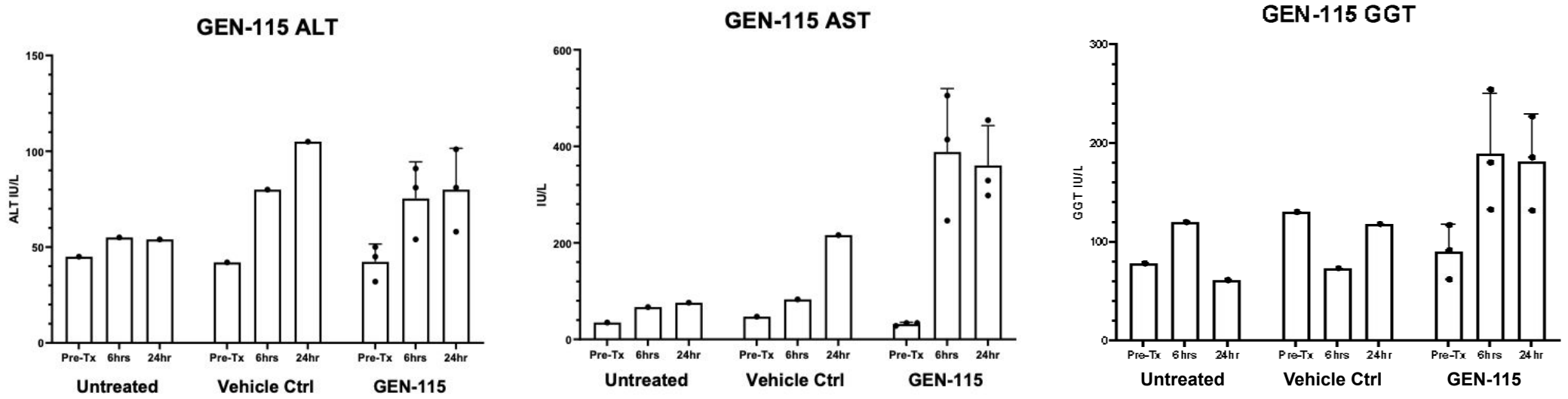


- Creatine kinase levels were mildly elevated 24 hours after GEN-115 injection. Injection of the vehicle induced a transient increase in CK.
- Phosphorus levels were decreased about 2X at 24 hours for both vehicle and GEN-115 groups

Vehicle control: buffer only IV administration
GFP mRNA (2 mg/kg dose) in Cyno (NHP)
Blood collection either 18hr or 24 hr after IV administration

GENEDIT

GEN-115 Showed Elevation of AST While ALT Is Stable



- AST showed elevation with both vehicle control and GEN-115
- GEN-115 injection did not significantly elevate levels of ALT and GGT

AST, aspartate aminotransferase
ALT, alanine aminotransferase
GGT, gamma glutamyl transpeptidase

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