



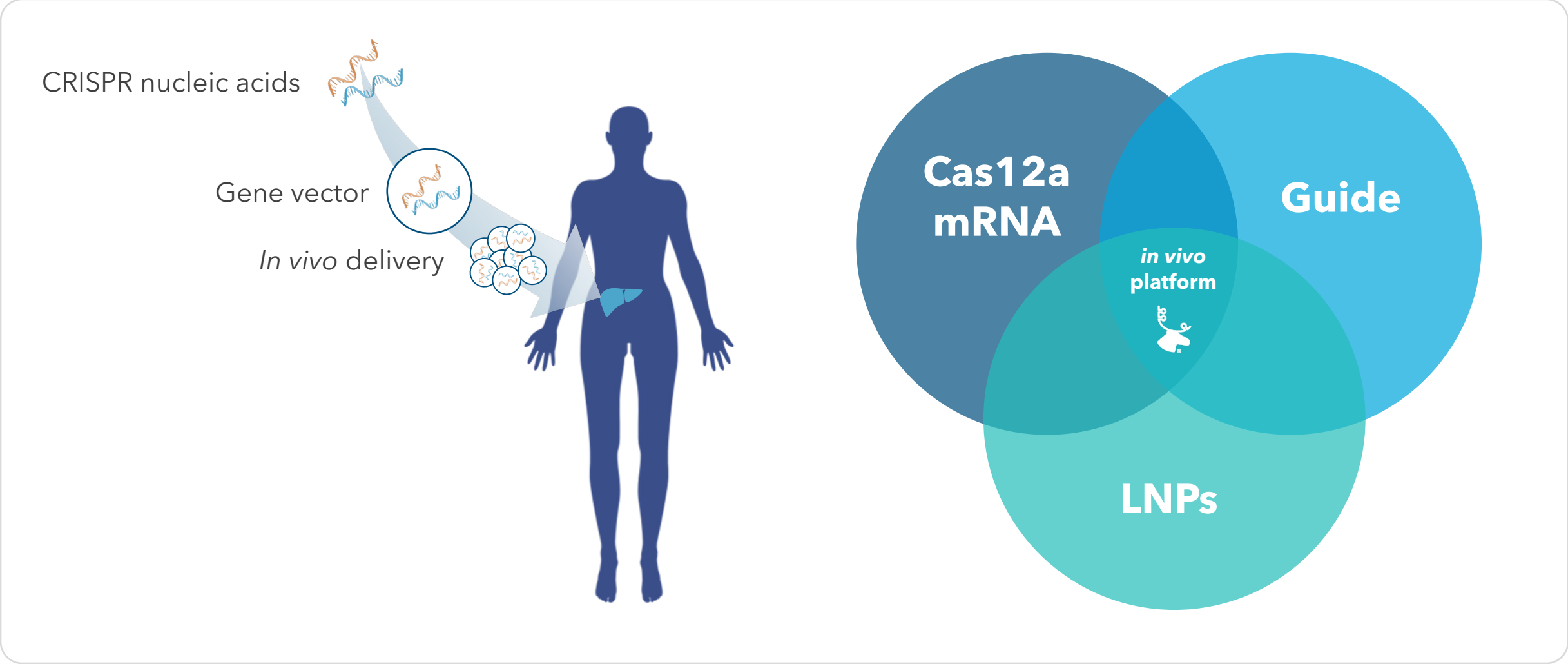
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May 10, 2024

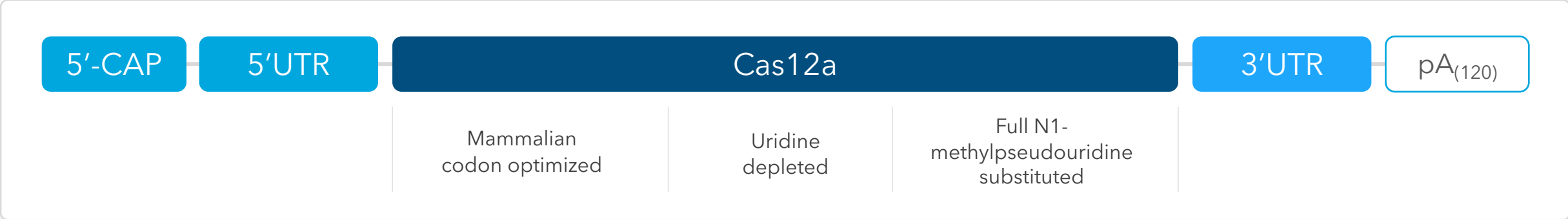
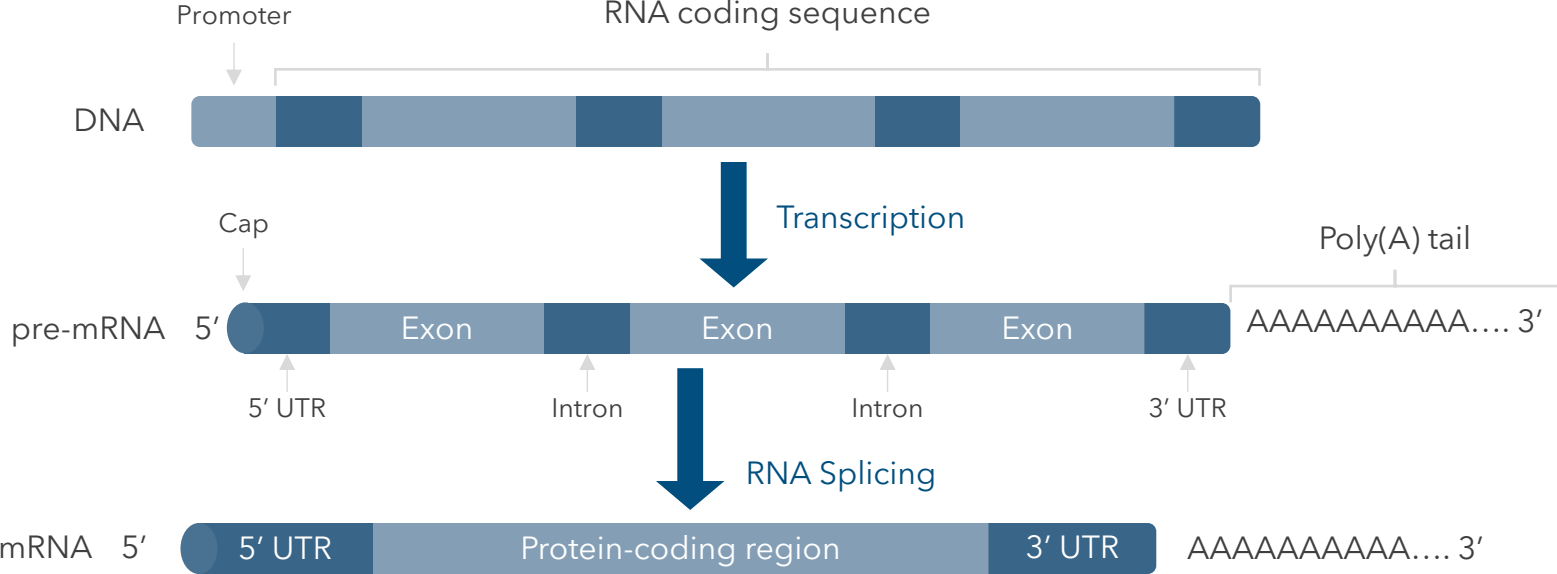
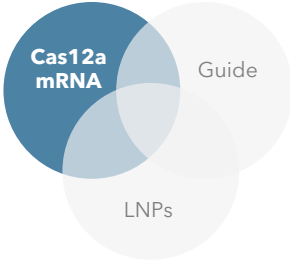
**Cas12a CRISPR Hybrid RNA-DNA (chRDNA)-  
Mediated *In Vivo* Genome-Editing Technology for  
Efficient and Functional Hepatic Gene Disruption**

27<sup>th</sup> American Society of Cell & Gene Therapy (ASGCT) Annual Meeting  
Baltimore, MD

# Development of a Cas12a-mediated *in vivo* editing platform



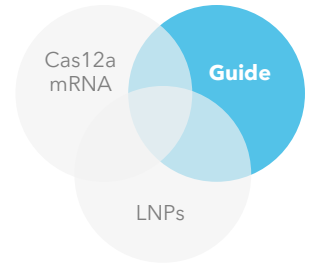
# mRNA optimization for *in vivo* applications



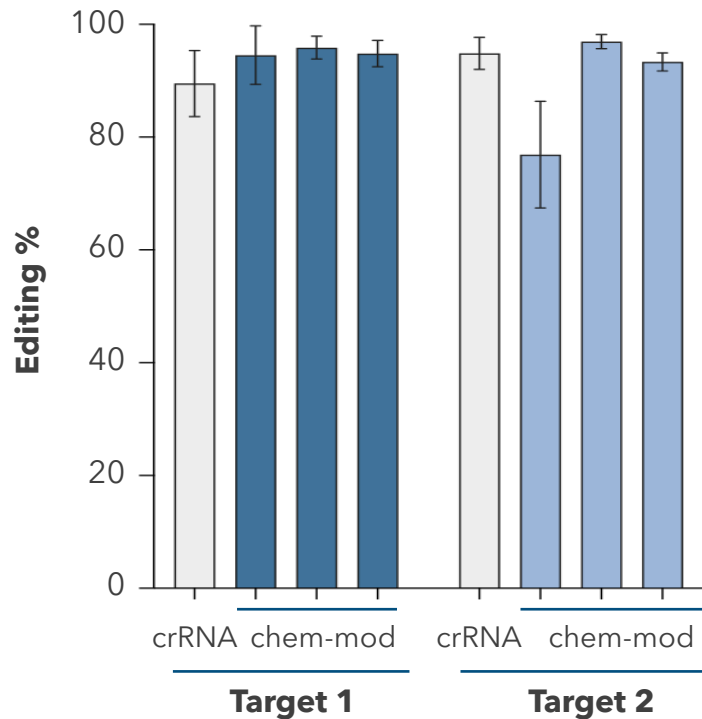
3 UTR: untranslated region; pA<sub>(120)</sub>: 3'-end poly(A) tail, 120 nucleotides



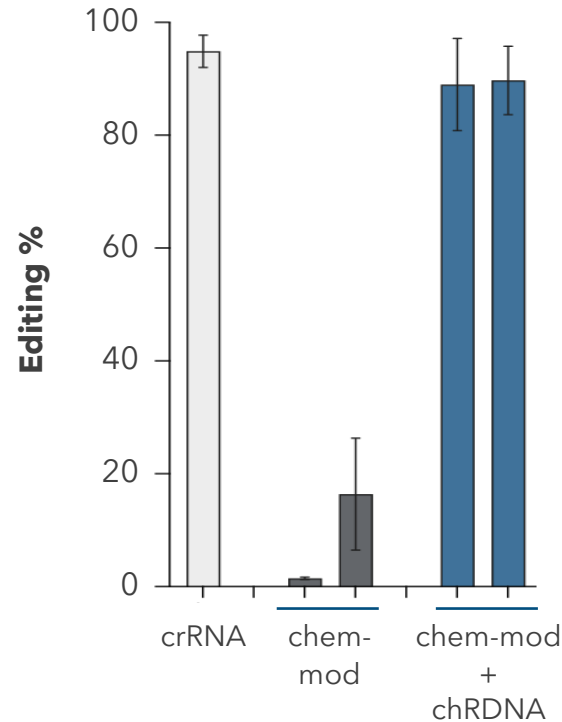
# Cas12a + CRISPR hybrid RNA-DNA (chRDNA) have improved specificity *in vitro*



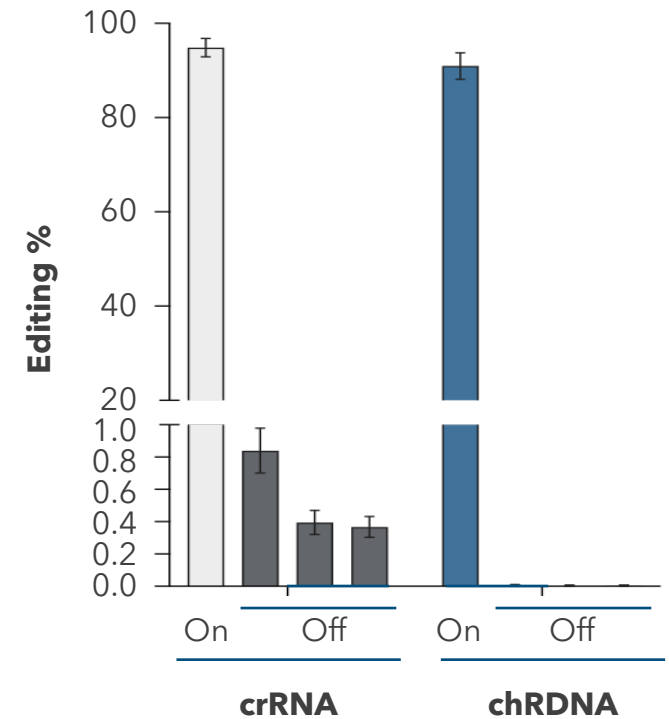
## Cas12a guides tolerate multiple types of chemically-modified bases



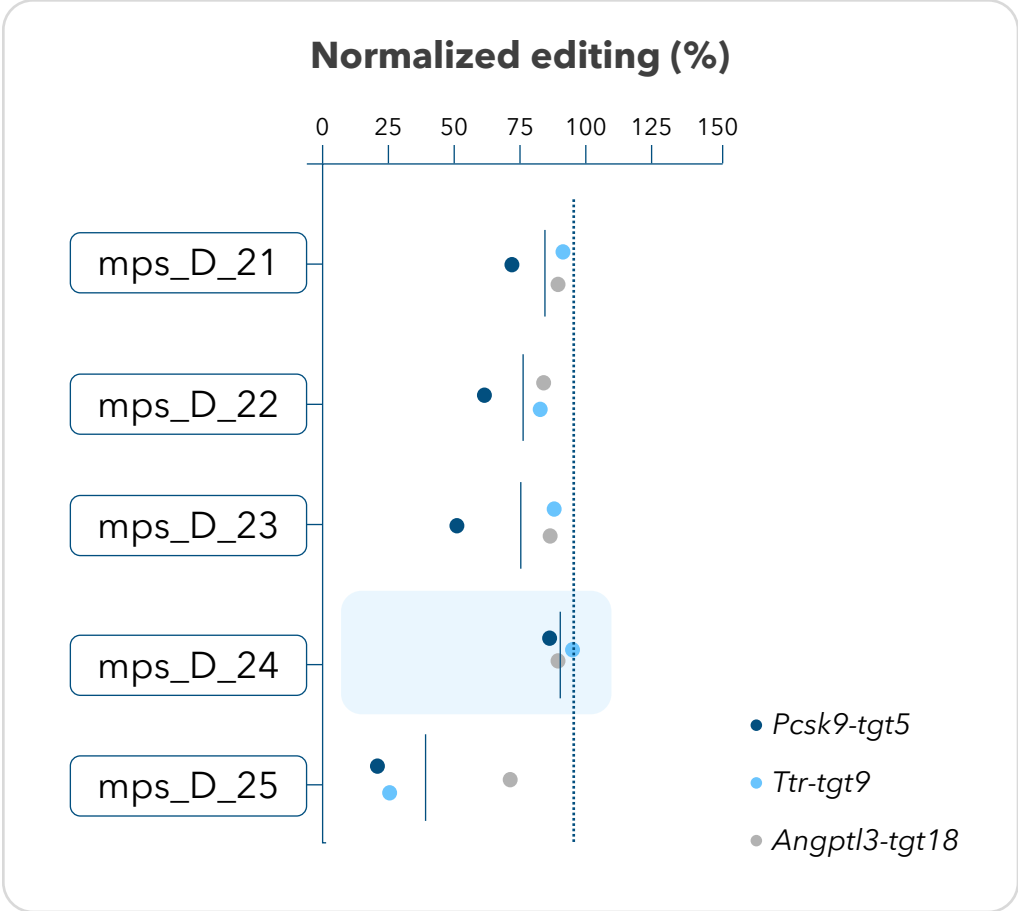
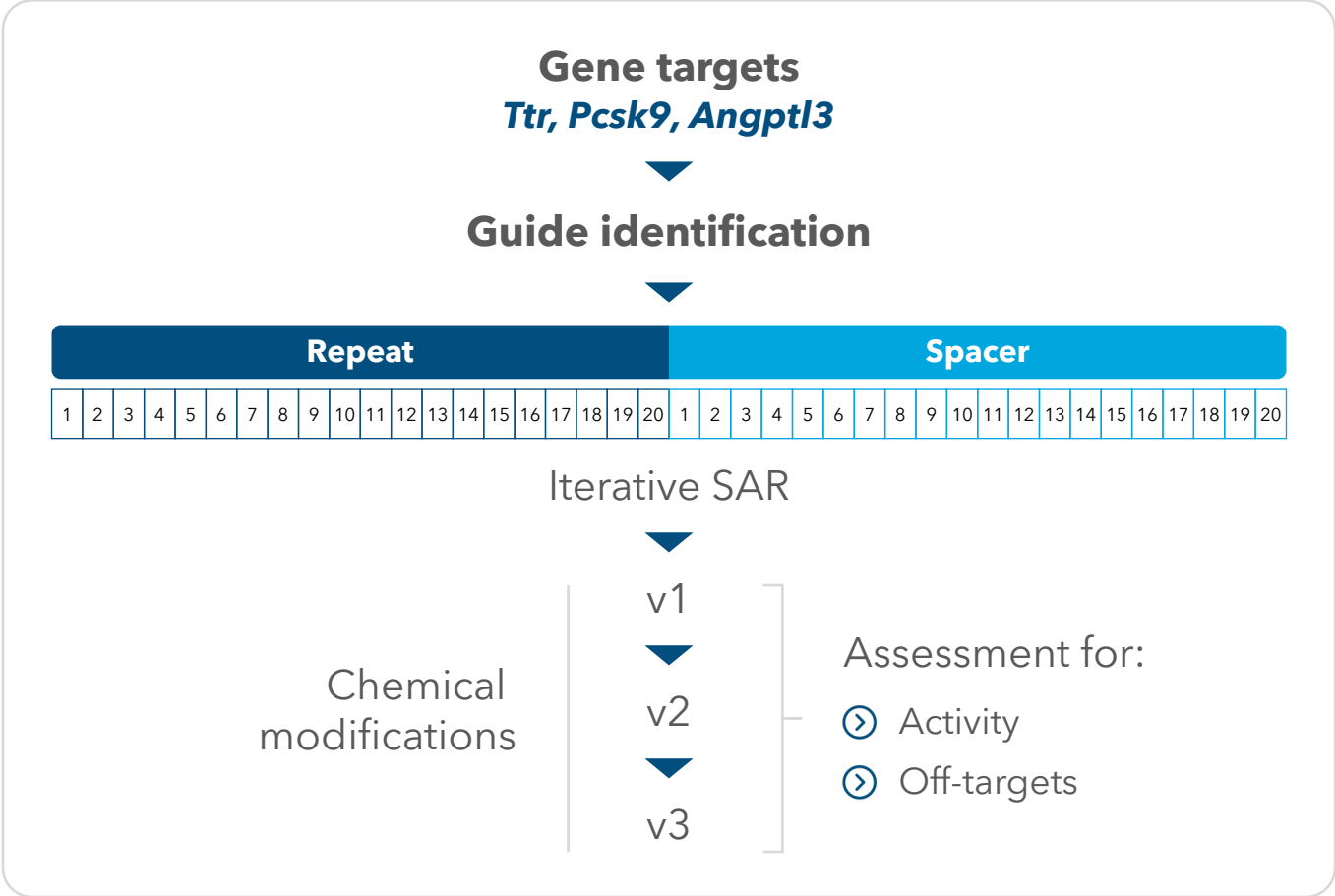
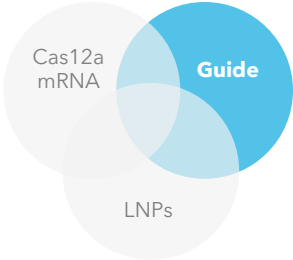
## Incorporation of DNA can rescue the activity of certain chemically-modified designs



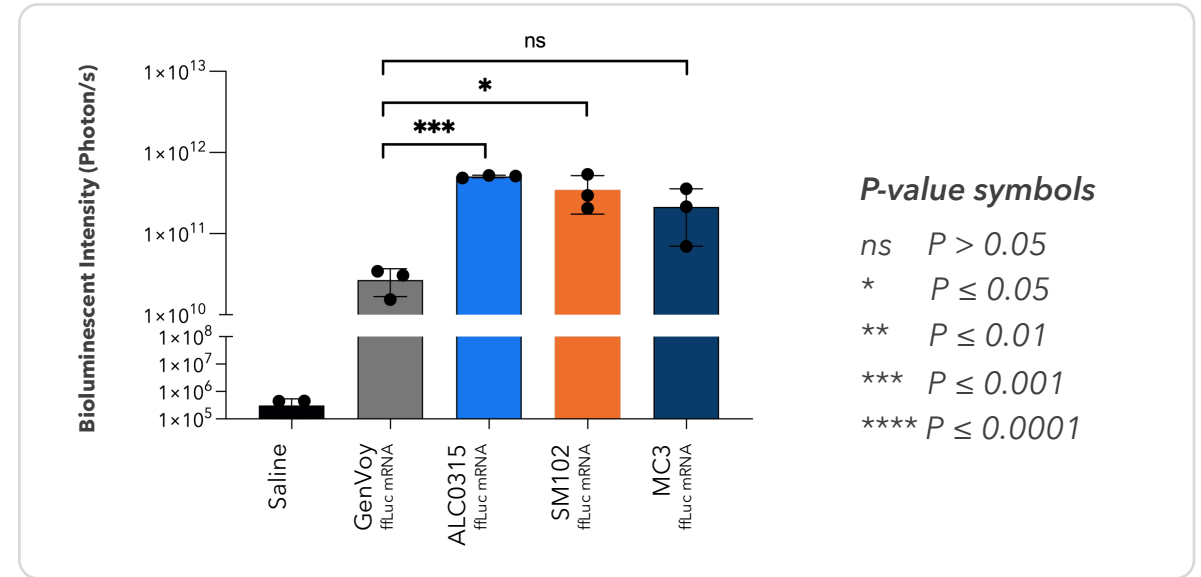
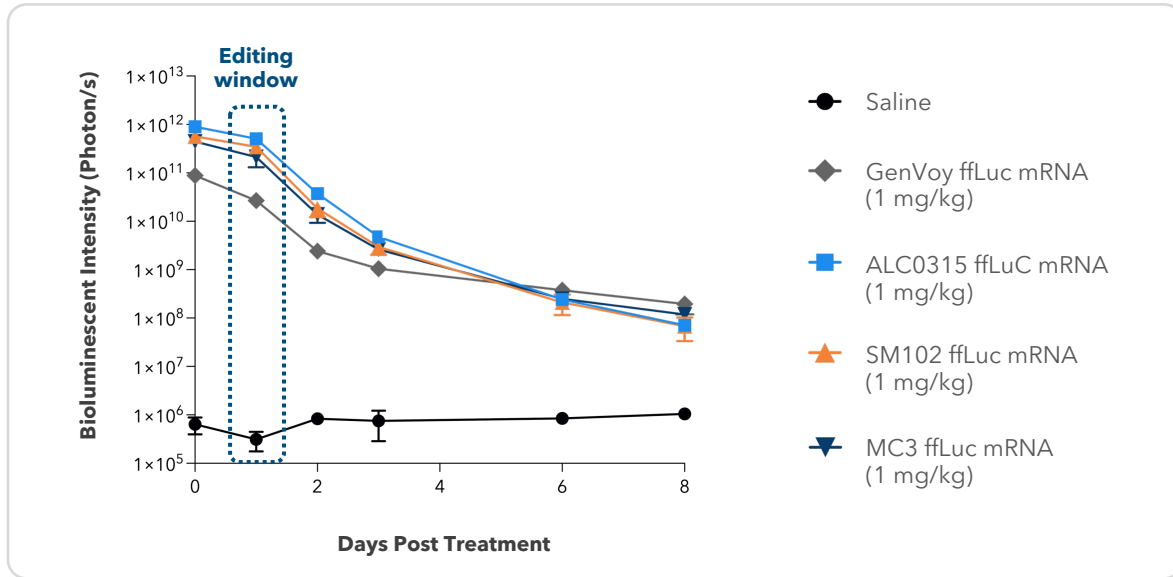
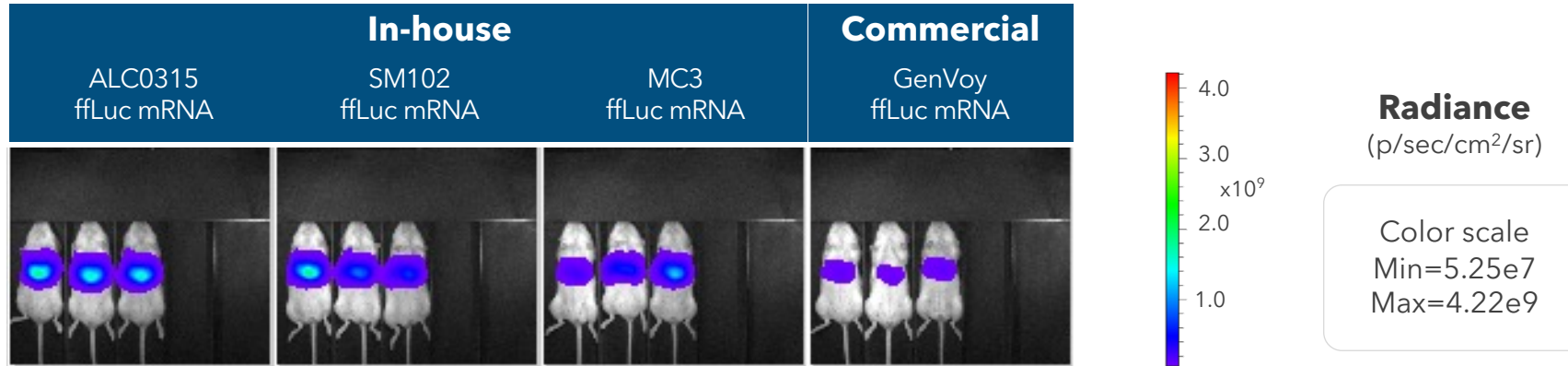
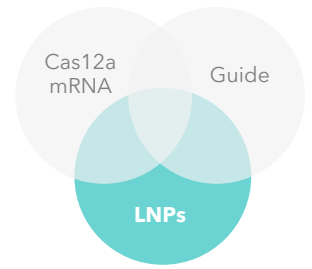
## Cas12a chRDNA guides have improved specificity compared to all-RNA guides



# Optimized chRDNA guides achieve peak activity across validated targets



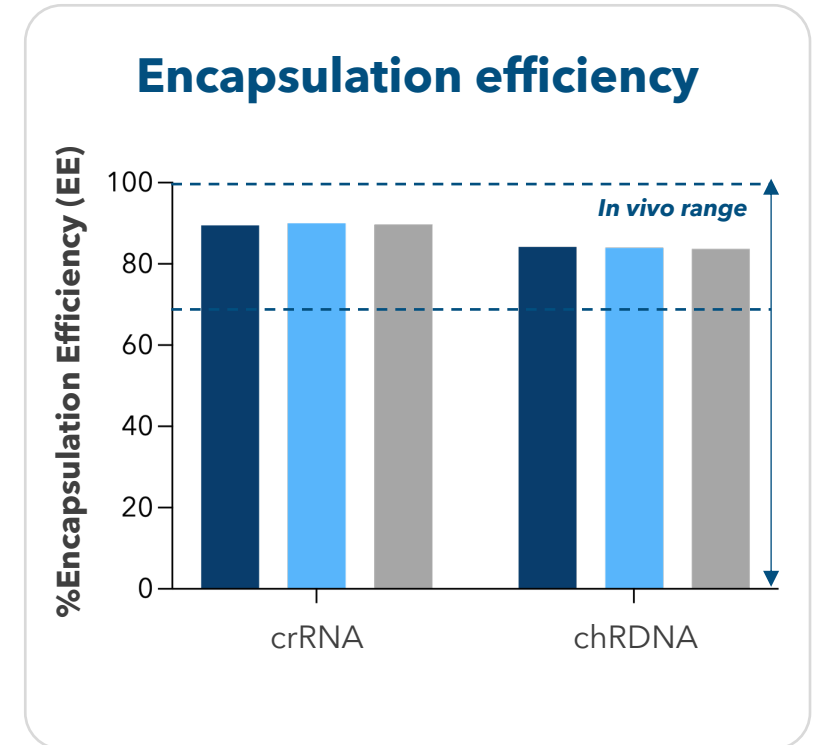
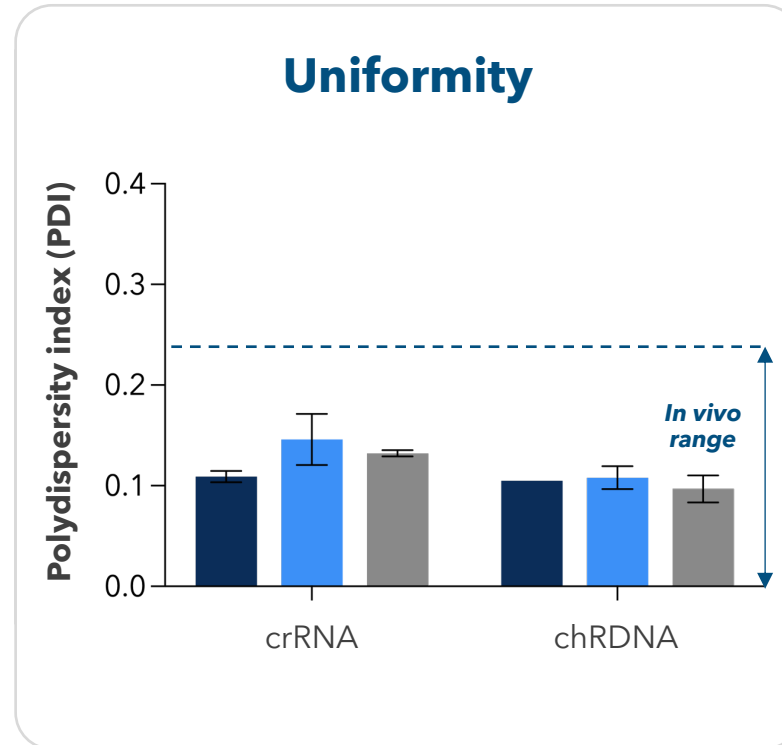
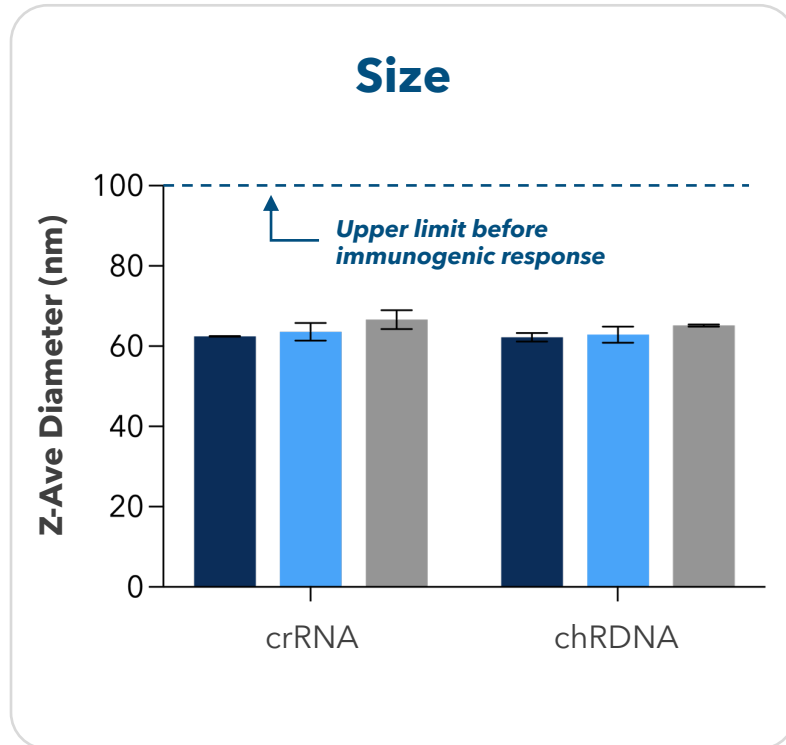
# In-house LNP formulation of ALC0315 drive higher expression at 24h compared to commercial formulation



# Reproducible platform for encapsulating mRNA + guides in LNPs

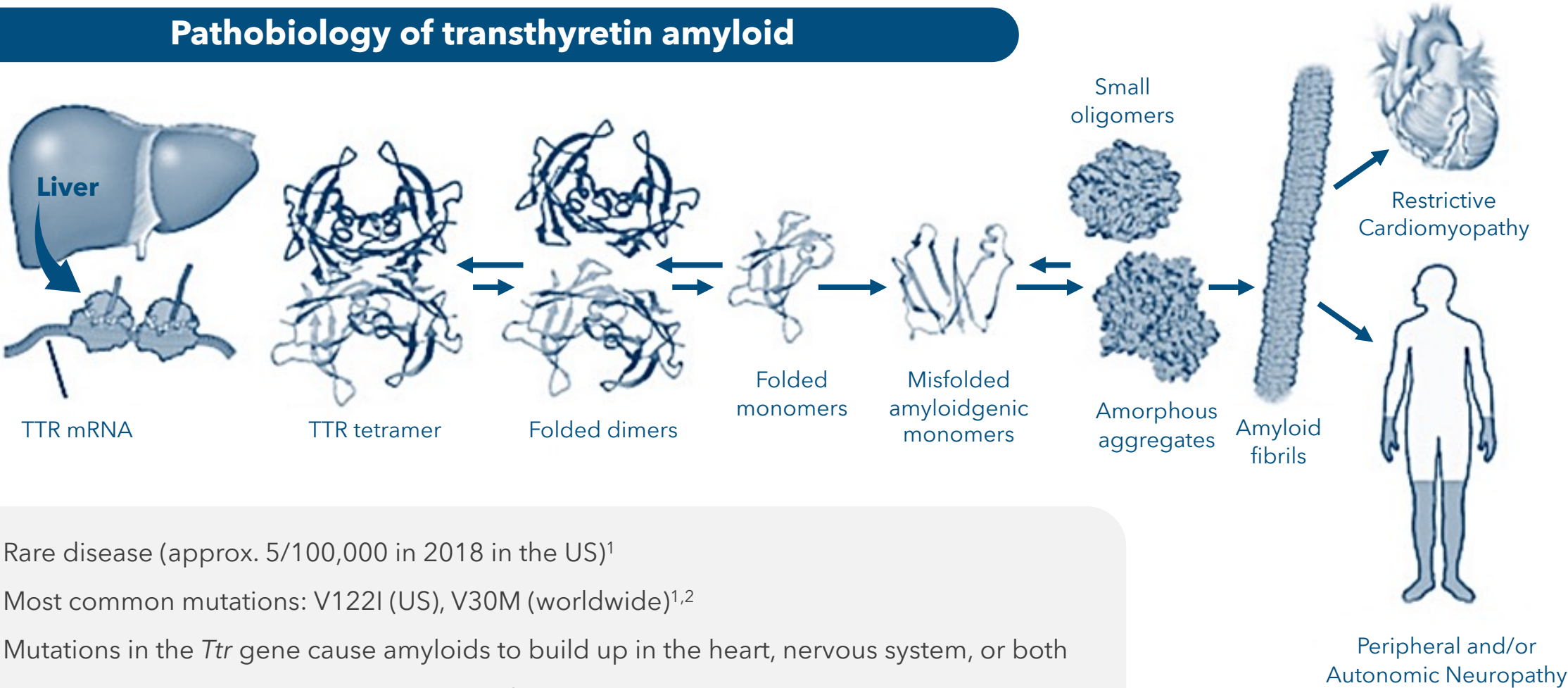
Cas12a mRNA was successfully encapsulated in LNPs with either crRNA or chRDNA targeting *Pcsk9*, *Ttr*, or *Angptl3* using ALC0315 in-house lipid mix

LNP size, polydispersity, and encapsulation efficiency were in range for *in vivo* administration



# Transthyretin (TTR) amyloidosis

## Pathobiology of transthyretin amyloid



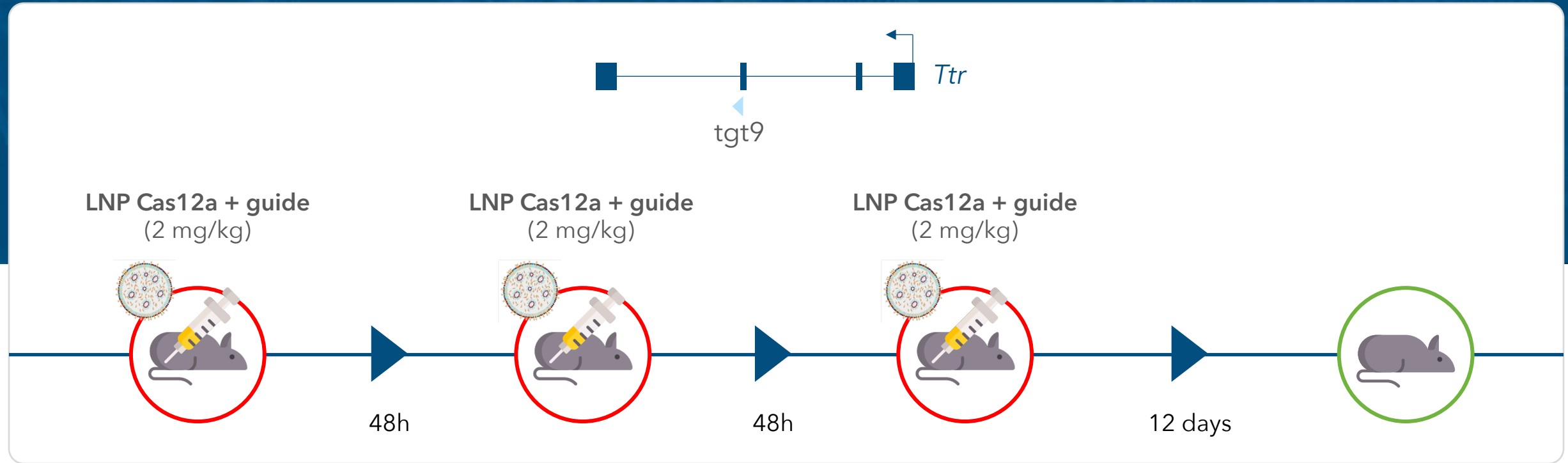
- > Rare disease (approx. 5/100,000 in 2018 in the US)<sup>1</sup>
- > Most common mutations: V122I (US), V30M (worldwide)<sup>1,2</sup>
- > Mutations in the *Ttr* gene cause amyloids to build up in the heart, nervous system, or both
- > Survival post-diagnosis approx. 25 months<sup>2</sup>

<sup>1</sup>Cleveland Clinic 2019, Amyloidosis Research Consortium, 2024  
<sup>2</sup>Ruberg, F. L., et al. Journal of the American College of Cardiology, 73(22), 2872-2891





# Ttr multi-dose study design



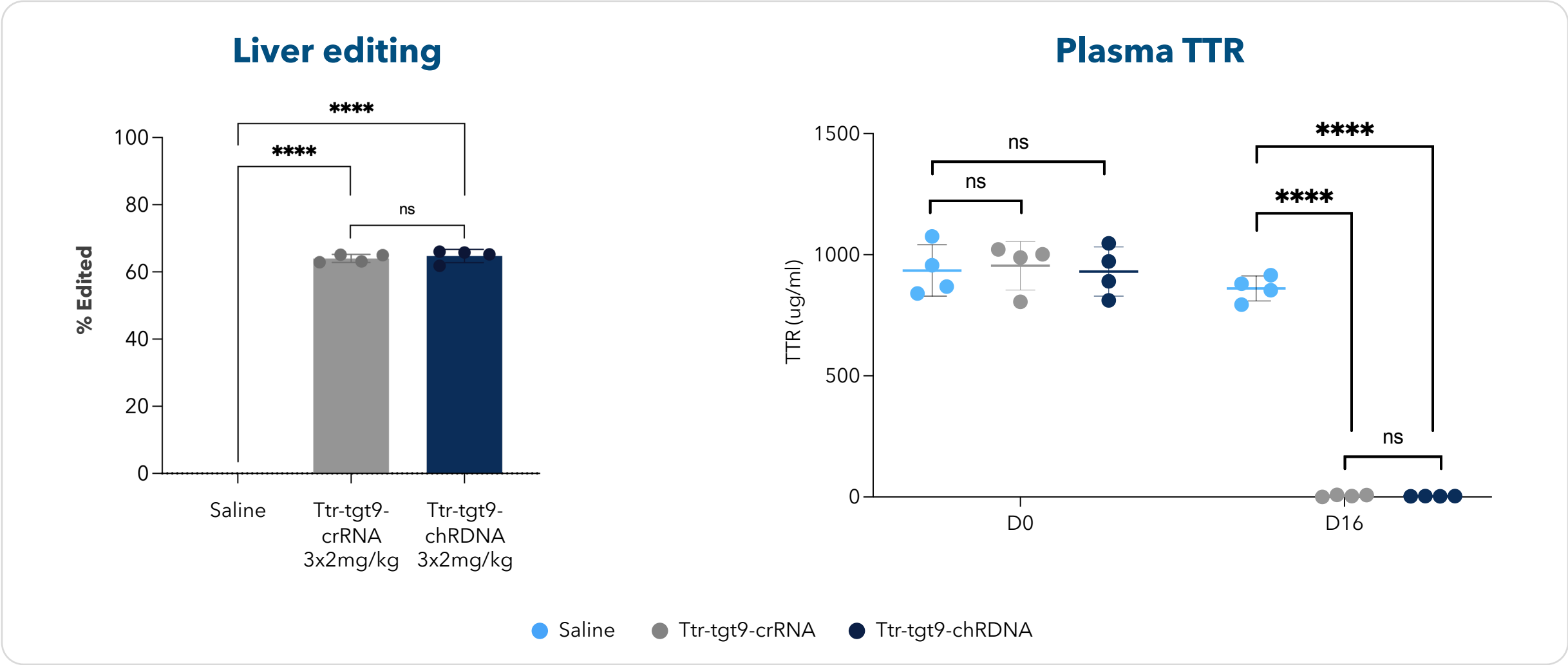
## Study groups

- 1 Saline (4 mice)
- 2 Cas12a mRNA + Ttr-tgt9-crRNA (4 mice)
- 3 Cas12a mRNA + Ttr-tgt9-chRDNA (4 mice)

**Mouse strain:** Balb/c  
**Age:** 7 weeks  
**Dose:** 3 x 2 mg/kg

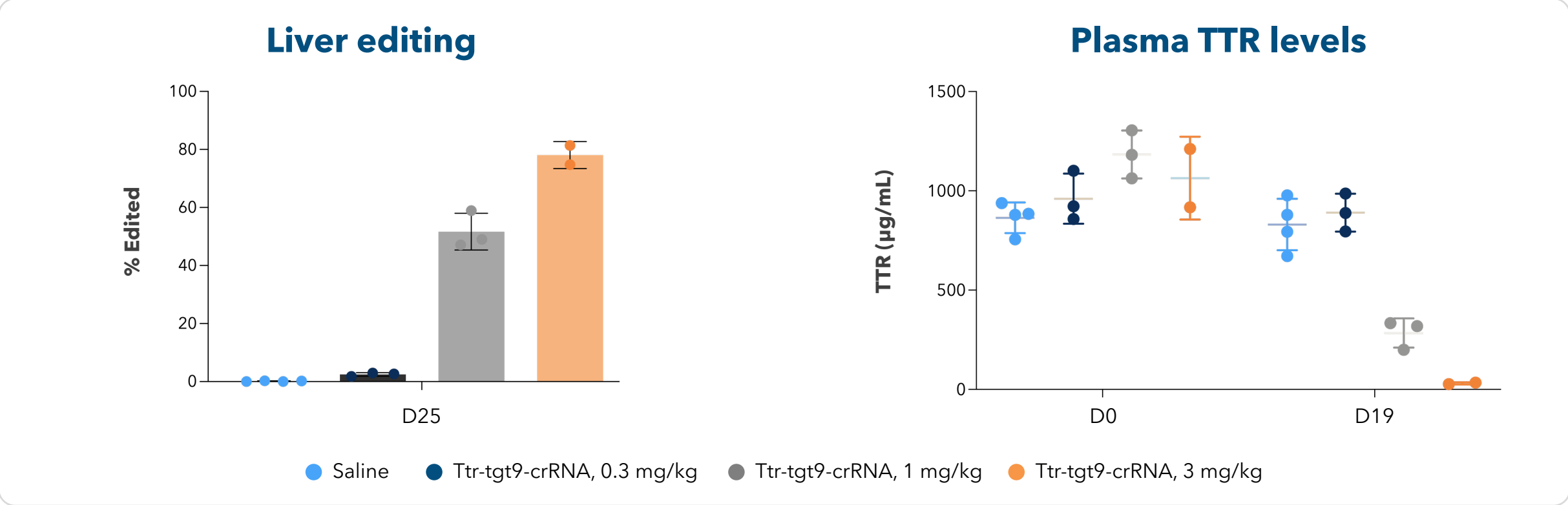
**Injection volume:** 400  $\mu$ L  
**Administration:** Primary intravenous (IV)

# In vivo liver editing of *Ttr* resulted in >98% knockdown of plasma TTR levels using both crRNA or chRDNA guides



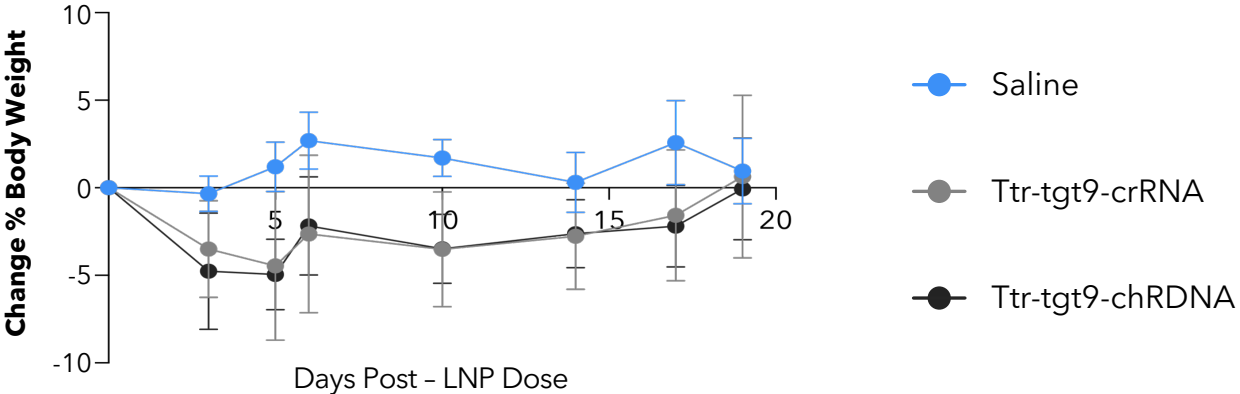
# Single-dose administration with all-RNA guides results in functional reduction of TTR

Previous studies addressing tolerability, toxicity, and immunogenicity were assessed using aggressive multi-dose challenges

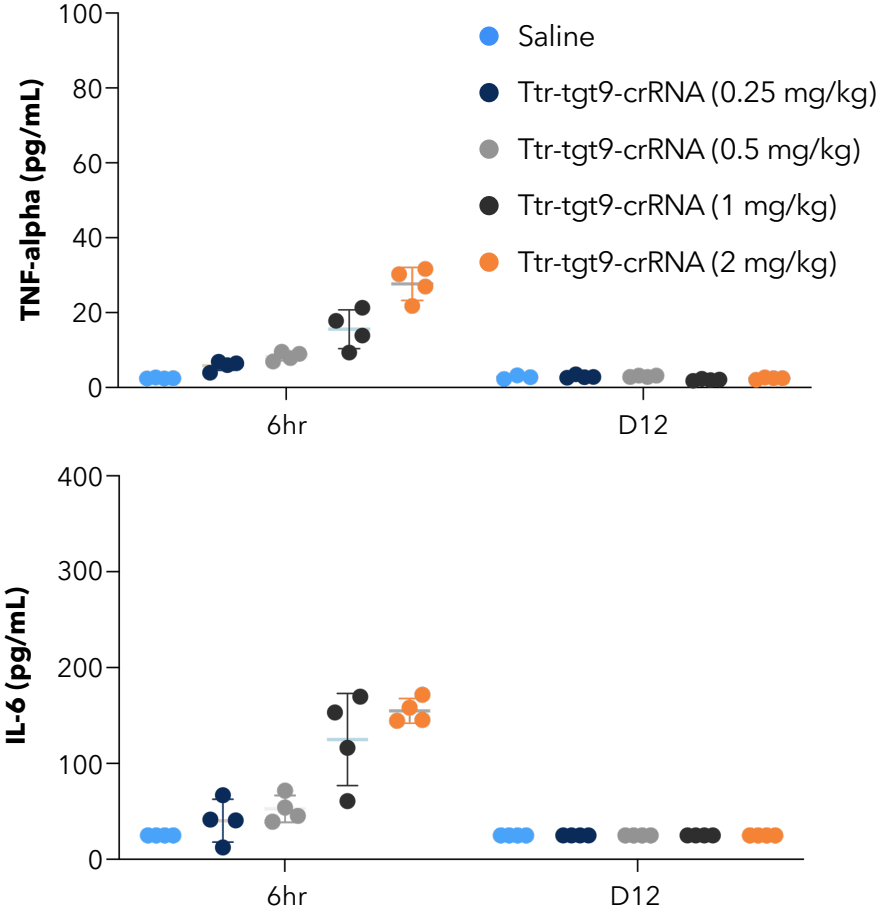


# Treatment generally well tolerated in preclinical models with both all-RNA and chRDNA guides

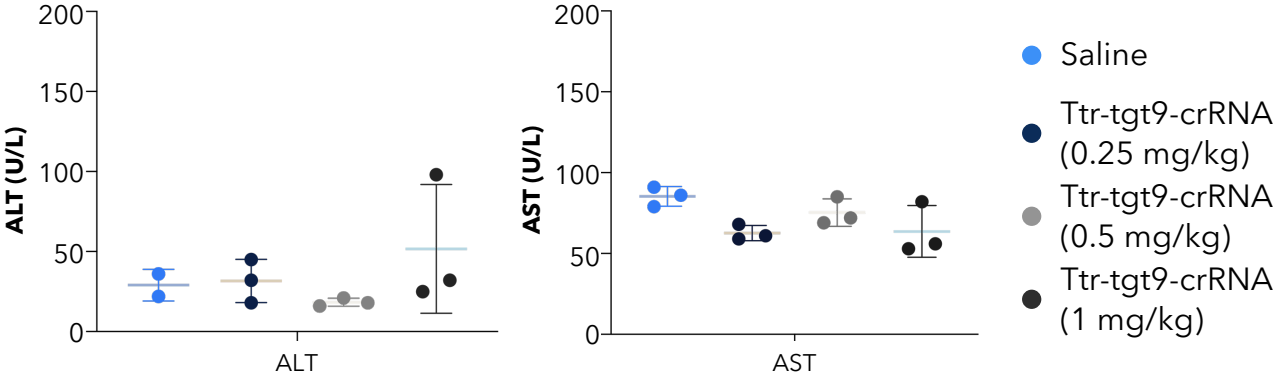
## Normal body weight change



## Immunogenicity

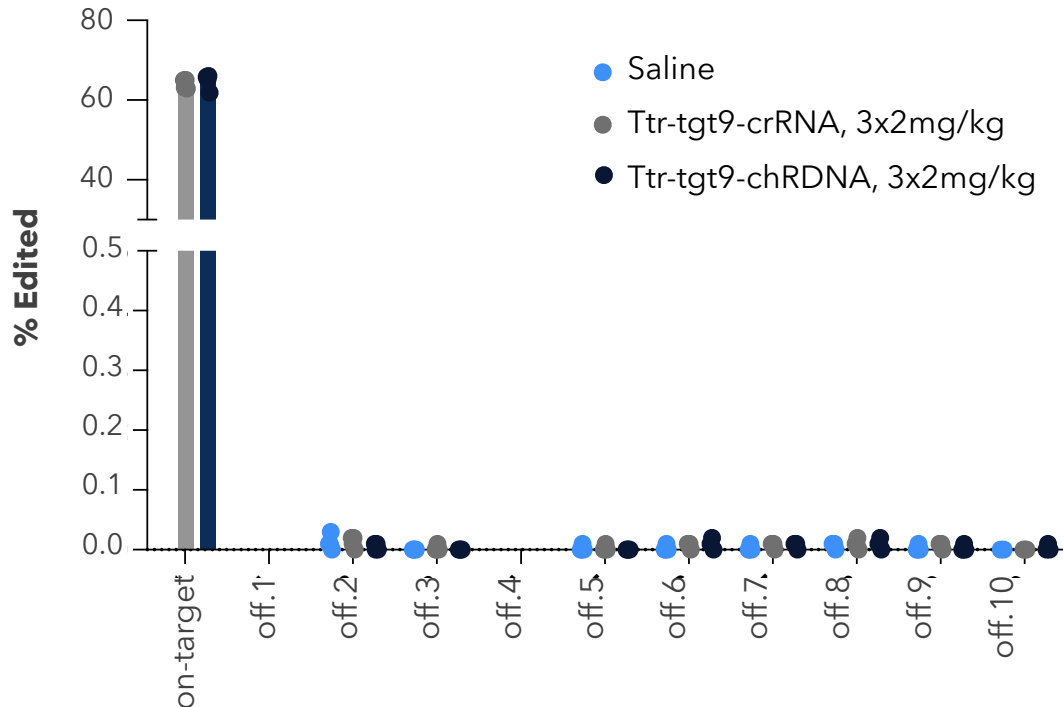


## Liver enzymes 24hrs post administration

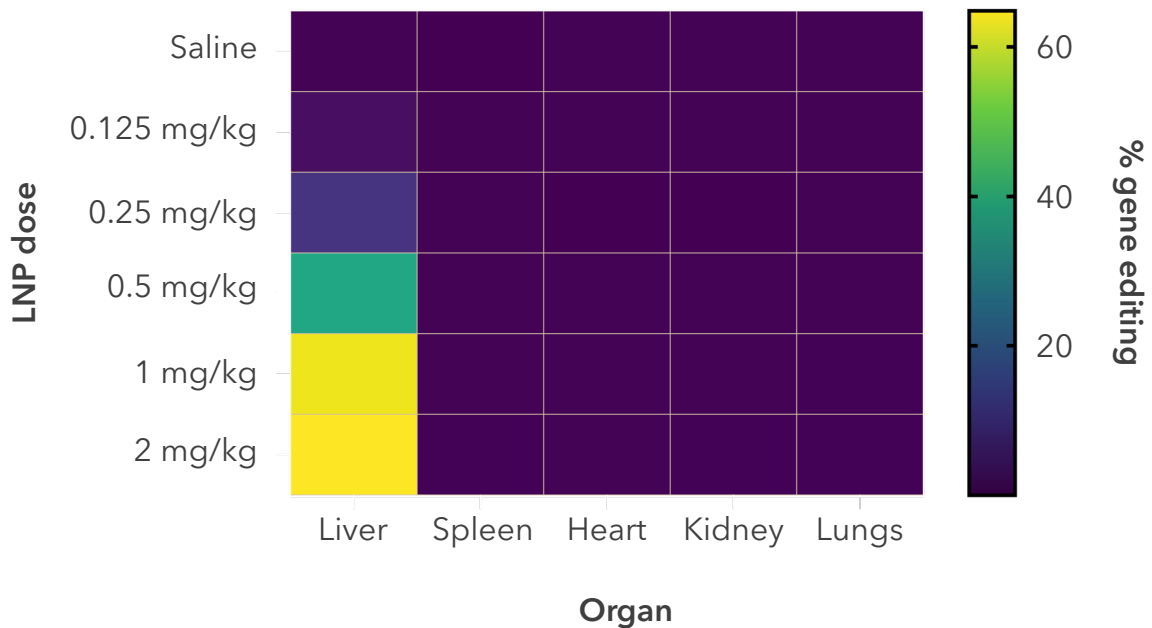


# No significant editing was detected at predicted off-target sites

## Liver analysis of *in silico* predicted off-target sites (high dose)

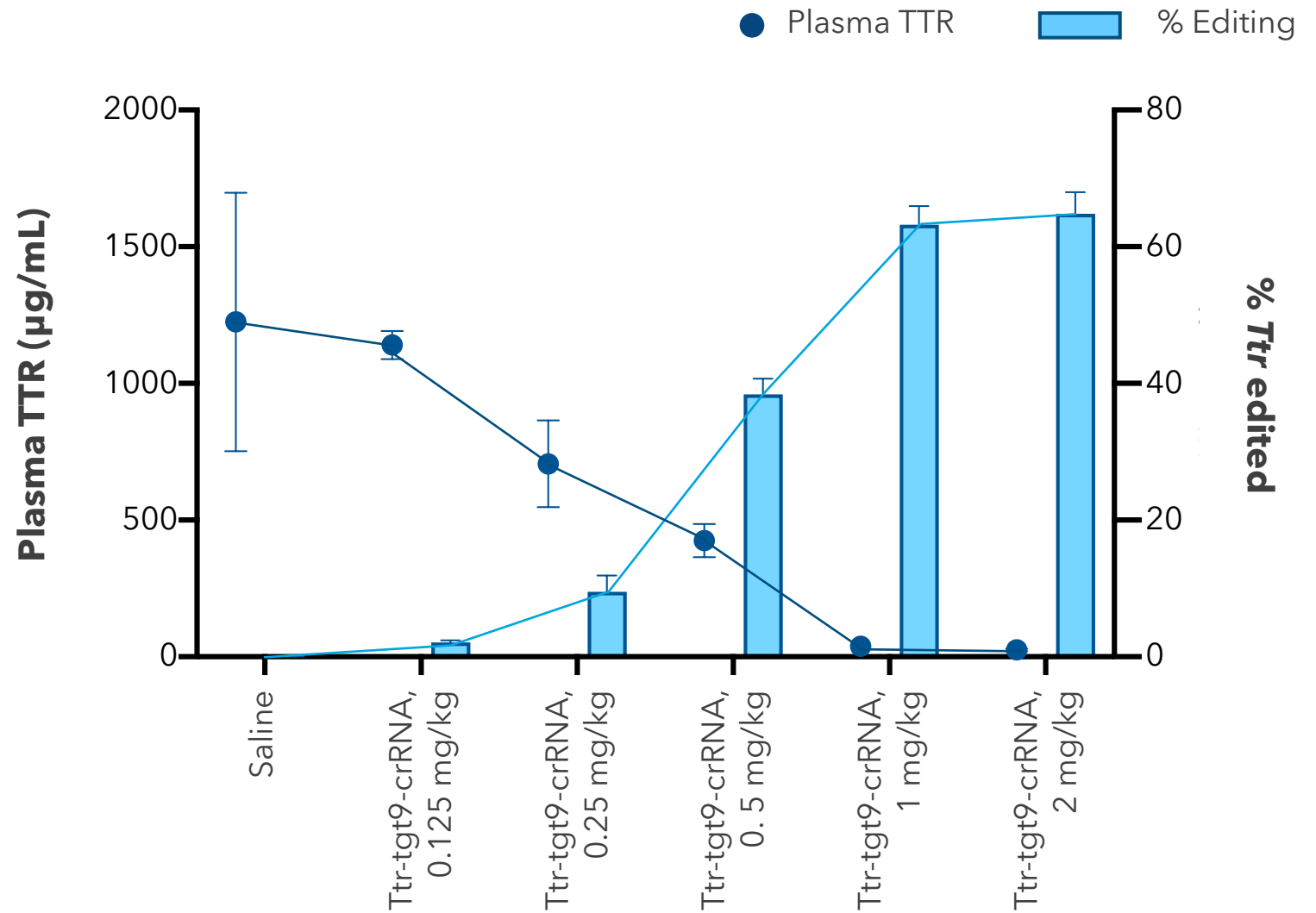


## Off-organ gene disruption



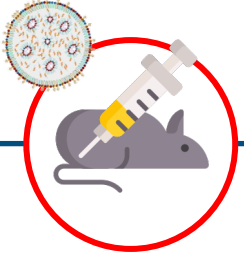
**Animal:** wild-type BALB/c      **LNP:** mRNA      **Guide:** crRNA or chRDNA      **Administration:** intravenous (tail)

# Cas12a + all-RNA guide demonstrate a dose-dependent reduction of plasma TTR levels *in vivo*



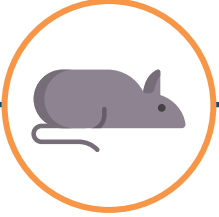
# Disruption of *Ttr* leads to durable near elimination of TTR

LNP Cas12a + guide  
(1 or 2 mg/kg)

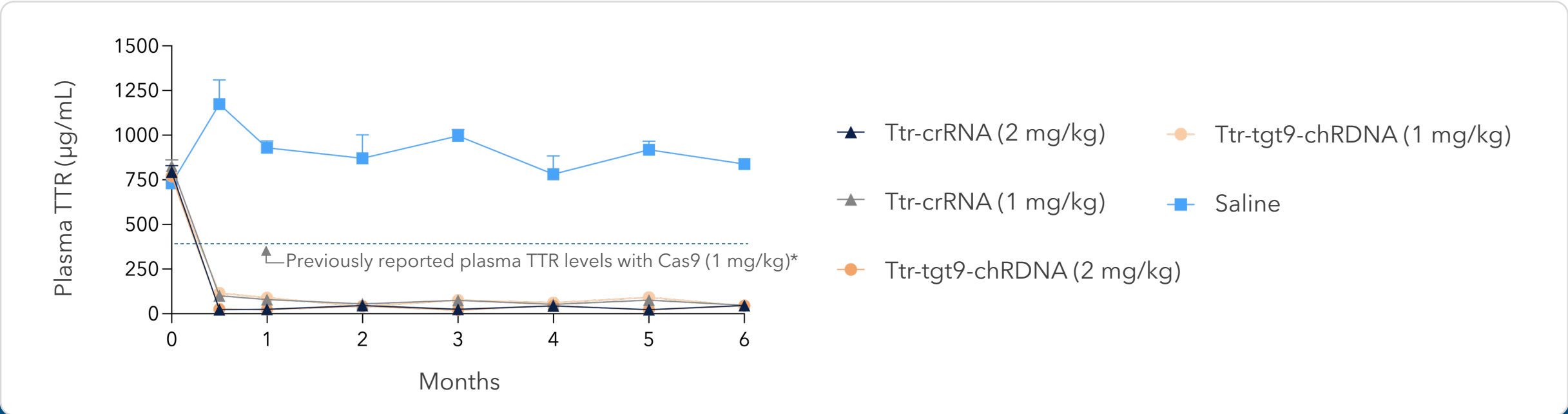
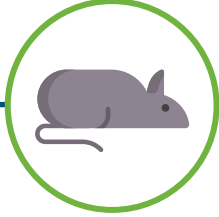


Bleed for ELISA every month

6 months



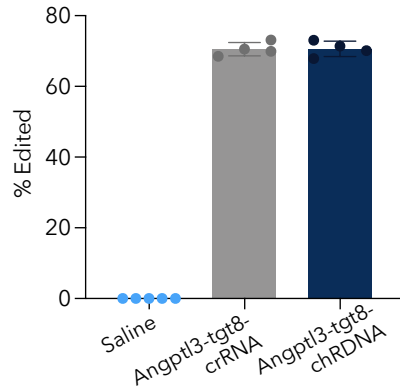
12 months



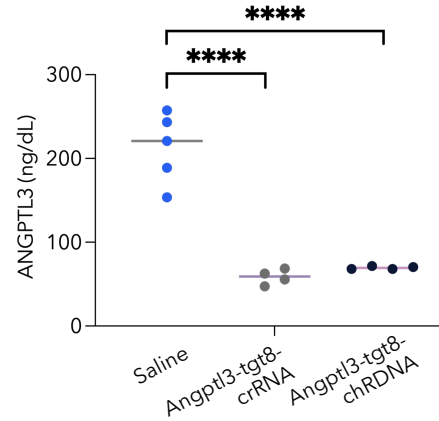
# Cas12a-mediated functional disruption of *Angptl3* and *Pcsk9* in vivo

## In vivo gene editing

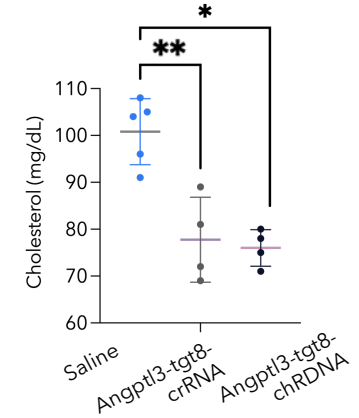
*Angptl3*



## Plasma protein levels

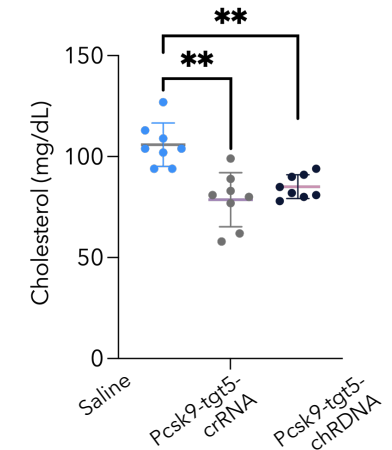
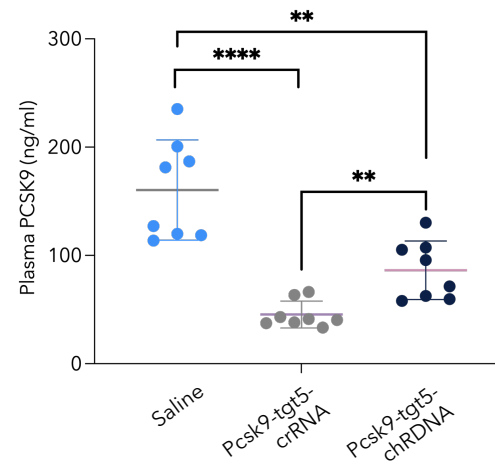
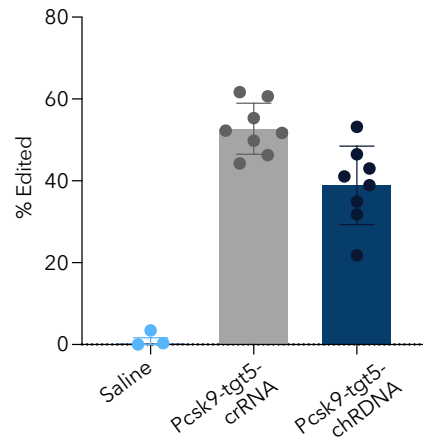


## Plasma cholesterol levels



Day 20

*Pcsk9*

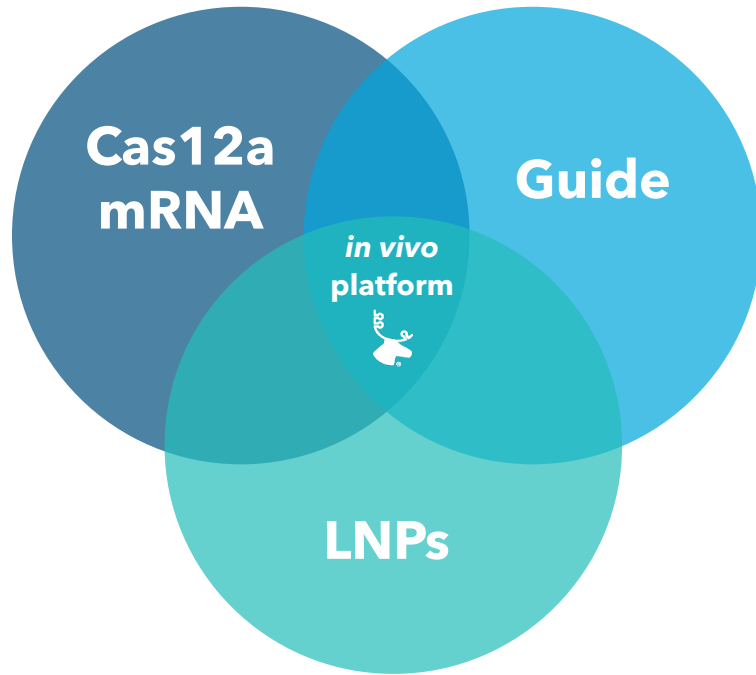


Day 22





# Cas12a chRDNA-mediated *in vivo* genome-editing technology for efficient and functional hepatic gene disruption



- Cas12a mRNA was successfully encapsulated in LNPs with either crRNA or chRDNA targeting *Ttr*, *Pcsk9*, or *Angptl3*
- LNP size, polydispersity, and encapsulation efficiency were in range for *in vivo* administration
- chRDNA designs achieve peak activity across all validated targets
- Achieved a functional gene disruption of 3 POC liver targets: *Pcsk9*, *Angptl3*, *Ttr*
- No acute toxicity observed *in vivo* in preclinical models
- No significant editing was detected at predicted off-target sites

**LNP delivery of Class 2 Type V Cas12a mRNA in conjunction with an optimized chRDNA guide is a powerful tool for specific, efficient, and functional genome editing *in vivo***



# Acknowledgements



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Tony Munoz



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Arthur Owen  
Jack Taylor



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Liz Stanaway  
Art Aviles  
Zili An



## Operations

Casey Cottrell



# The Herd!

