Targeted delivery of base editors to hepatocytes in vivo

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TIDES USA

September 23, 2021

Disclosure

I am an employee of Verve Therapeutics

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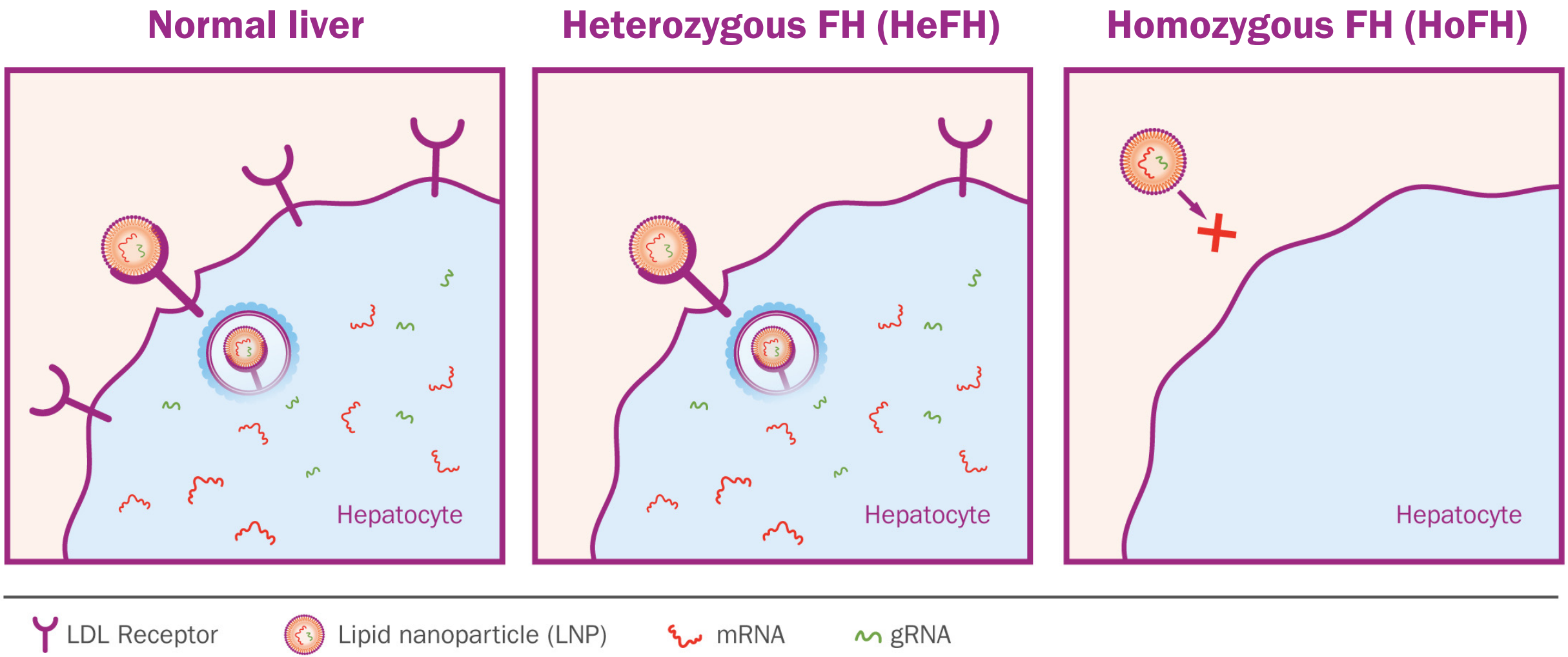
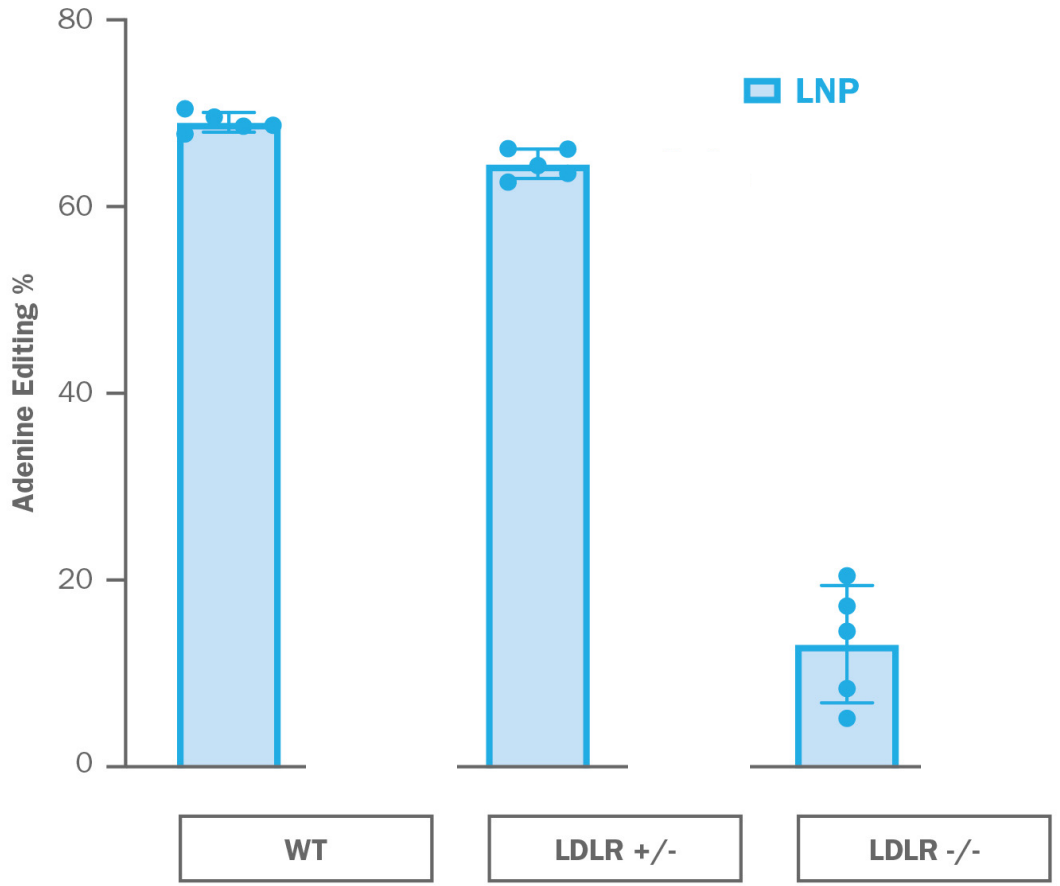
Homozygous familial hypercholesterolemia (HoFH) patients completely lack LDL Receptor; in this setting, LNP delivery to liver challenging

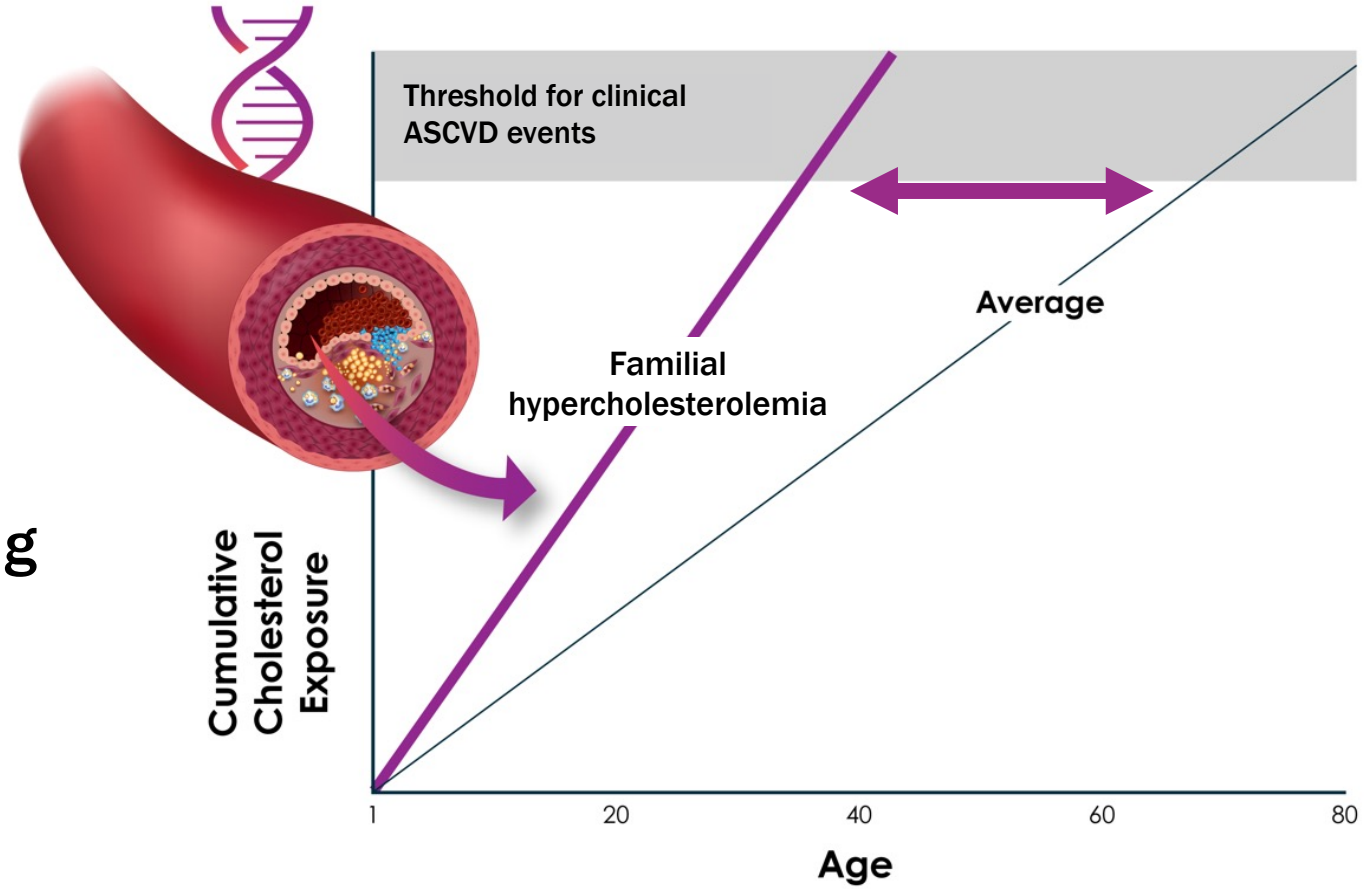


In mouse models of FH, LNPs deliver drug efficiently to livers of HeFH but fail to deliver to HoFH LDLR knockout (ldlr -/-) mice



Homozygous familial hypercholesterolemia (HoFH): a life-threatening genetic disease with very high cumulative exposure to LDL-C

- Usually caused by mutations in both copies of the LDLR gene, ~ 1,300 people in U.S.
- Lack of LDLR on hepatocytes leads to poor clearance of LDL-C from the blood
- LDL-C levels >500 mg/dL starting early in life
- Myocardial infarction (heart attack) common in 20s and 30s



Adapted from Horton et al. J Lipid Res., 2009

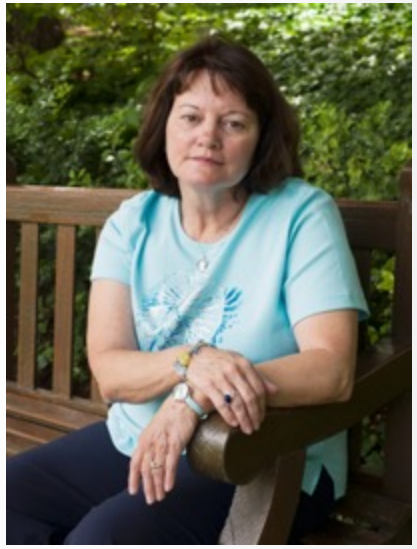
Individuals who naturally lack ANGPTL3 gene have lifelong low blood lipids, are healthy and resistant to heart attack

Target gene validated by human genetics

Rare Gene Mutations Inspire New Heart Drugs

By Gina Kolata
May 24, 2017

Anna Feurer learned she had unusually **low triglyceride levels** after having bloodwork at a corporate health fair. The discovery prompted researchers to recruit her and her family for a research study of their genetic makeup.



Credit. *Jess T. Dugan for The New York Times*

Human knockout:

Extremely low blood lipids in an individual

Triglycerides: **19 mg/dL**

LDL-C: **37 mg/dL**

Heterozygous deficiency:

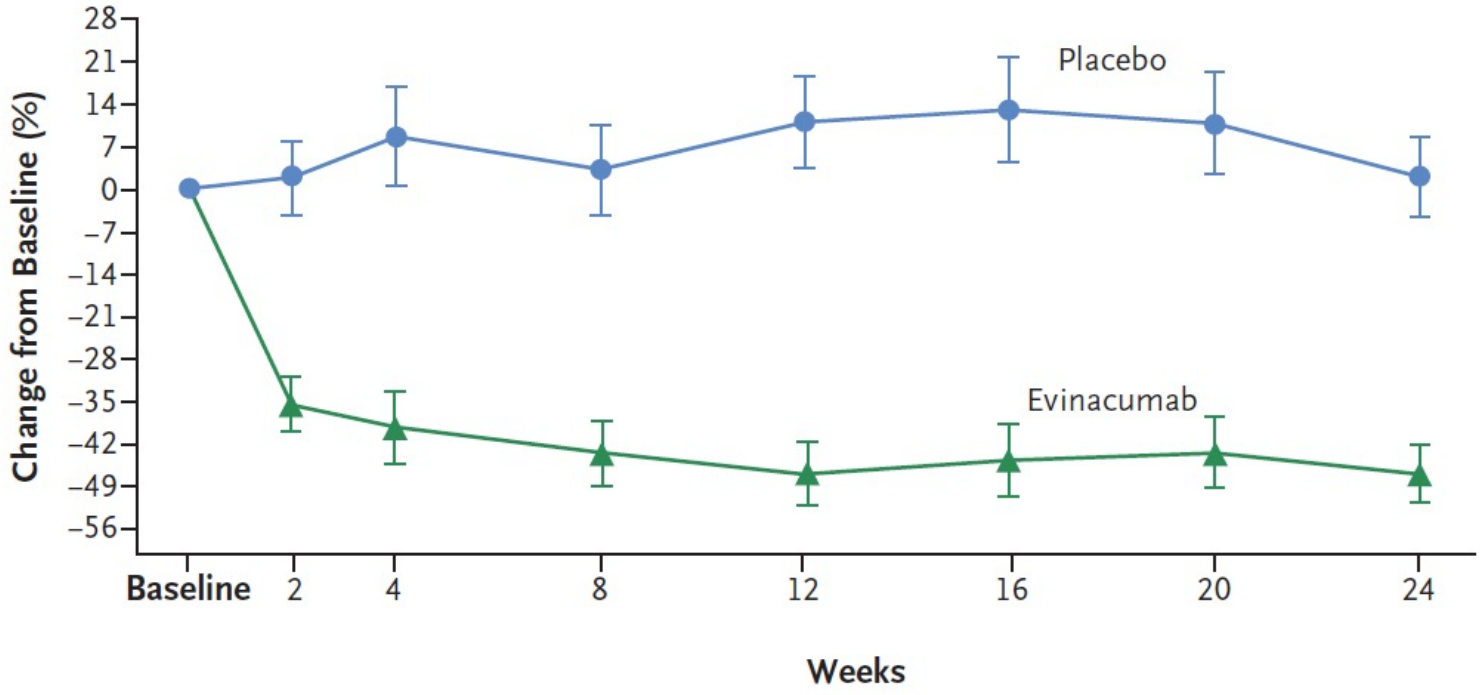
Low lipids in population

Resistant to heart attack

Musunuru et al., *N Engl J Med* 2010

ANGPTL3 inhibition benefits patients with HoFH

- ANGPTL3 inhibition with evinacumab lowered LDL-C by about 47% in a pivotal phase 3 trial in patients with HoFH
- Evinacumab now approved for HoFH



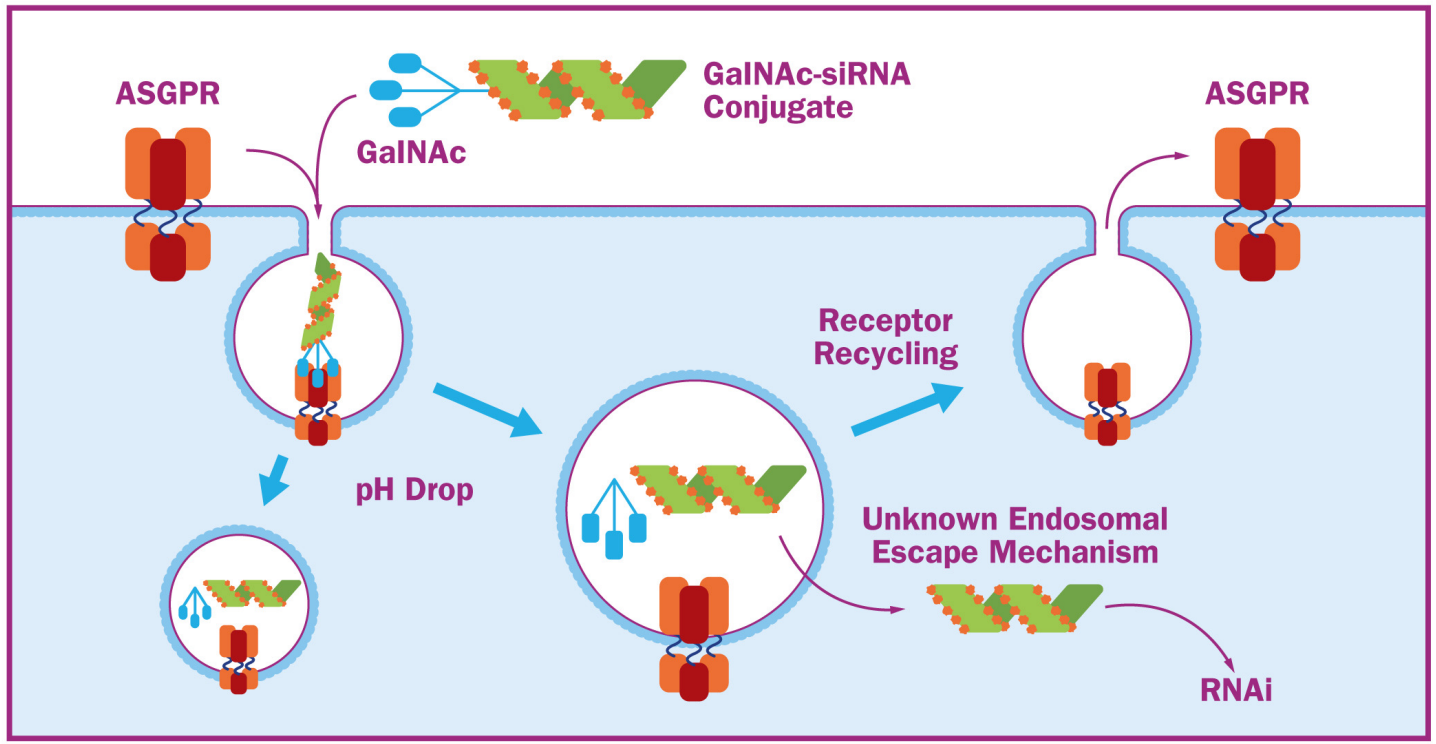
Raal, *N Engl J Med* 2020

Goal: an LNP delivery system that would enable ANGPTL3 editing in both patients with HeFH and HoFH

PROGRAM	INITIAL INDICATION	DEVELOPMENT STATUS			
		Research/ Lead optimization	IND-Enabling	Clinical	Development Milestones
Low-density lipoprotein cholesterol (LDL-C)					
VERVE-101 ABE-PCSK9	Heterozygous familial hypercholesterolemia	→			• IND Submission (2022) • Phase 1 Initiation (2022)
LDL-C and triglyceride-rich lipoprotein (TRL)					
ANGPTL3	Familial hypercholesterolemia	→			• Candidate selection (2022) • Begin IND-enabling studies (2022)

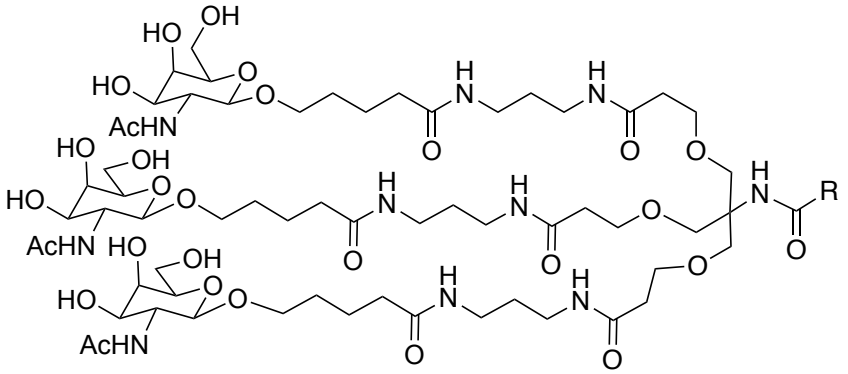
Liver ASGPR is an alternative pathway for entry into hepatocytes

ASGPR has been successfully targeted to deliver siRNAs-GalNAc conjugates to the liver in NHPs and humans



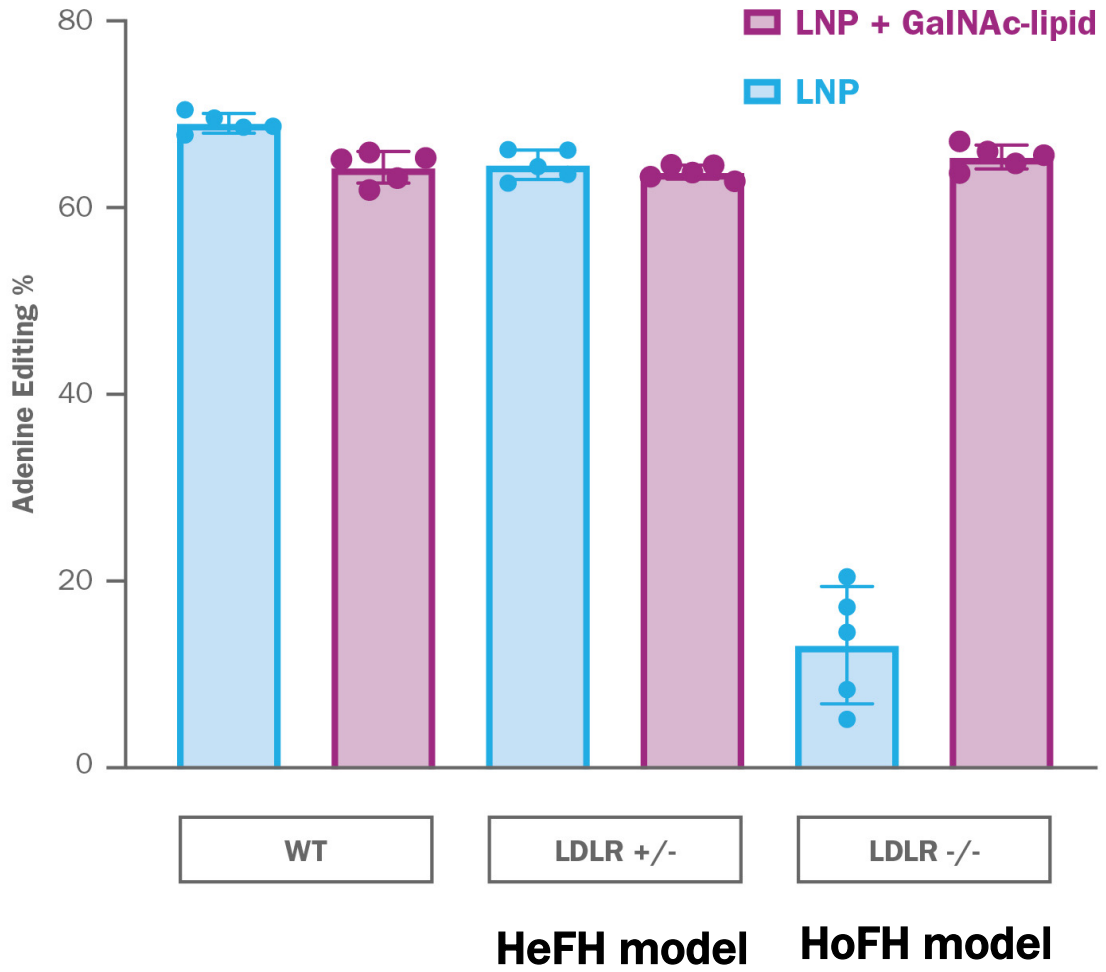
Adapted from Springer and Dowdy, Nucleic Acid Therapeutics 2018, 28, 109

Alnylam siRNA GalNAc ligand



Mol. Therapy, 2010, 18, 1357,
JACS, 2014, 136, 16958

Verve solution: ASGPR targeting proprietary GalNAc ligand that, when added to LNP, enables liver delivery in HoFH mouse model



Editing data are from analyses of liver necropsy specimens at 1 week

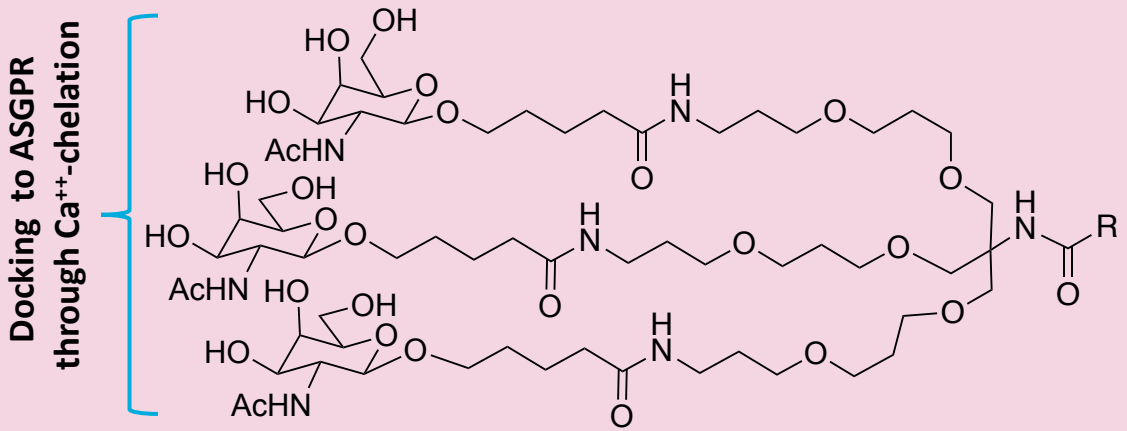
In order to achieve this, we developed and optimized five potential aspects of an ASGPR-targeted GalNAc-LNP

1. Design and screen novel GalNAc-based ASGPR ligand
2. Optimize chemical composition of the linker and lipid tail
3. Formulation design to develop a scalable GalNAc–LNP process
4. Optimize LNP surface density of GalNAc ligand
5. Develop analytical methods for characterizing GalNAc-LNPs

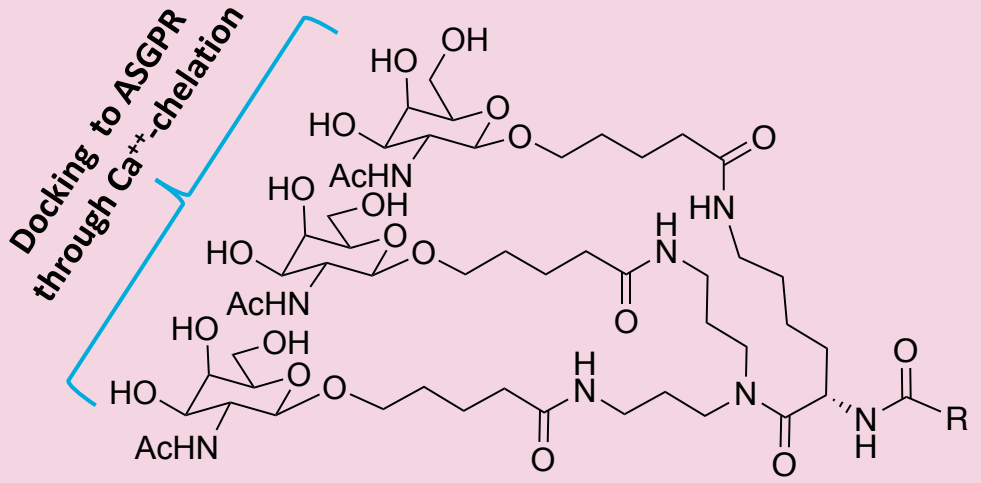
Rational design of novel Verve ASGPR ligands

1 ASGPR Ligand Design

Design 1



Design 2



- Valency
- Proximity effect

- Simpler chemistry
- Cost effective, manufacture friendly

Evaluation of GalNAc-Lipid LNPs in mouse models of homozygous familial hypercholesterolemia (HoFH)

1 ASGPR Ligand Design

Intravenous administration to *ldlr -/-* mice

GROUP 1
GalNAc Ligand design 1
Reference LNP
ABE mRNA
gene target 1 gRNA

GROUP 2
GalNAc Ligand design 2
Reference LNP
ABE mRNA
gene target 1 gRNA

GROUP 3
GalNAc Ligand design 1
Reference LNP
ABE mRNA
gene target 2 gRNA

GROUP 4
GalNAc Ligand design 2
Reference LNP
ABE mRNA
gene target 2 gRNA

Primary endpoints

- Whole liver DNA editing
- Target protein reductions

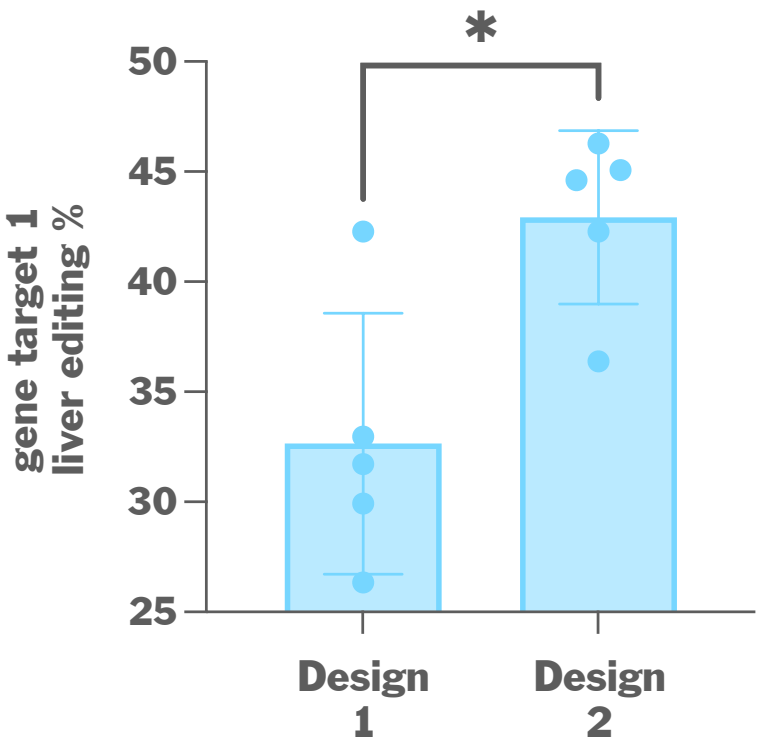
T = 0 T = 5 - 7 days

necropsy

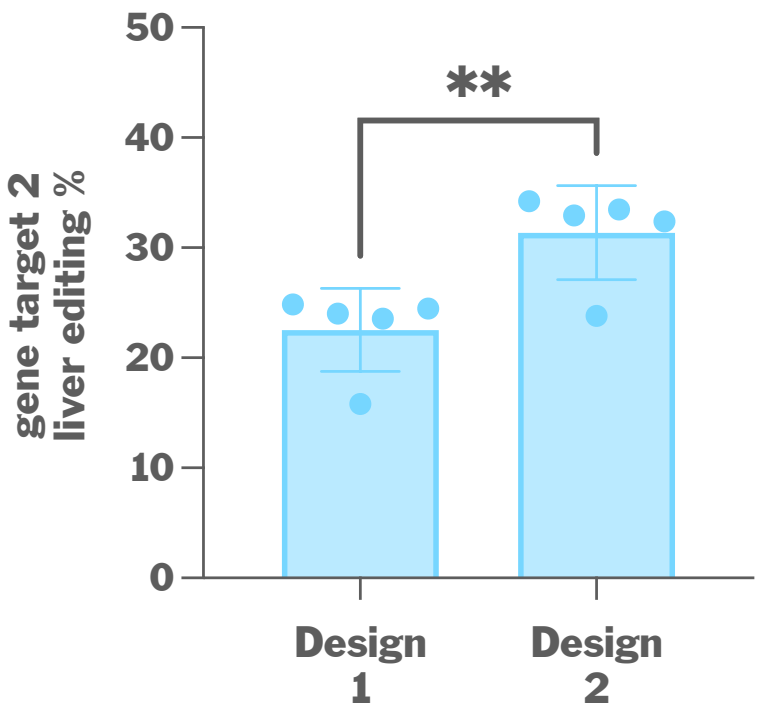
ASGPR ligand design 2 outperformed design 1 in delivery of base editing mRNA/gRNA to ldlr -/- mice

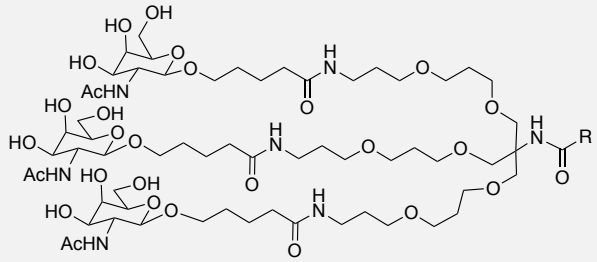
1 ASGPR Ligand Design

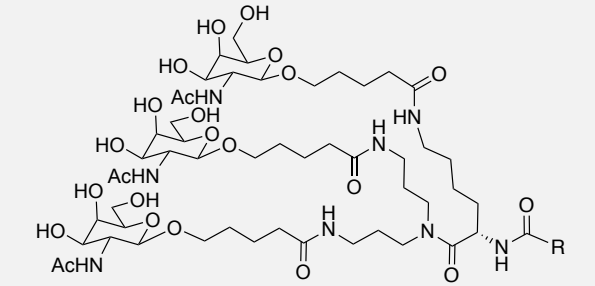
Gene Target 1



Gene Target 2





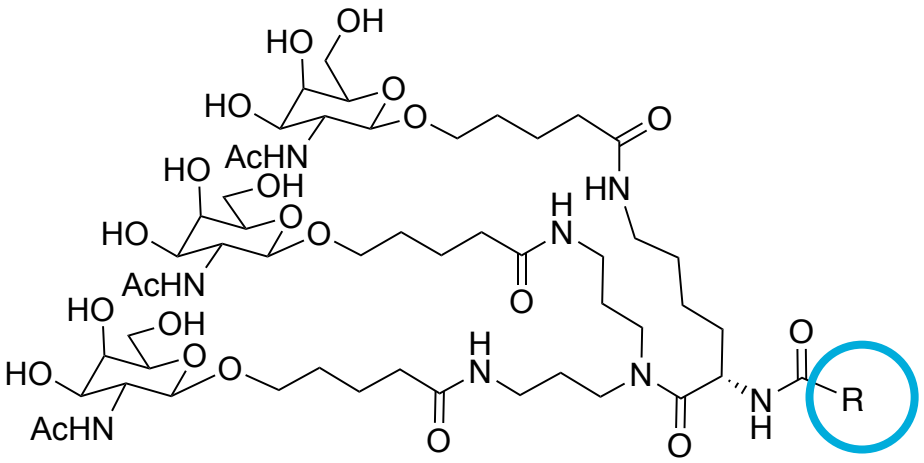


ldlr -/- mice
N = 5 per group
Dose: 0.1 mg/kg

Identifying the optimal lipid anchor and spacing between trivalent GalNAc and lipid tail

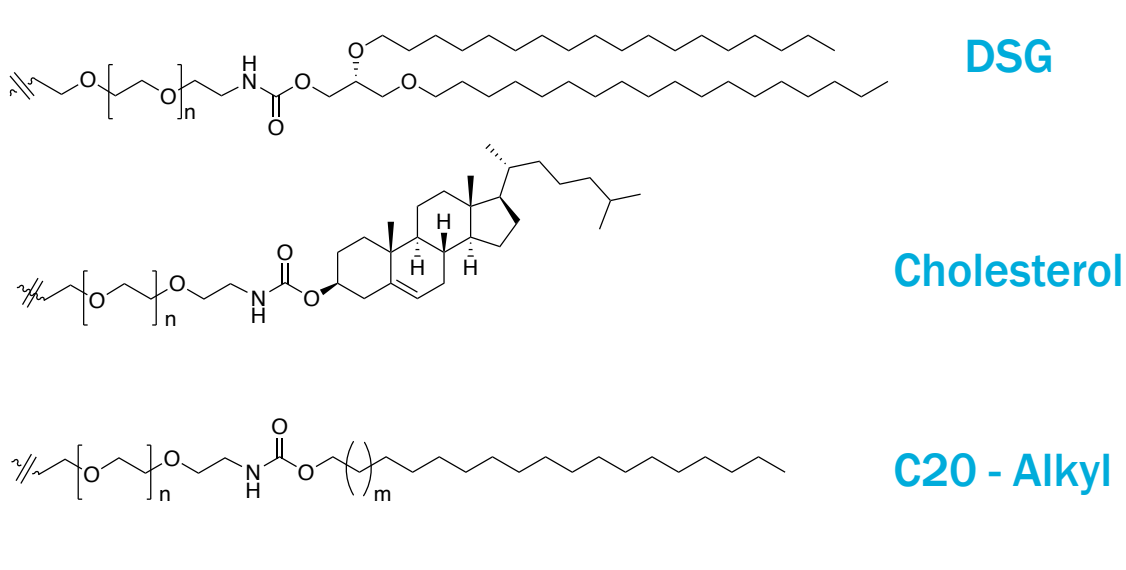
2 Lipid anchor and spacer

- What length PEG spacer maximizes ligand engagement with the ASGPR?
- How do hydrophobicity and structural features of the lipid anchor impact LNP formulation, particle morphology and in vivo performance?



Ligand design 2

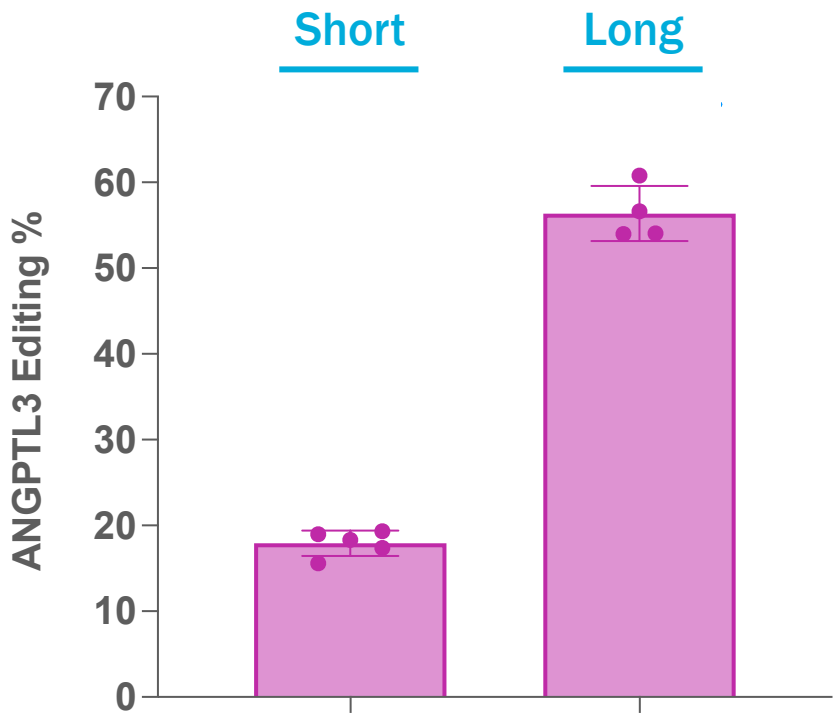
R =



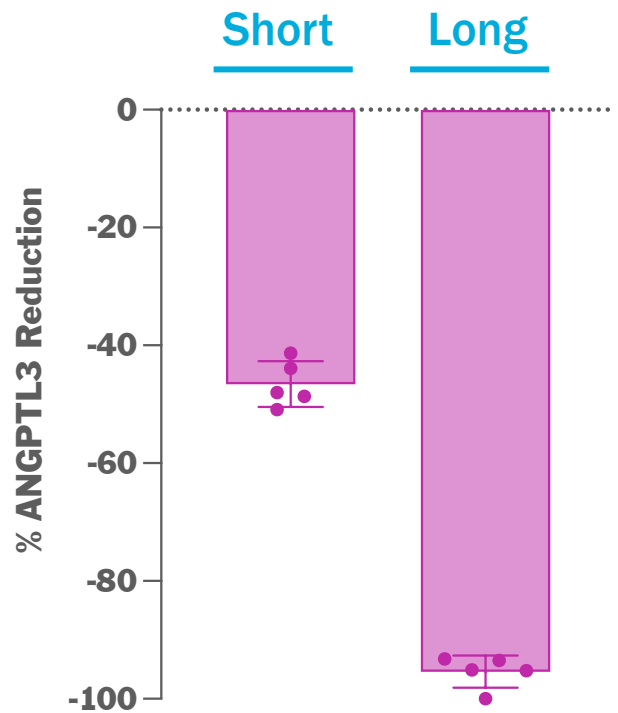
Longer spacer improved GalNAc-mediated liver delivery

2 Lipid anchor and spacer

Liver ANGPTL3 editing Day 9



ANGPTL3 protein reduction Day 9

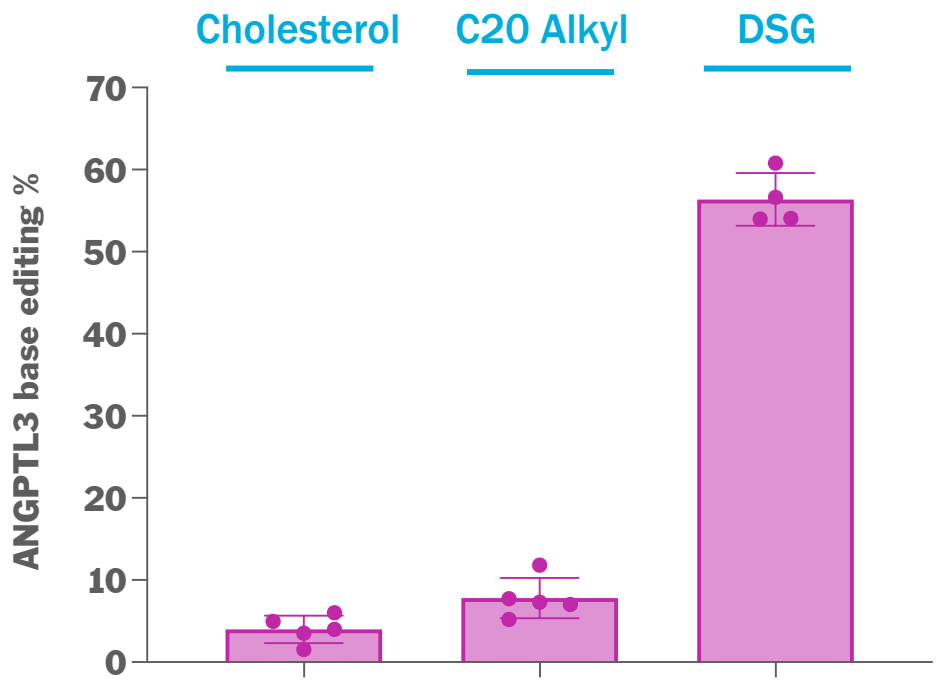


Single 0.3 mg/kg dose in ldlr -/- mice

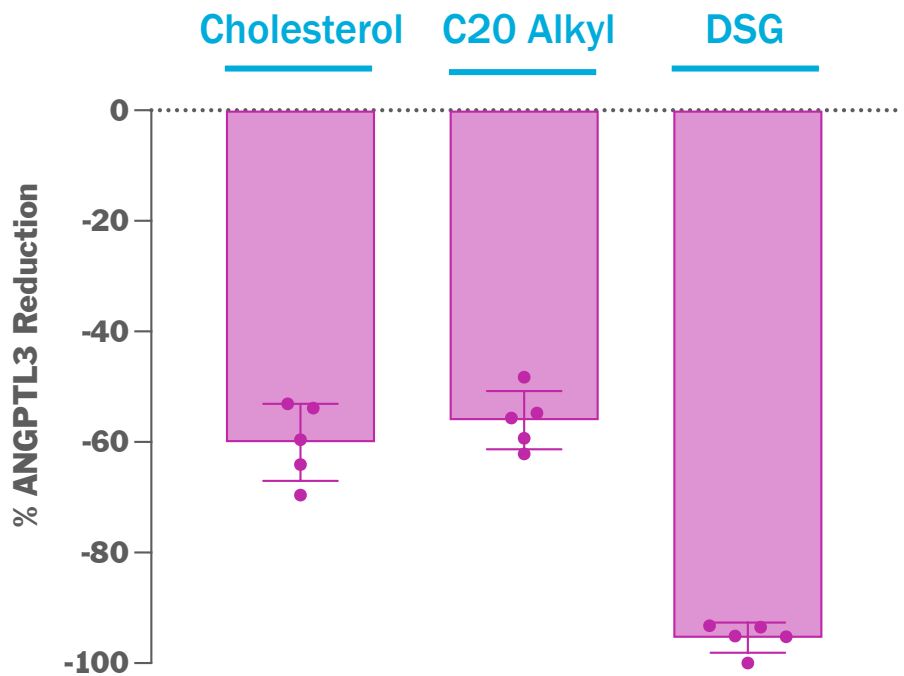
DSG lipid anchor outperformed alternative lipid anchors in liver delivery to ldlr -/- mice

2 Lipid anchor and spacer

Liver ANGPTL3 editing Day 9



ANGPTL3 protein reduction Day 9



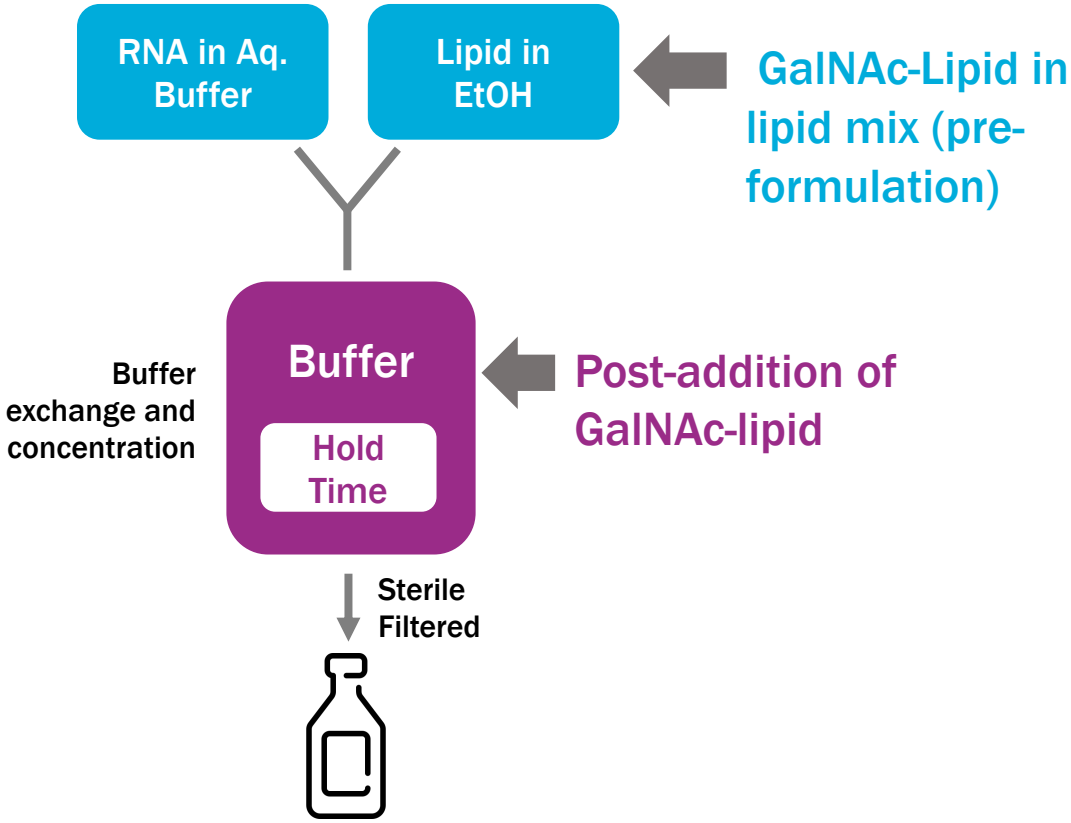
Single 0.3 mg/kg dose in ldlr -/- mice

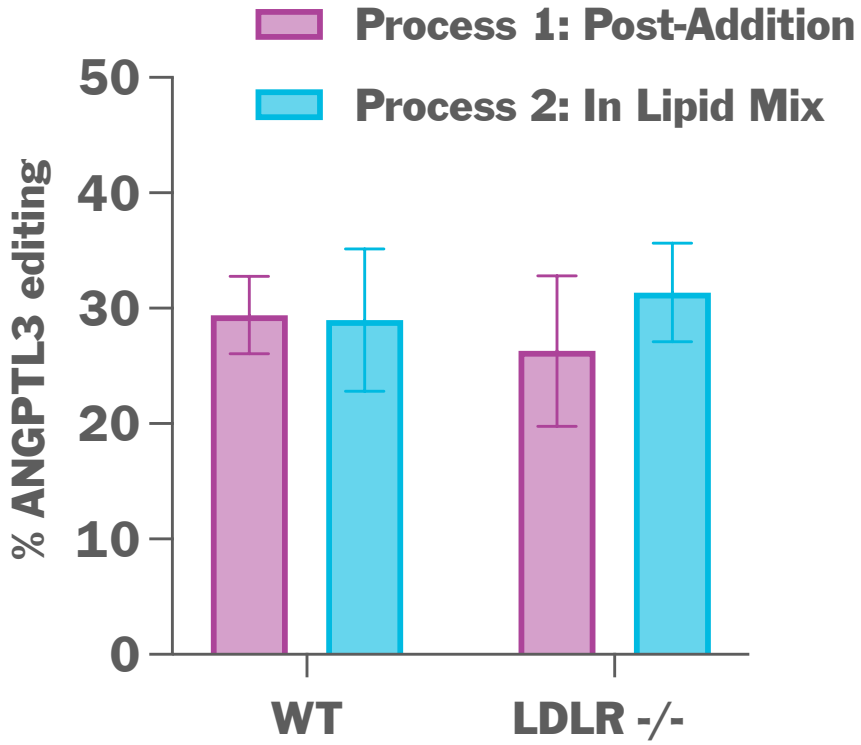
GalNAc-lipid can be added directly to the lipid mix and formulated without any additional post-processing steps

3 LNP Process Optimization

In lipid mixing process offers:

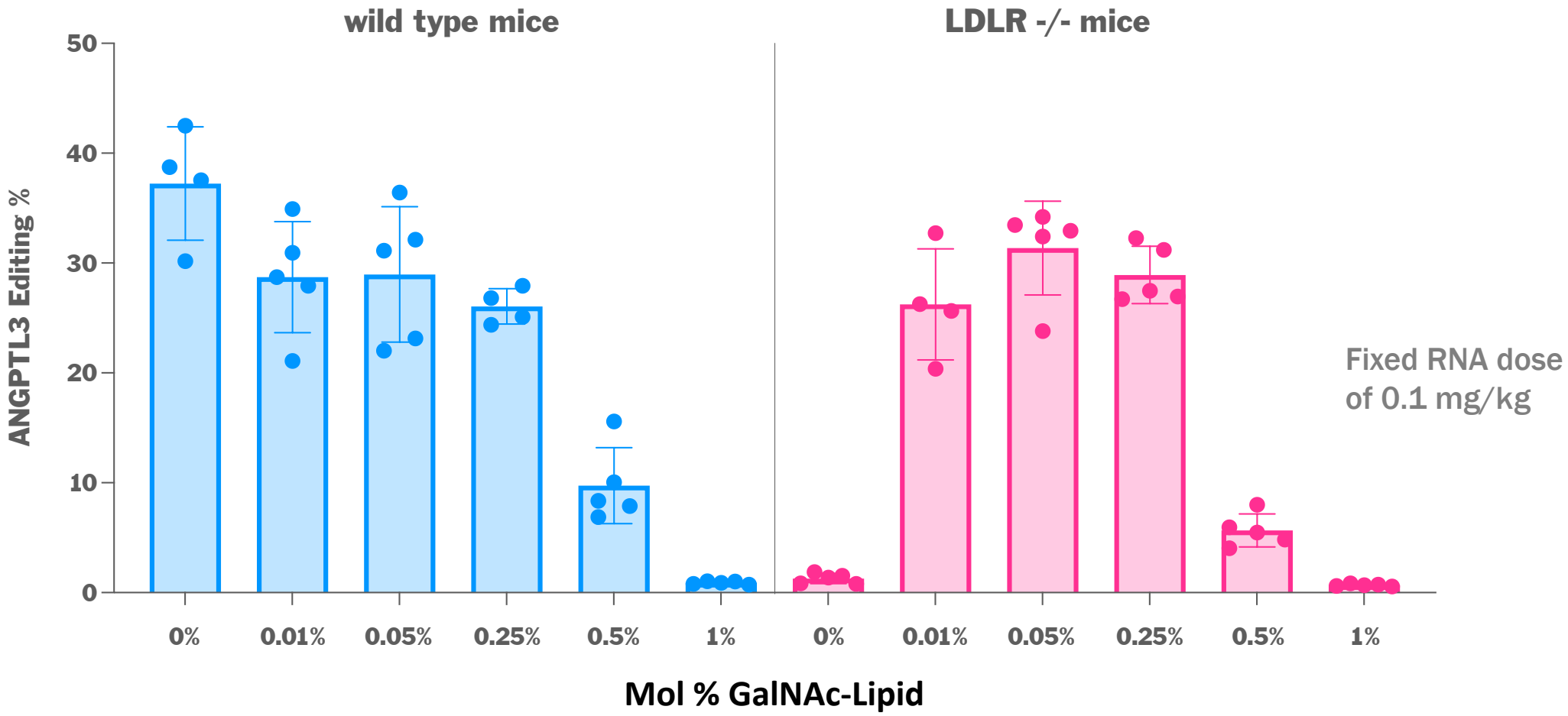
- **Near-homogenous distribution of GalNAc ligand across all LNP particles**
- **Scalable with CMC risk similar to conventional LNP & low COG**





Low GalNAc-Lipid content of 0.05 mol% achieved maximal liver editing activity

4 GalNAc surface density



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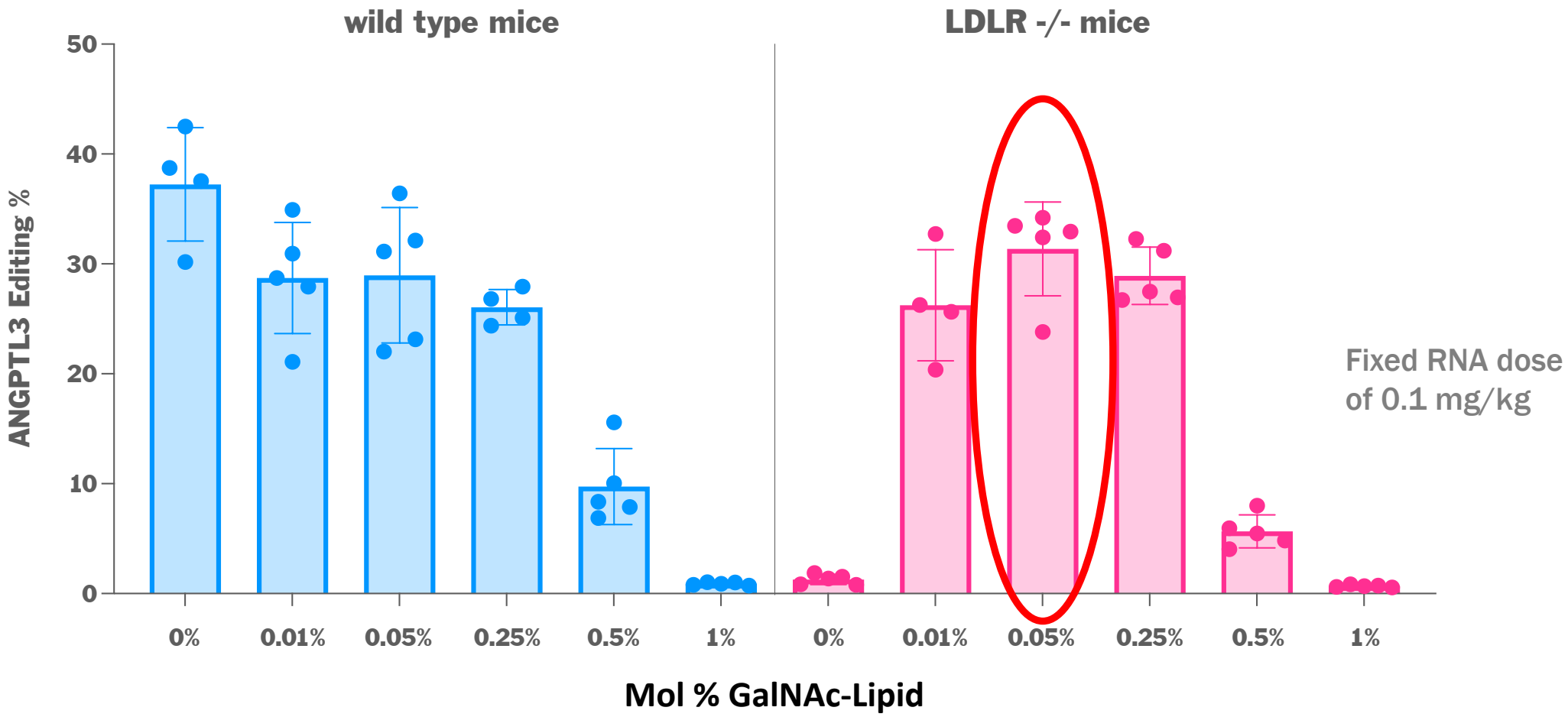
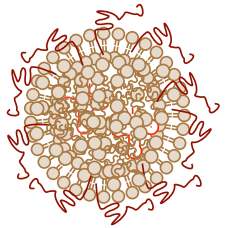
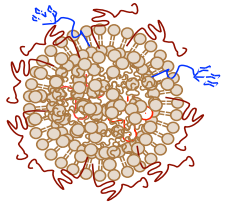


Developed an analytical method to differentiate populations of LNPs with and without GalNAc ligands on the surface

5 Analytical Methods

LNPs without GalNAc Ligand

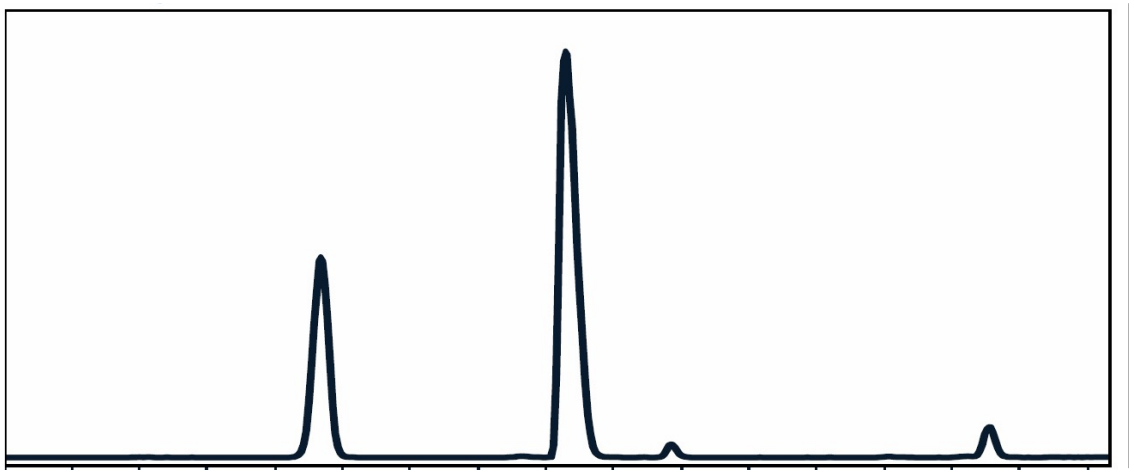




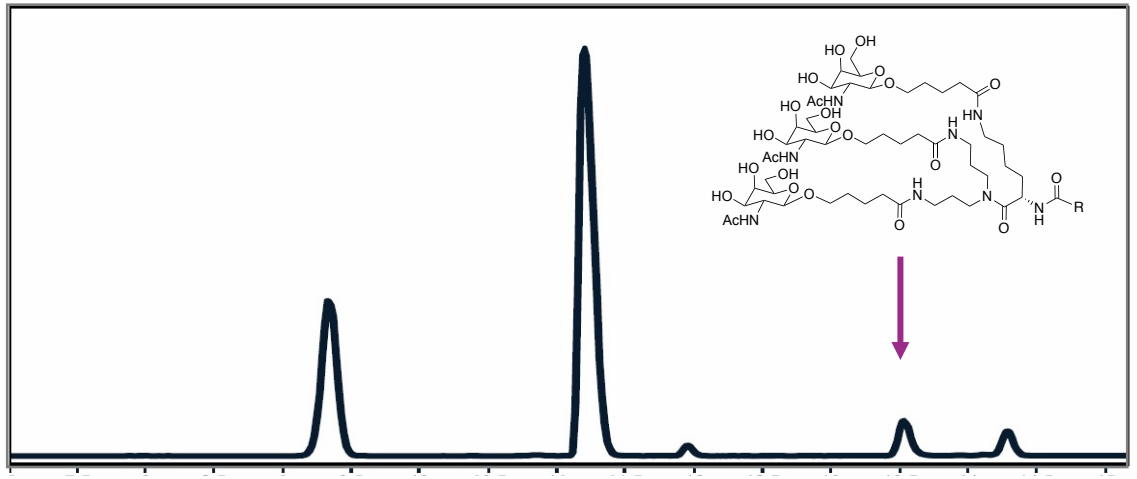
LNPs with GalNAc Ligand

Lectin Affinity Column

Flowthrough



Elution

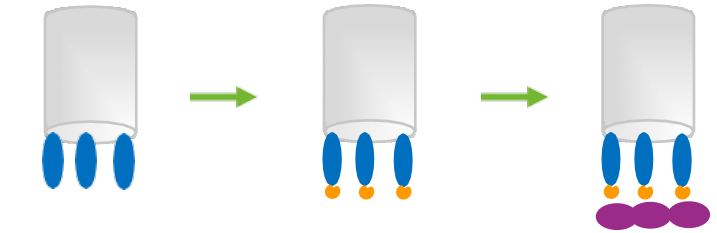


LNPs without GalNAc do not bind to lectin affinity column

LNPs with GalNAc-lipid bind and elute from the lectin affinity column

Confirmation that GalNAc-LNPs specifically bind to ASGPR

5 **Analytical Methods**



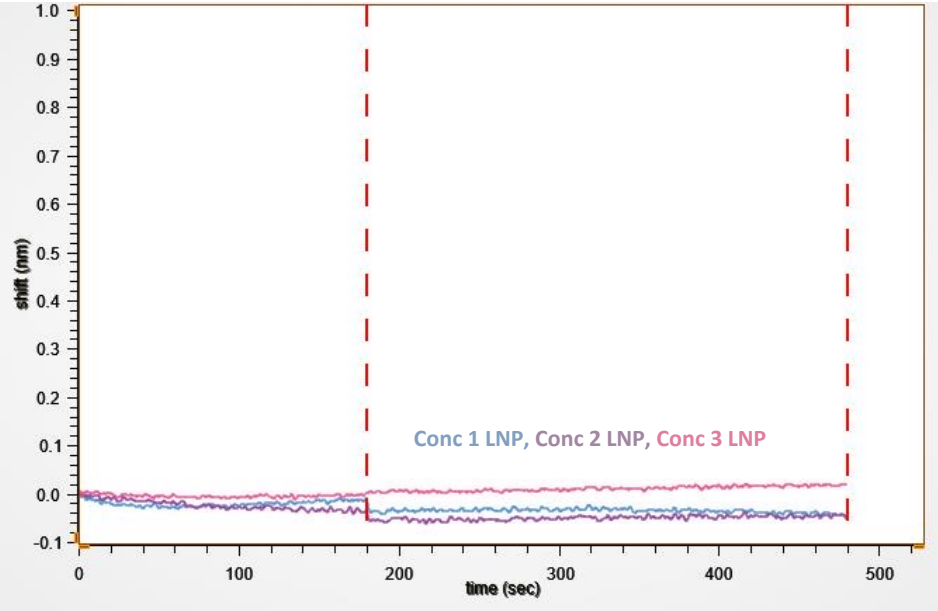
Biolayer Interferometry (BLI)

Anti-His

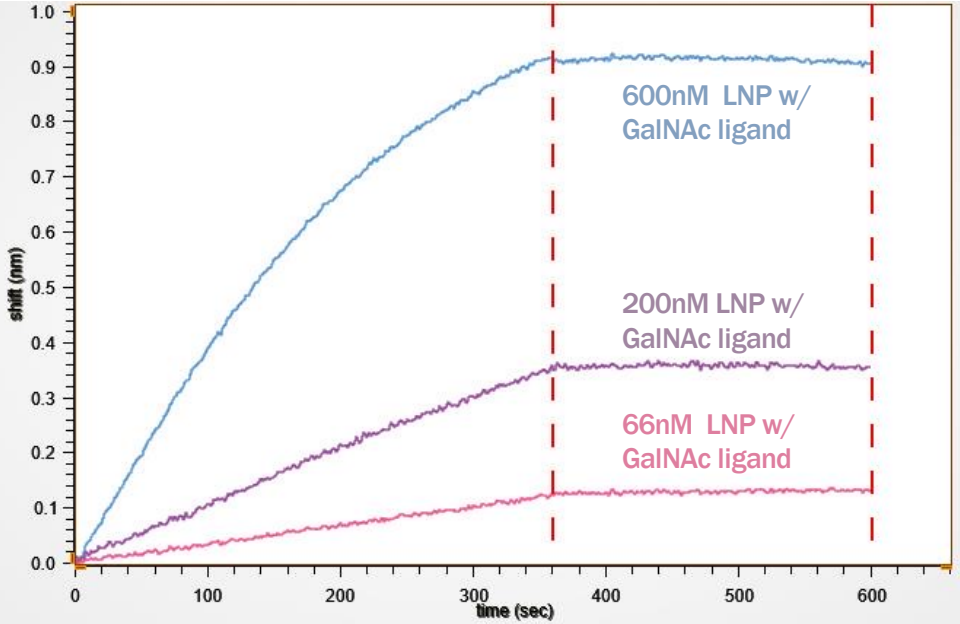
His-tagged ASGPR

GalNAc - LNP

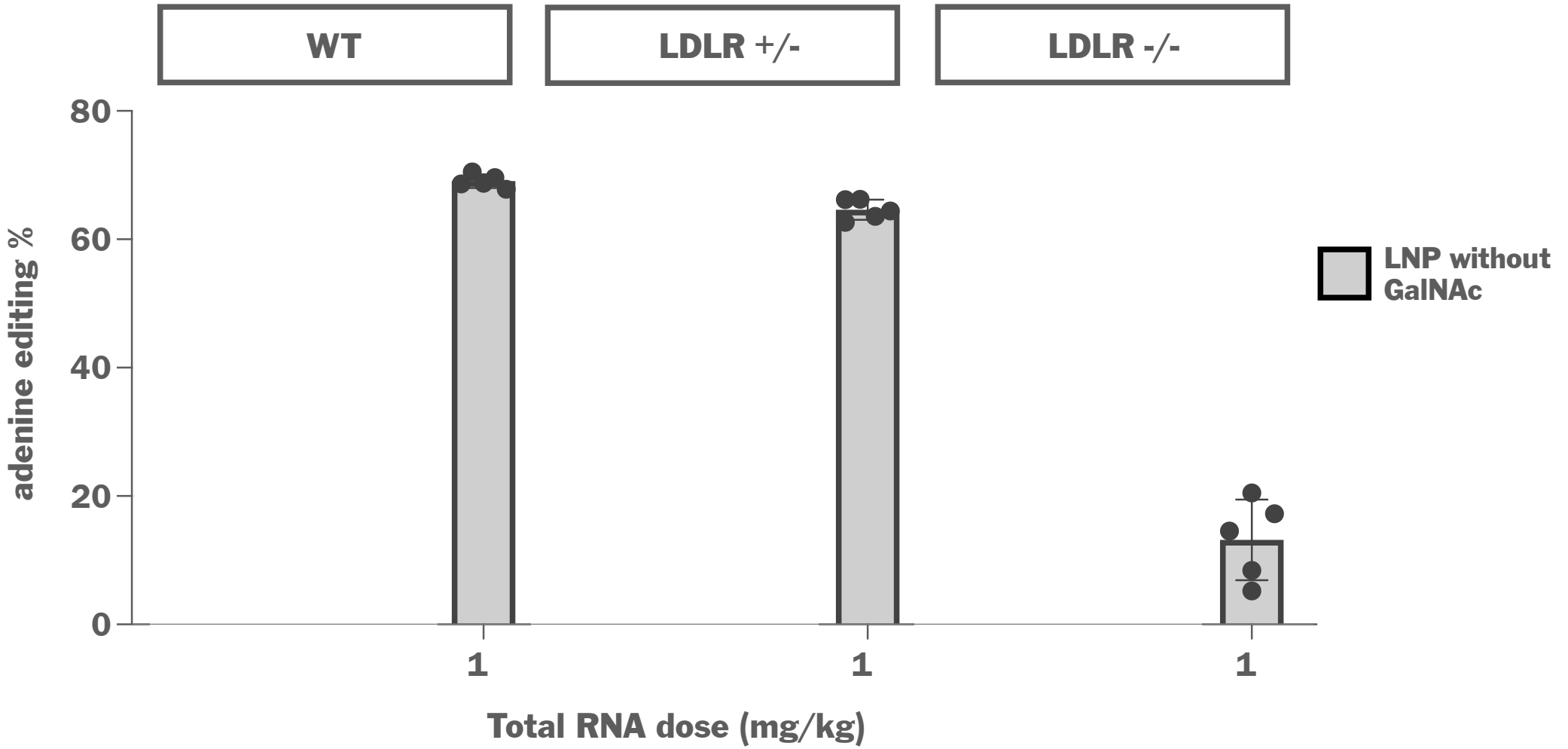
LNP without GalNAc ligand



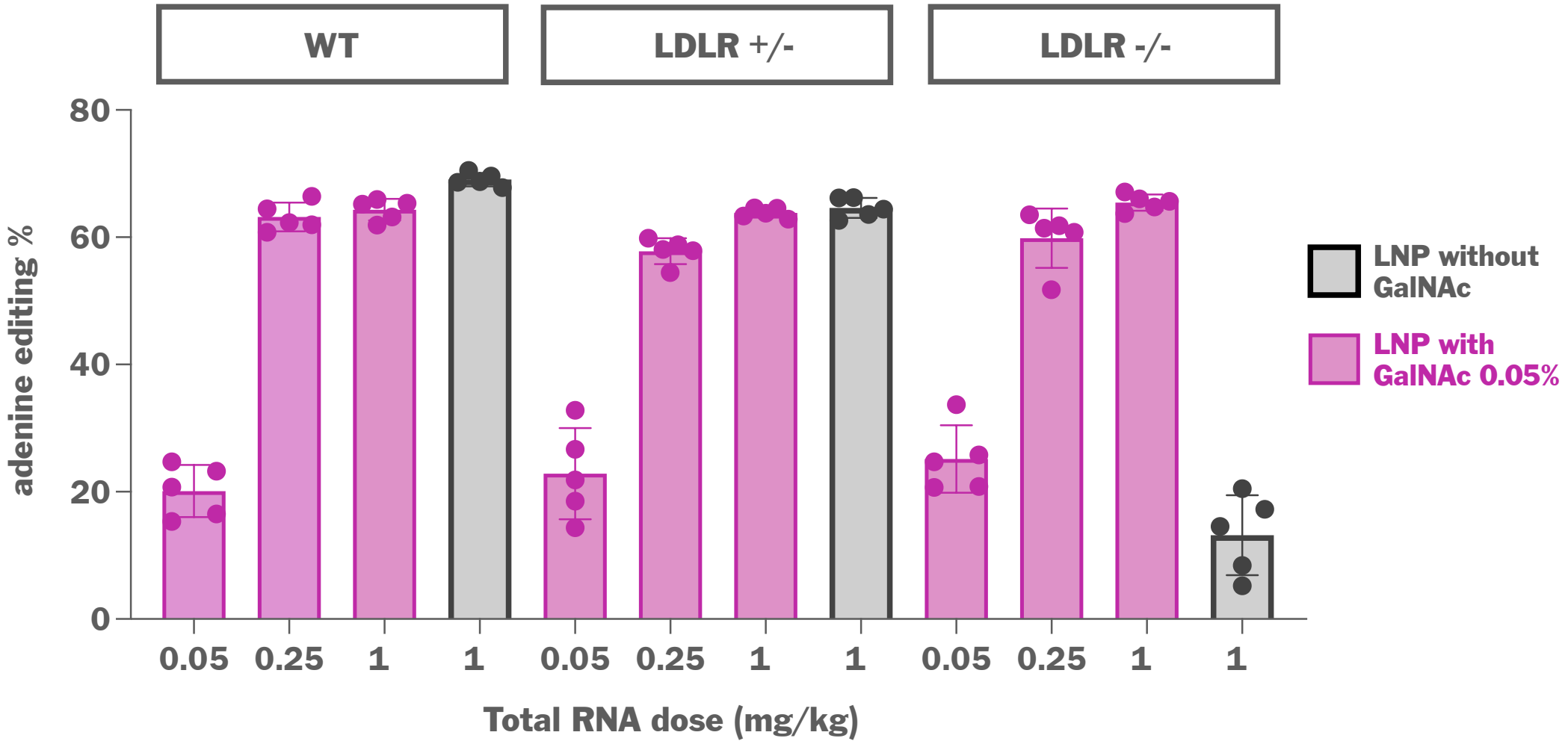
LNP with GalNAc ligand



Recall the problem: standard LNPs do not deliver well to hepatocytes completely lacking LDLR



Optimized GalNAc-LNPs have nearly identical effective doses in mice irrespective of LDLR status



Summary: Verve's proprietary GalNAc LNP achieved high efficiency liver delivery and base editing in mouse model of HoFH

- ✓ Proprietary ASGPR ligand design using GalNAc
- ✓ Ligand potency 10x greater than any reported before with LNPs
- ✓ GalNAc-LNP yielded equal potency regardless of LDLR status in mice
- ✓ Developed scalable formulation process for stable GalNAc-LNPs
- ✓ Developed analytical methods to assess GalNAc-LNPs

We have established a proprietary GalNAc-LNP delivery system that will enable ANGPTL3 editing in patients with HoFH (as well as HeFH)

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		Research/ Lead optimization	IND-Enabling	Clinical	Development Milestones
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LDL-C and triglyceride-rich lipoprotein (TRL)					
ANGPTL3	Familial hypercholesterolemia	→			• Candidate selection (2022) • Begin IND-enabling studies (2022)

Next Steps

- Demonstration of GalNAc-LNP delivery to non-human primates
- Scaling up production of GalNAc-Lipid and GalNAc-LNP
- Additional analytical method development

Thank you to the world-class team of problem solvers at Verve

Key Contributors

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