Beam THERAPEUTICS

Optimization of LNP for in vivo base editing

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TIDES USA

Disclosure

- I am a Beam employee and shareholder

Overview

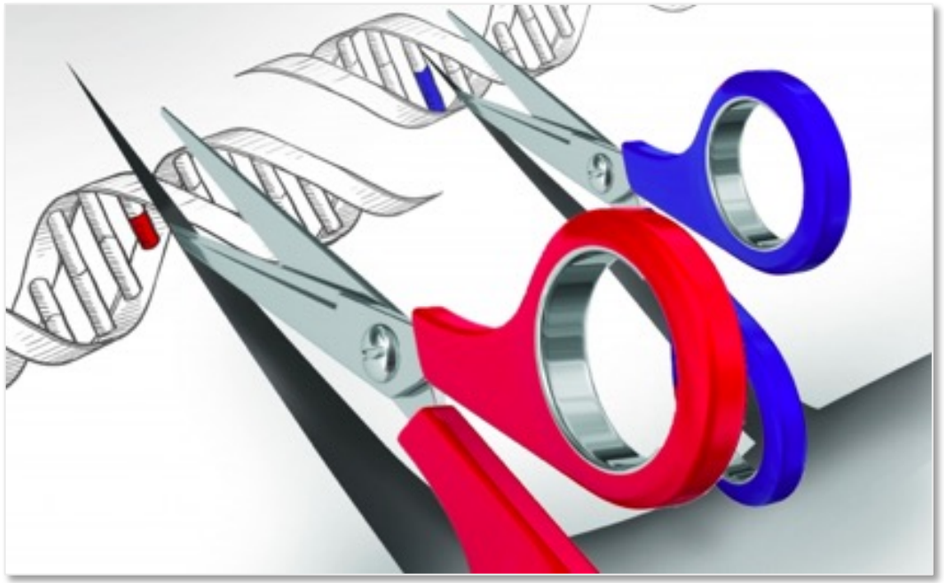
- Introduction to Base Editing and Beam's program portfolio
- Optimization of LNP platform for potent in vivo base editing in the liver of NHPs
- Develop LNPs for in vivo delivery outside the liver

Base editing is a new approach to gene editing

Nuclease editing

Creation of double-stranded breaks in DNA at a target location to **disrupt, delete, insert, or modify** genes

CRISPR, Zinc Finger Nucleases, TALEN, ARCUS



Base editing

Direct conversion of one base pair to another at a target location, without double-stranded breaks

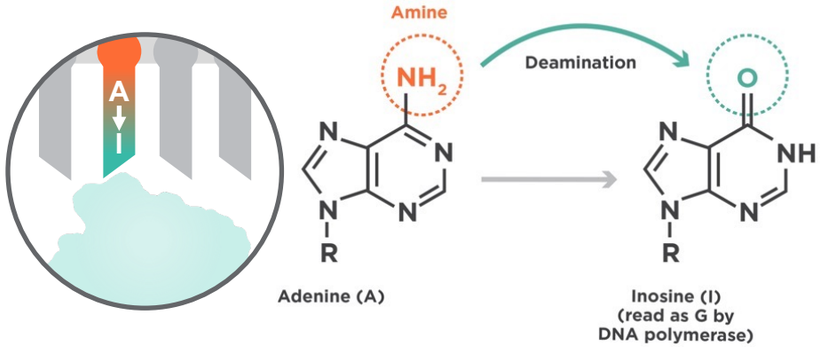
Base editors chemically modify target bases, permanently and predictably

CRISPR – established guide RNA-driven DNA targeting:

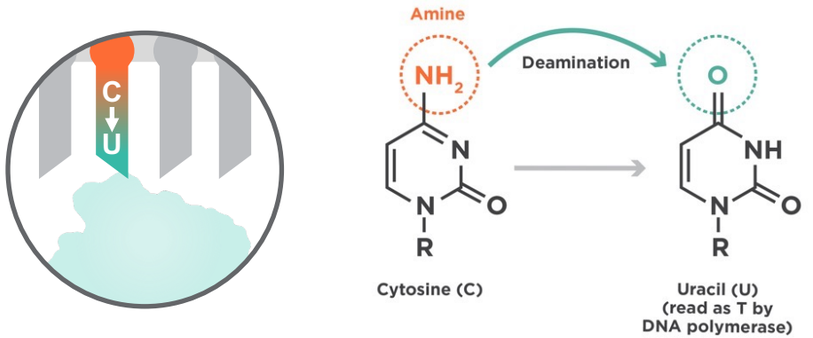
- Opens a short stretch of single strand DNA window
- Modified to not cause double stranded breaks

Deaminase – operates on single stranded DNA to completes chemical modification at predictable target DNA base

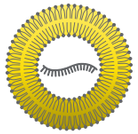
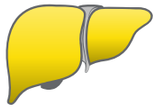
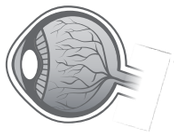
A-to-G base editor (“ABE”)



C-to-T base editor (“CBE”)

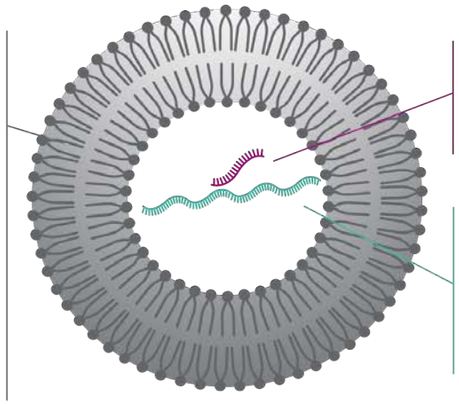


Diversified portfolio of wholly-owned base editing programs

DELIVERY	THERAPEUTIC AREA	PROGRAM / DISEASE		APPROACH	RESEARCH	LEAD OPTIMIZATION	IND ENABLING	PHASE I/II	PIVOTAL
ELECTRO-PORATION 	Hematology 	BEAM-101	Sickle Cell Disease Beta Thalassemia	Fetal hemoglobin activation					
		BEAM-102	Sickle Cell Disease	Direct correction of sickle-causing mutation					
	Oncology 	BEAM-201	T-cell Acute Lymphoblastic Leukemia	Multiplex silenced CD7 CAR-T					
		Acute Myeloid Leukemia		Multiplex silenced CAR-T					
NON-VIRAL (LNP) 	Liver Diseases 	Alpha-1 Antitrypsin Deficiency		Precise correction of E342K					
		Glycogen Storage Disorder 1a		Precise correction of Q347X					
				Precise correction of R83C					
		Undisclosed		Multiplex editing					
VIRAL (AAV) 	Ocular and CNS 	Stargardt Disease		Precise correction of G1961E					
		Undisclosed		Precise correction					
		Undisclosed		Gene silencing					

LNP = Lipid Nanoparticle; AAV = Adeno Associated Virus; CNS = Central Nervous System

We deliver base editor mRNA and gRNA using LNP to enable in vivo base editing



Surrogate payload

target: CAGGATCCGCACAGACTCCA GGG

- Rodent-NHP conserved region on liver-expressed ALAS1*
- 5A→G edit causes a I491T mutation of unknown functional consequences

Adenine base editor (ABE)

- Optimization of LNP components led to potent A→G editing in NHP liver
  - mRNA production process
  - gRNA chemical modification
  - LNP formulation

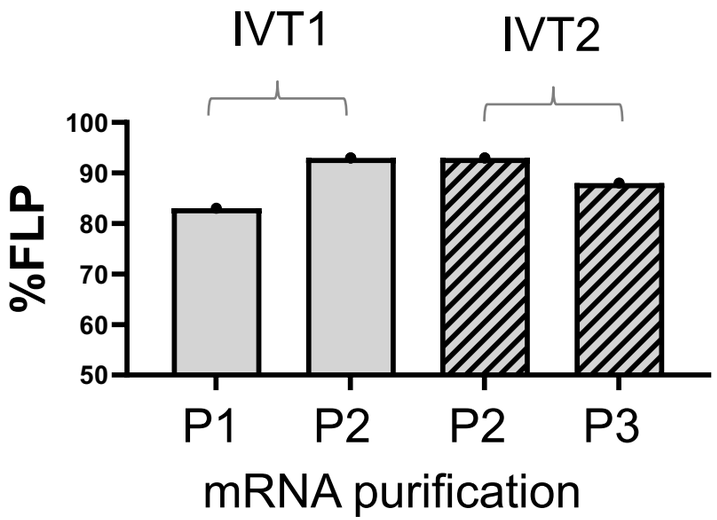
*ALAS1: 5'-aminolevulinate Synthase 1

Goal of mRNA process optimization is to improve activity & reduce immune stimulation

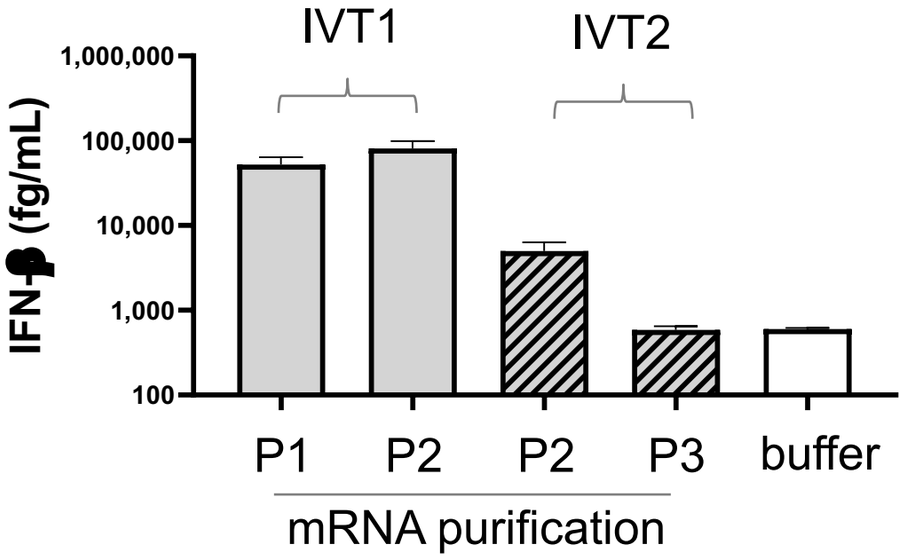
Goal	↑ Translation: higher base editor expression, higher editing ↓ Immune stimulation: reduce toxicity			
Process steps	design mRNA construct	Plasmid production	In vitro transcription	Purification
Purpose	• ↑ Translation • ↓ Immune stimulation	• ↑ full length product • ↑ capping • ↓ dsRNA	• ↓ short transcripts • ↓ dsRNA • ↓ process residuals	
Tunable parameters	• 5'cap • 5'/3'-UTR • codon optimization • poly(A) • modified nucleosides	• cap • modified NTP • reaction condition • other…	• oligo dT • IPRP • HIC • other…	

Optimization of IVT and purification increased full-length product and eliminated immune stimulation in vitro

% Full length mRNA

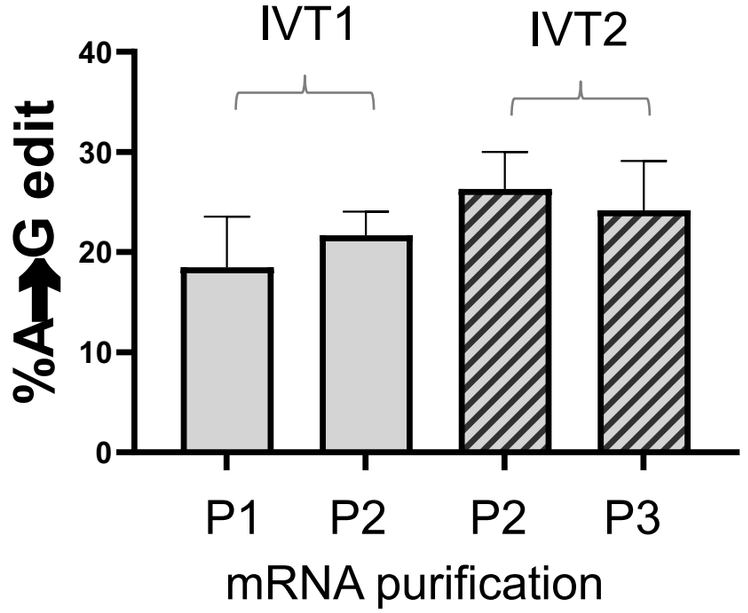


In vitro immune stimulation

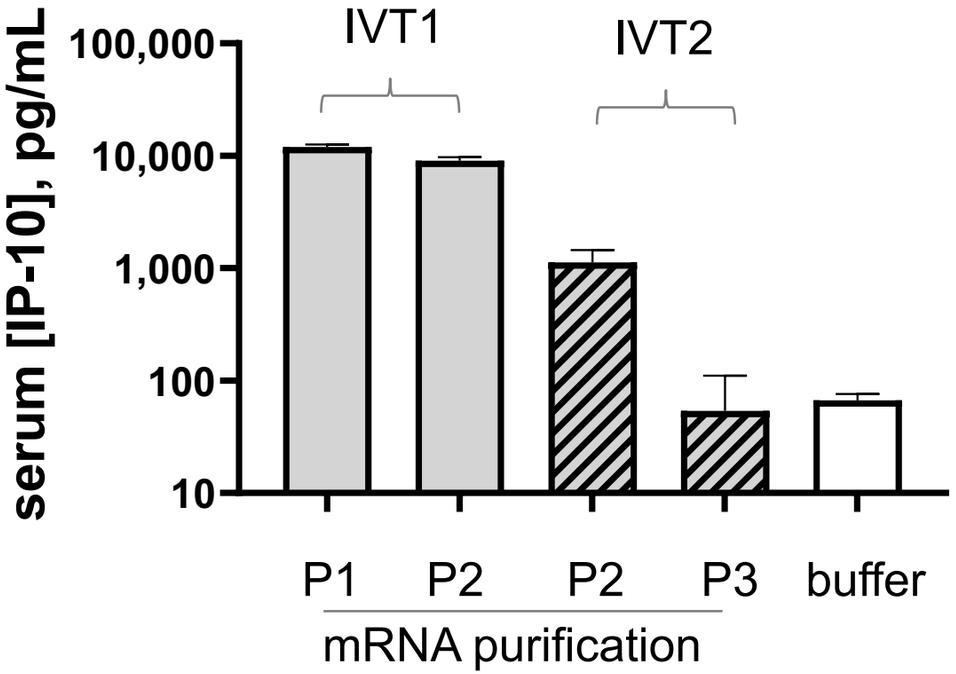


Optimized mRNA is active and does not induce inflammatory response in vivo

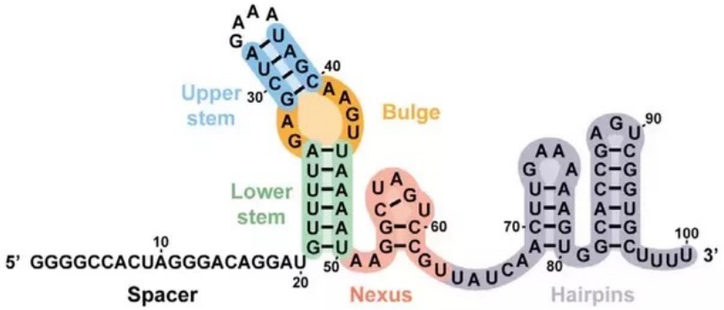
ALAS1 edit in mouse liver
(0.1mg/kg total RNA)



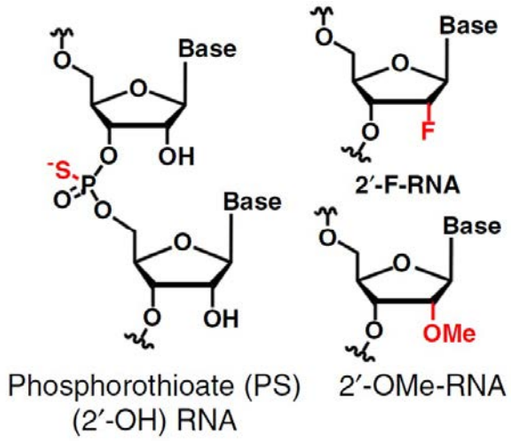
Mouse serum [IP-10]
6hr-post injection



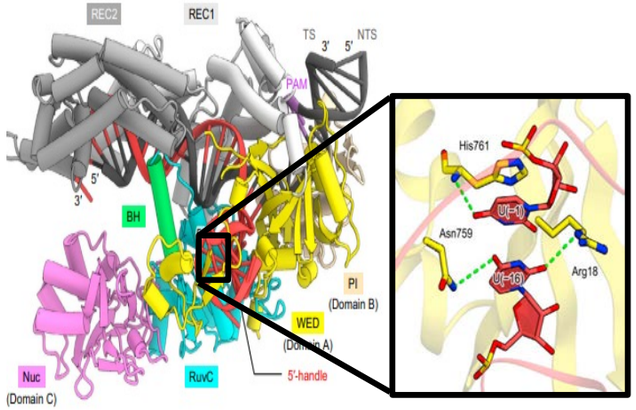
gRNA can be chemically modified to increases its stability



Modifications



Internal mods are positioned via a structure-guided approach

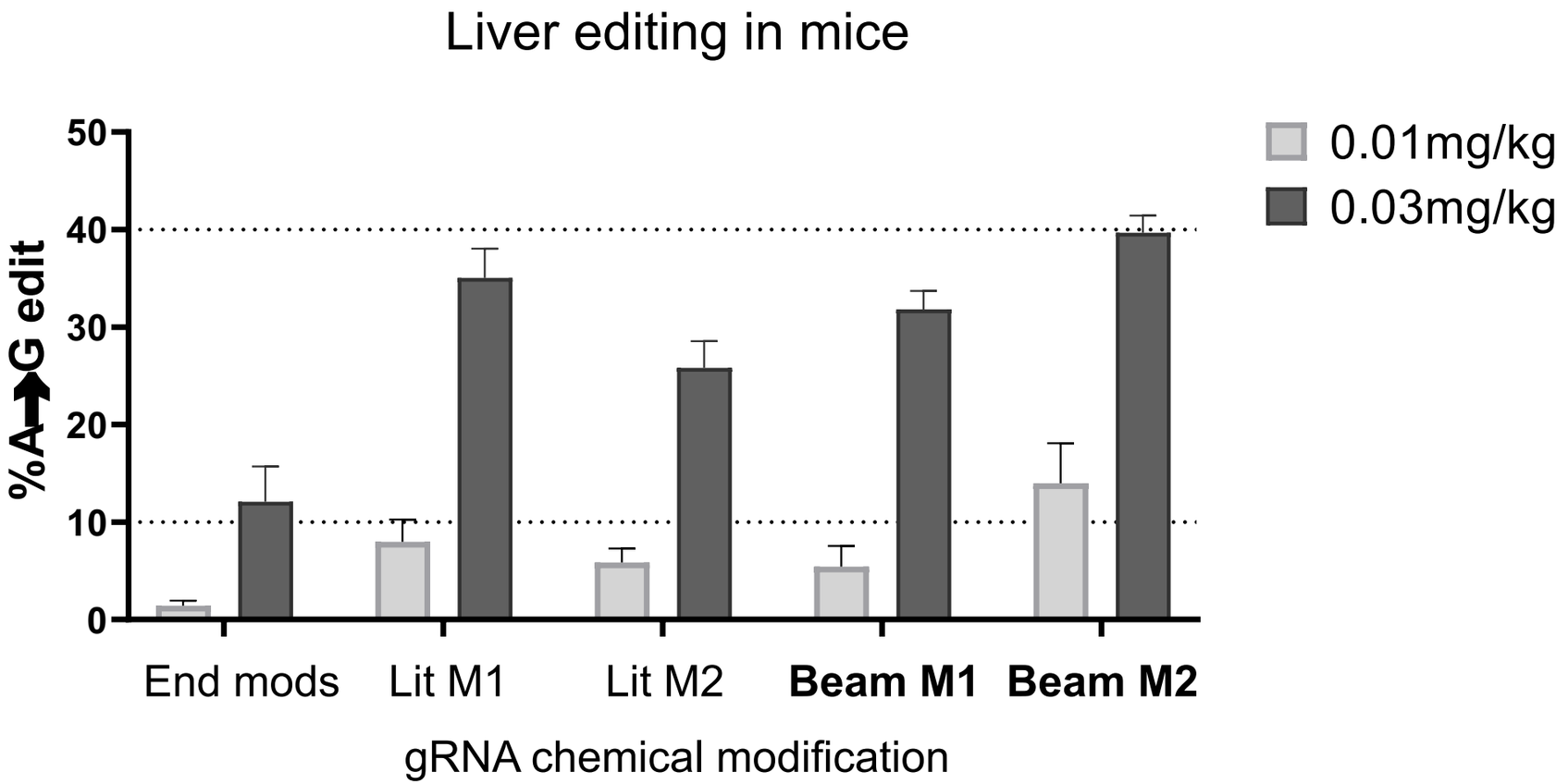


Stabilizing modification types

- **End mods**: Stabilize gRNA against **exo**nucleases
  - Modifications at the first three nts of the 5’ end and first three nts of the last four at the 3’ end
- **Heavy (internal) mods**: Stabilize gRNA against **endo**nucleases
  - Can inhibit Cas activity and thus must be placed at specific locations
  - Particularly important for in vivo studies

Yamano, Takashi, et al. *Cell* 165.4 (2016): 949-962; Hendel, Ayal, et al*Nature biotechnology* 33.9 (2015): nbt-3290; Yin, Hao, et al *Nature biotechnology* 35.12 (2017): 1179-1187; Finn, Jonathan D., et al. Cell reports 22.9 (2018): 2227-2235.

Beam proprietary sgRNA modifications increase base-editing potency in vivo



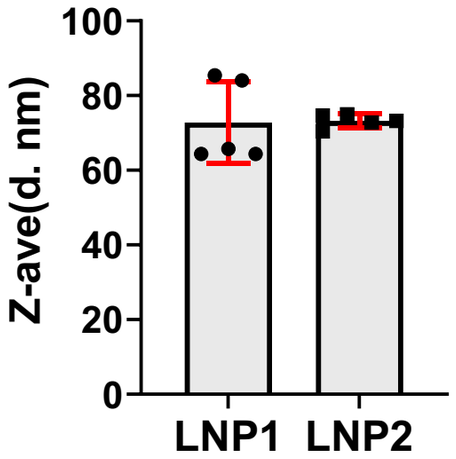
Produce potent, stable, and consistently manufactured LNP

- Scope of process optimization
  - Lipid composition
    - Helper lipid components
    - Molar % of lipids
    - N:P ratio
  - Formulation process
    - Mixing of components
    - Purification and concentrating
  - Buffer and excipients

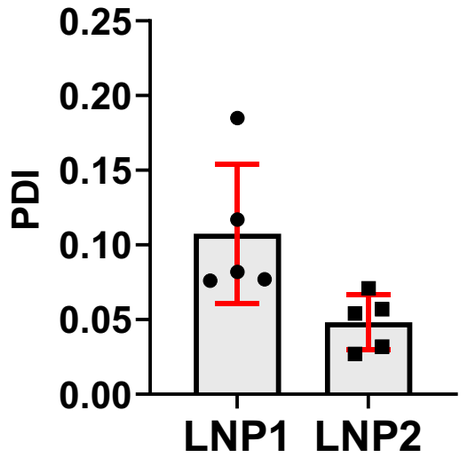
- In this work, mRNA and sgRNA are co-encapsulated in the same LNP at 1:1 mass ratio

Consistency of LNP formulations was improved through process optimization

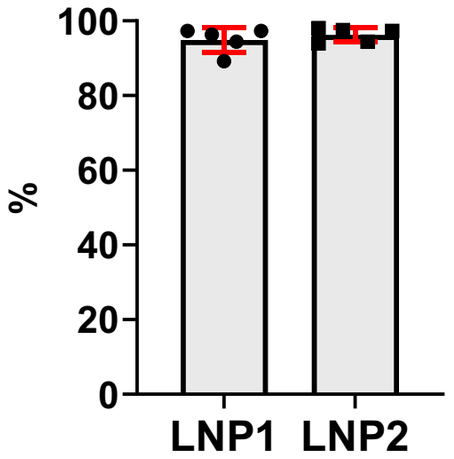
Size



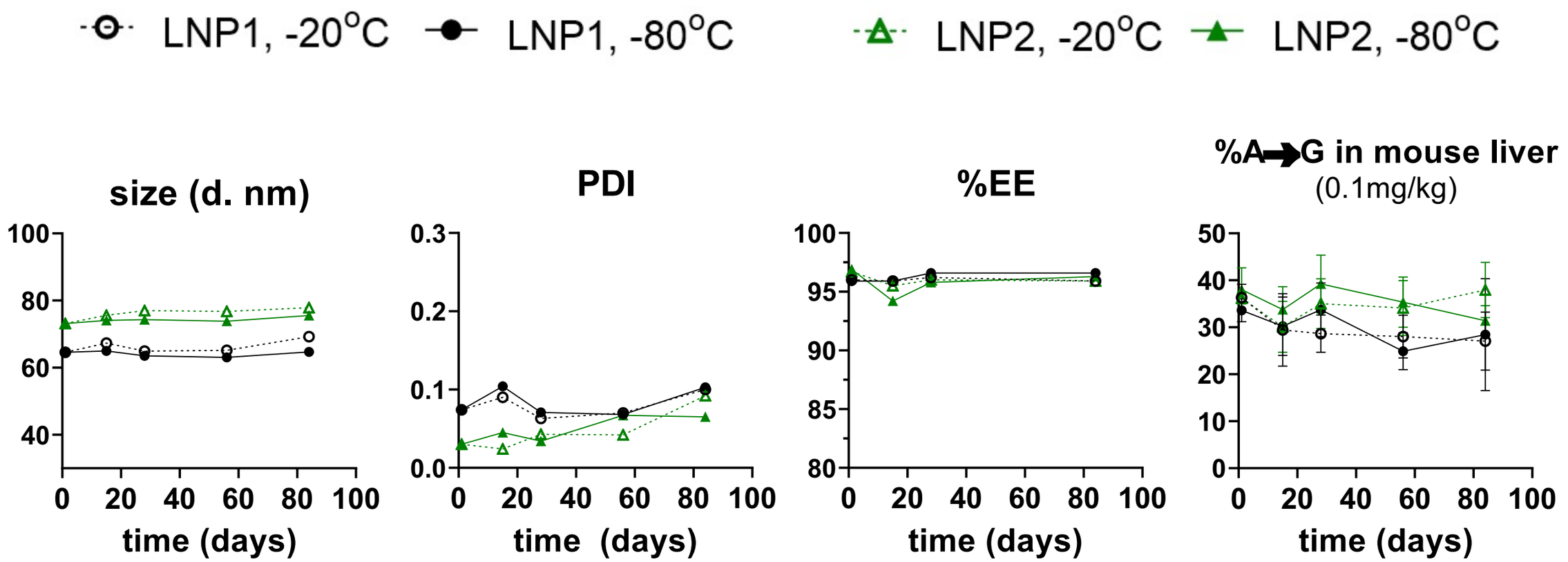
Polydispersity index



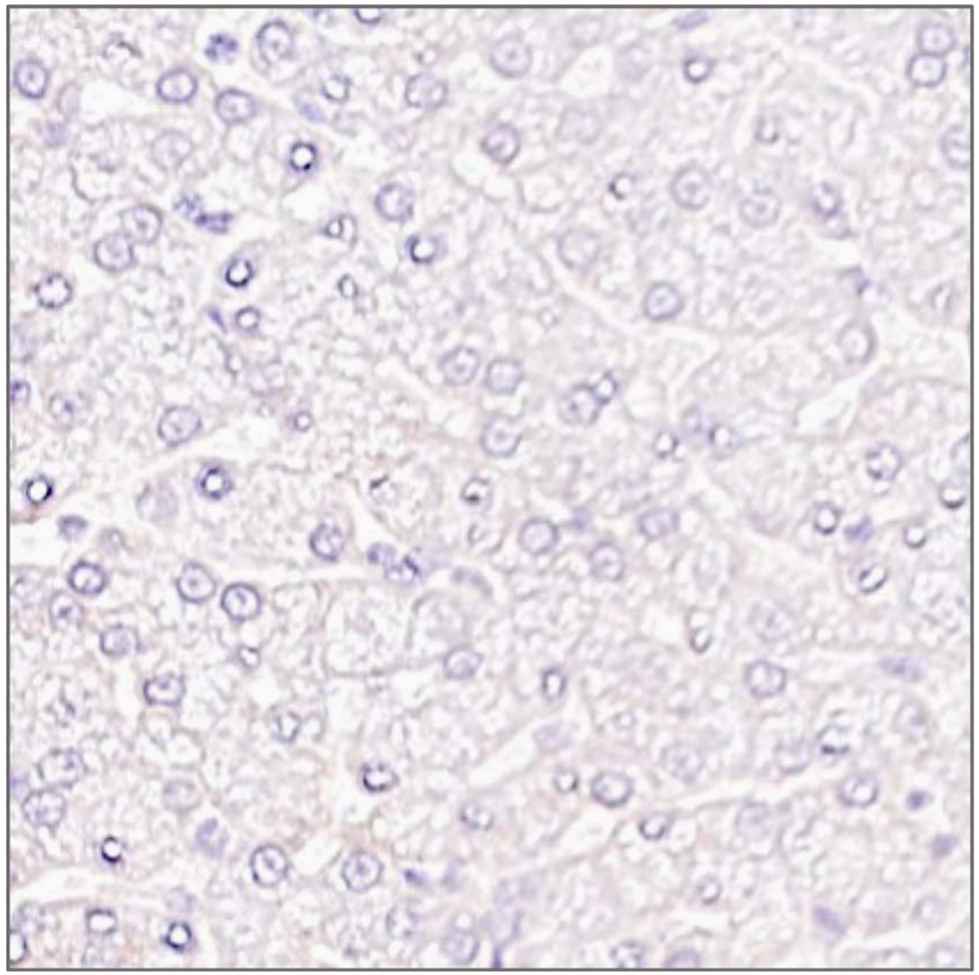
Encapsulation efficiency



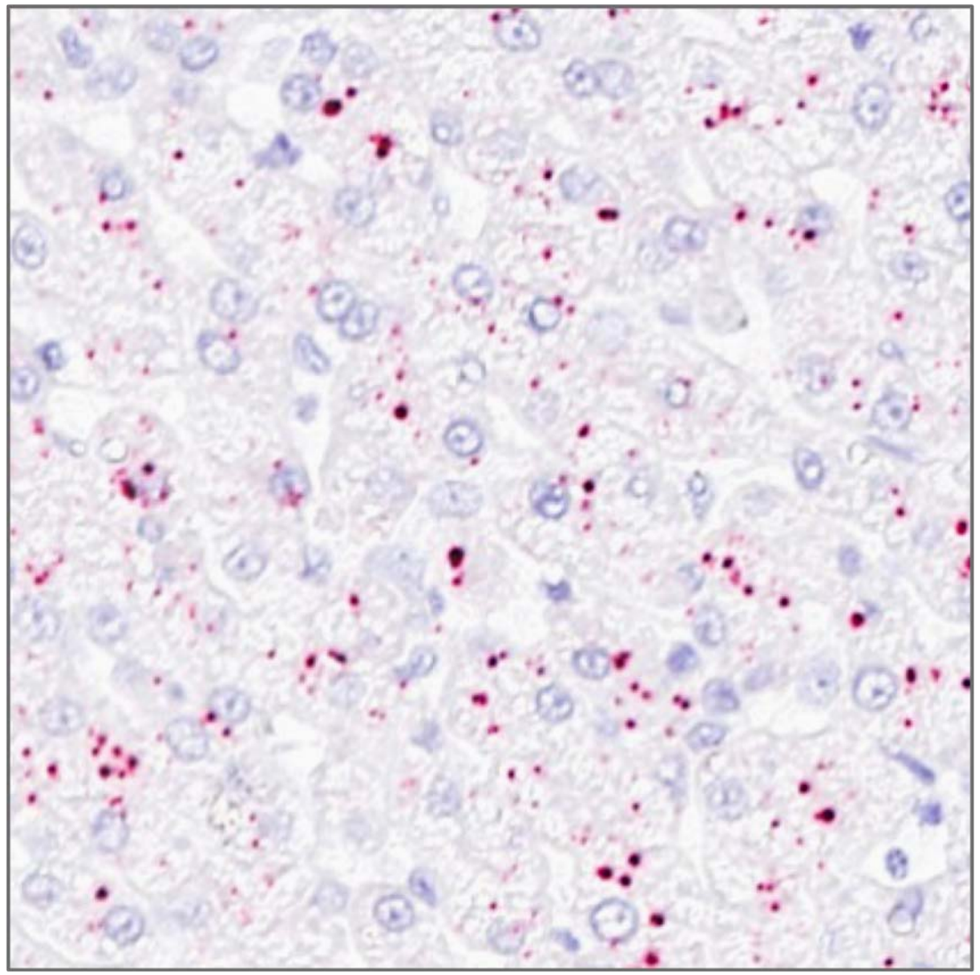
LNPs remain stable after 3-month storage at -80°C and -20°C



High degrees of hepatocyte editing is detected via BaseScope in liver of LNP-treated NHP



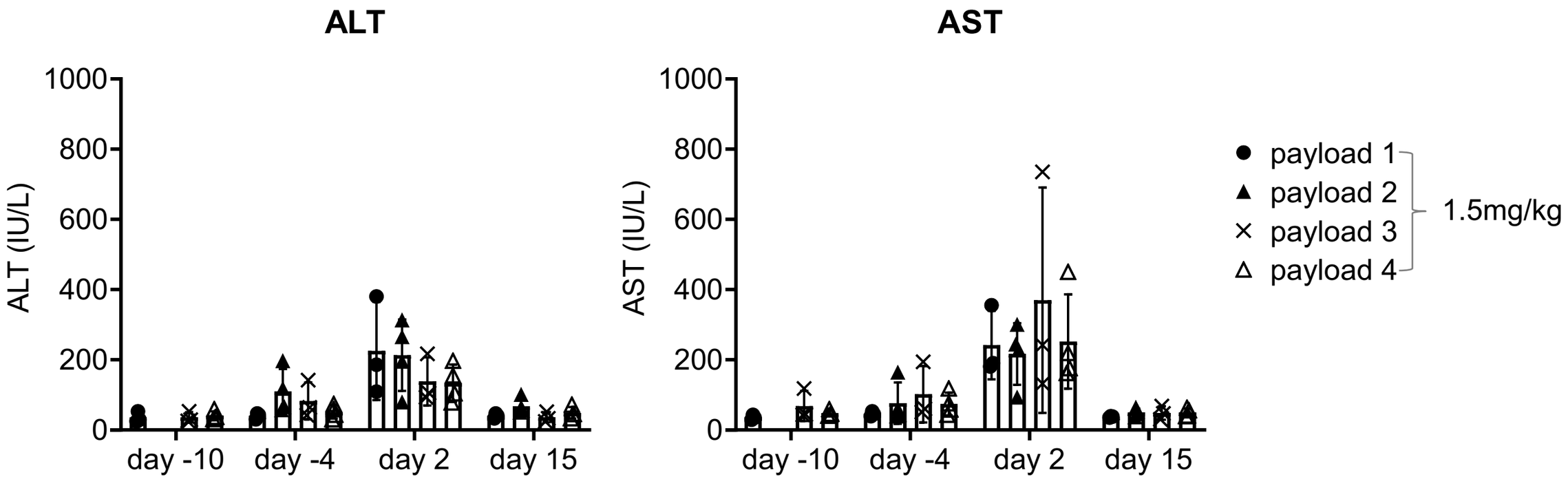
Untreated NHP liver



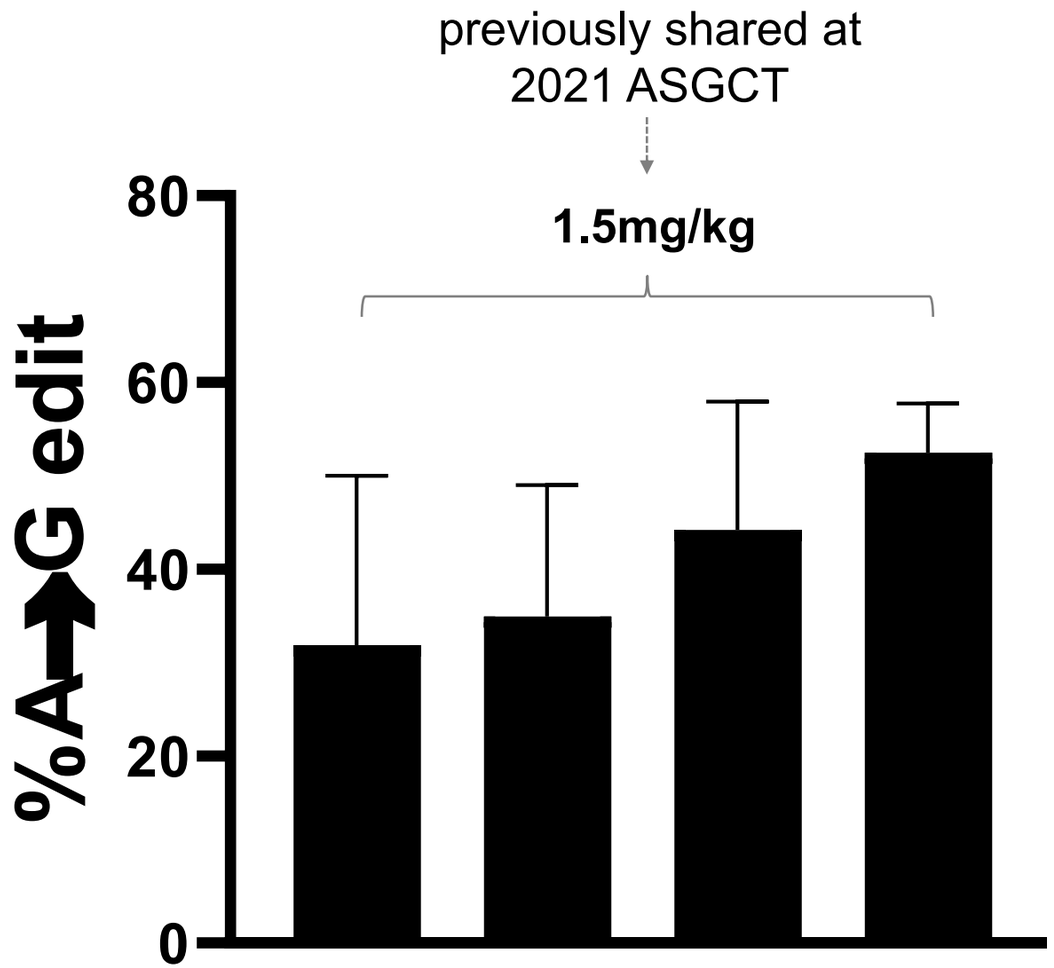
LNP-treated NHP liver
(47% whole liver editing)

LNPs appear well tolerated in NHPs based on clinical pathology

- Minimal to mild transient increases in AST and/or ALT at 24hr (Day 2) post-dose resolving by Day 15
- No other significant changes in clinical pathology parameters were observed

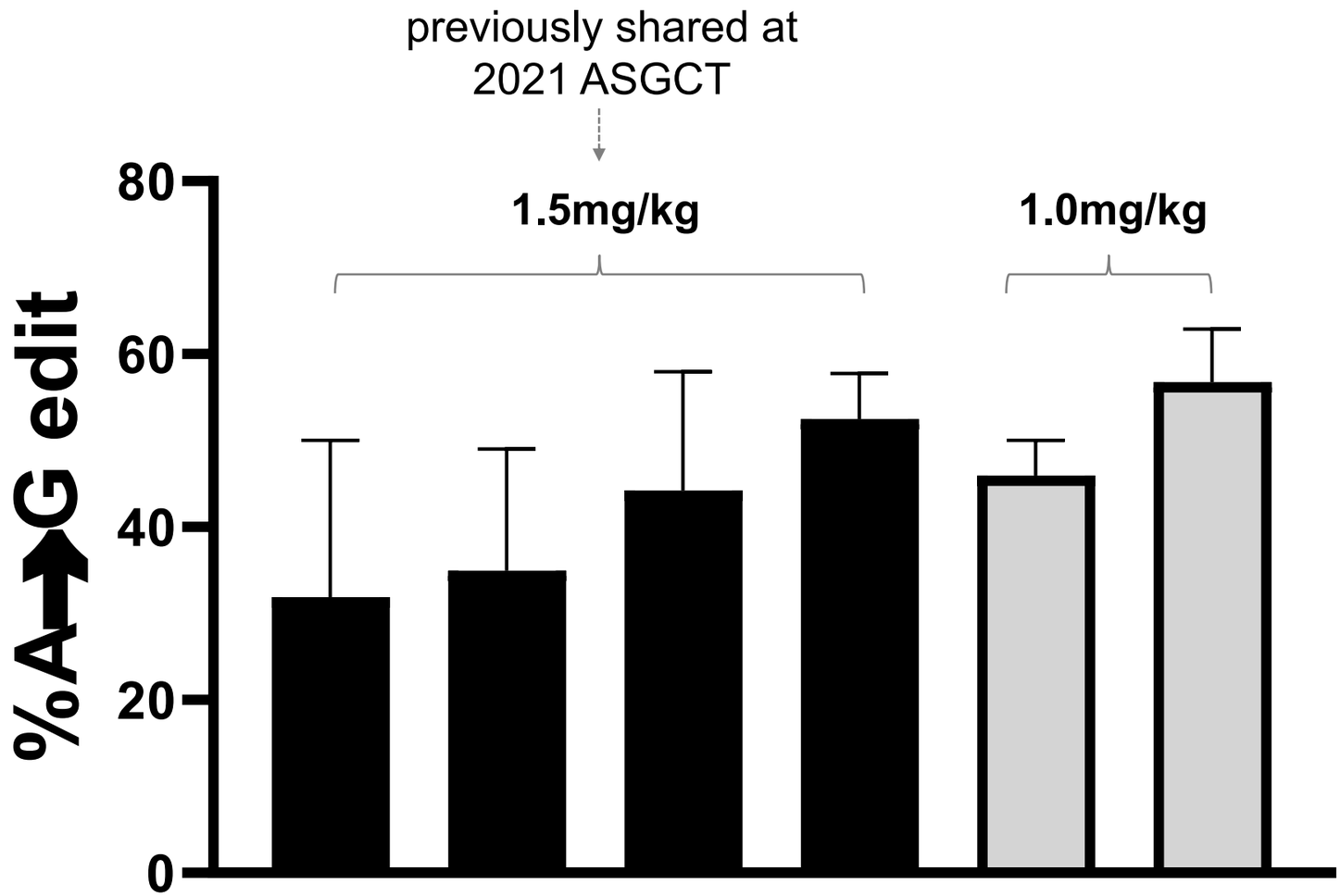


Improvements to LNP processes increase LNP potency up to 60% editing at clinically relevant dose



n=2 or 3
Bars represent mean +/- standard deviation

Improvements to LNP processes increase LNP potency up to 60% editing at clinically relevant dose



n=2 or 3
Bars represent mean +/- standard deviation

Summary of Beam liver LNP development

- We optimized the LNP platform for in vivo base editing in the liver
- The optimized platform consists of
  - Potent, immunosilent mRNA
  - Chemically modified sgRNA
  - Consistent, stable LNP
- Optimized LNP produced up to 60% A→G editing in NHP liver at 1.0mg/kg
- Optimization is a continuous journey

Developing LNPs for extrahepatic tissues

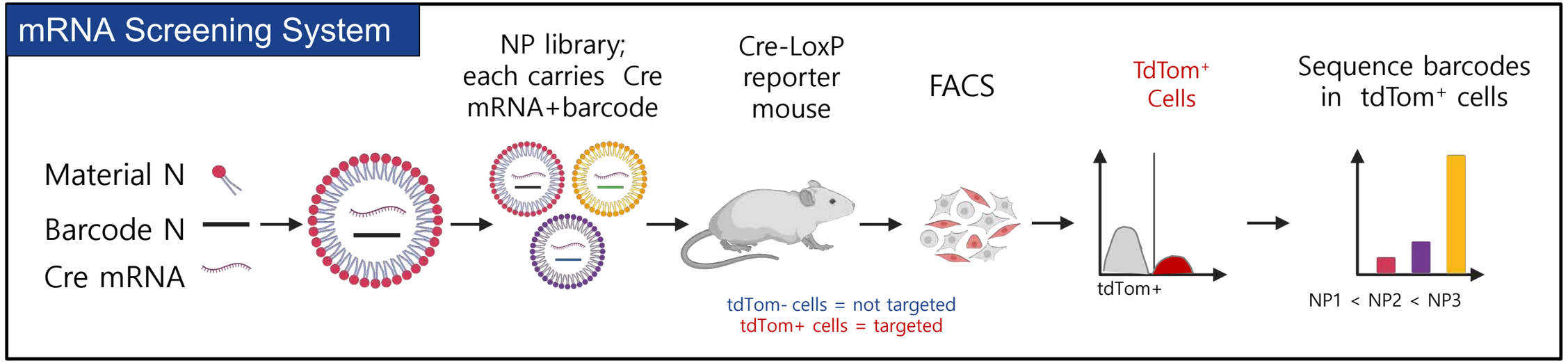
Physiology-directed accumulation in the Liver

Liver-centric LNP discovery and development

Low-throughput, high-cost & complexity discovery assays

An ideal LNP discovery process would be (i) very high throughput, (ii) *in vivo* (mice →NHPs), and (iii) analyze delivery to any desired combination of on- / off-target cell types.

High-throughput *in vivo* screening of LNPs using DNA barcodes



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Article
Cite This: J. Am. Chem. Soc. XXXX, XXX, XXX−XXX
pubs.acs.org/JACS

Nanoparticles That Deliver RNA to Bone Marrow Identified by in Vivo Directed Evolution

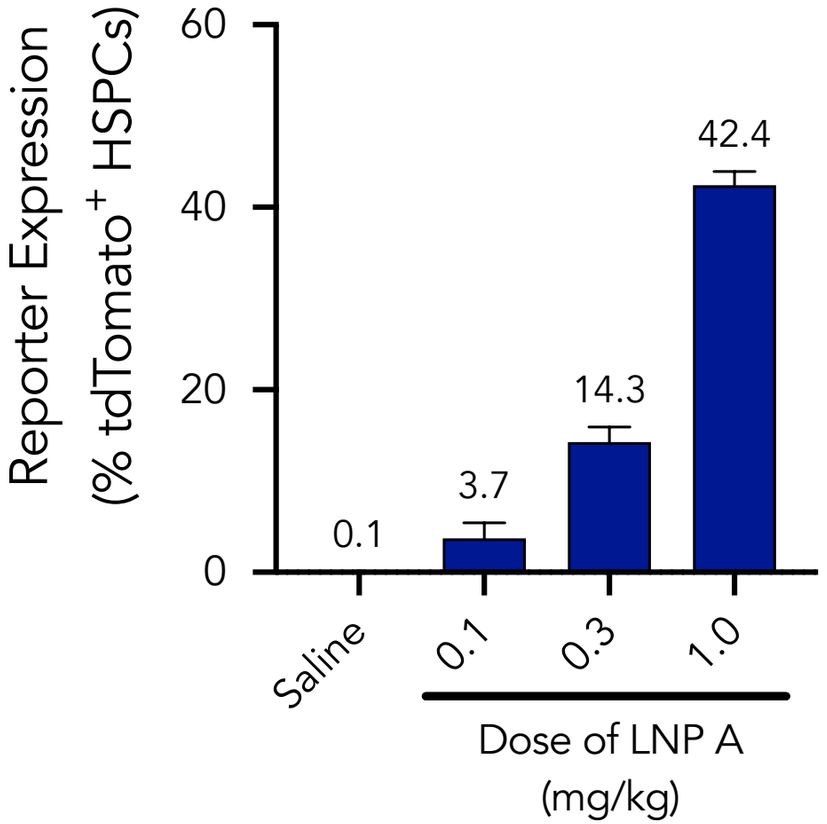
Cory D. Sago, Melissa P. Lokugamage, Fatima Z. Islam, Brandon R. Krupczak, Manaka Sato, and James E. Dahlman*

PNAS

High-throughput in vivo screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing

Cory D. Sago[a], Melissa P. Lokugamage[a], Kalina Paunovska[a], Daryll A. Vanover[a], Christopher M. Monaco[b], Nirav N. Shah[b], Marielena Gamboa Castro[a], Shannon E. Anderson[a], Tobi G. Rudoltz[a], Gwyneth N. Lando[a], Pooja Mummilal Tiwari[a], Jonathan L. Kirschman[a], Nick Willett[a,c,d,e], Young C. Jang[b], Philip J. Santangelo[a], Anton V. Bryksin[c], and James E. Dahlman[a,1]

Developing LNPs for the delivery of mRNA to Hematopoietic Stem & Progenitor Cells (HSPCs)



- The development of LNPs for the targeting of HSPCs could meaningfully impact the treatment of hemoglobinopathies
- Using our DNA barcoding approaches, we identified a family of LNPs that delivers to HSPCs in mice.
- In Cre-reporter mice, hit ‘LNP A’ transfected in a dose-dependent manner with >40% HSPCs transfected at 1.0mg/kg

Thank you

mRNA
- Valentina McEneany
- Jason St. Laurent
- Krishna Sapkota
- Jeffrey Cataloni
- Shefal Parikh

gRNA
- Brian Cafferty
- James Tam
- Ho Yau

LNP
- Shailendra Sane
- Xiao Luo
- Dongyu Chen
- Raymond Yang
- Emma Wang
- Mihir Patel
- Cory Sago

In vivo
- Sarah Smith
- Krishna Ramanan
- Richard Dutko
- Dominique Leboeuf

Automation and NGS
- Jeremy Decker
- Colin Lazzara
- Bob Gantzer

Analytical Development
- Andrew Hashkes
- Jeff Marshall
- Carlo Zambonelli

Liver therapeutics
- Michael Packer
- Robert Dorkin

Cell Biology
- Deb Wysong

Toxicology
- Brian Johnson

Leadership
- John Evans
- Giuseppe Ciaramella
- Mano Singh
- Francine Gregoire
- Rodrigo Laureano
- Steve Prescott

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