Disclosure

David Morrissey is an employee of, and shareholder in Intellia Therapeutics

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Robust *In Vivo* Editing of Hepatocyte Target DNA Mediated by Lipid Nanoparticle Delivery of CRISPR/Cas9 Components

David V. Morrissey, Ph.D.
ASGCT Annual Meeting
May 13, 2017

Revolutionizing Medicine Through Genome Editing

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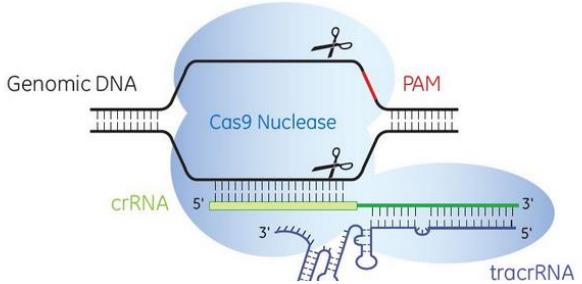
Overview

- Effective delivery is a critical challenge for therapeutic application of CRISPR/Cas9
- Lipid nanoparticles (LNPs) as a clinically viable delivery vehicle for *in vivo* CRISPR/Cas9 editing
- Efficacy and pharmacokinetics of LNP delivered Cas9 mRNA and sgRNA
- Chemical modification of sgRNA is critical for *in vivo* activity

CRISPR/Cas9

- Versatile and robust genome editing platform
- RNA guided endonuclease (REN)
  - S.py Cas9
    - 20 base + NGG PAM recognition sequence
- RNP (Cas9 + gRNA) induces double stranded breaks (DSB)
  - cell repair machinery dictates editing event



Dual guide (dgRNA): 42 and 74-mer

Figure 1a. Illustration of Cas9 nuclease programmed by the crRNA:tracr complex cutting both strands of genomic DNA 5' of the PAM

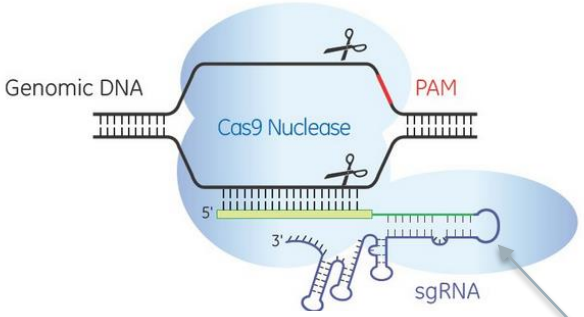


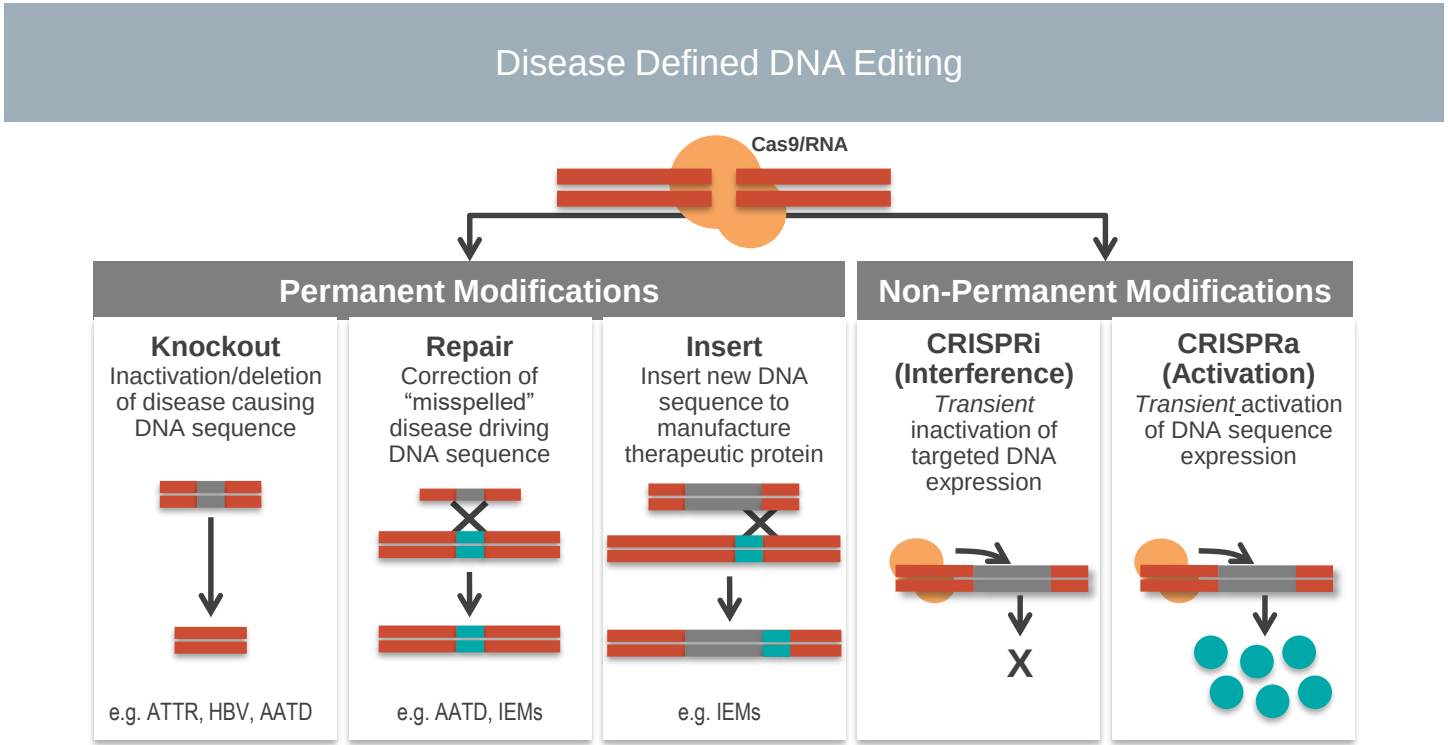
Figure 1b. Illustration of Ca
5' of the PAM

Single guide (sgRNA): 100-mer

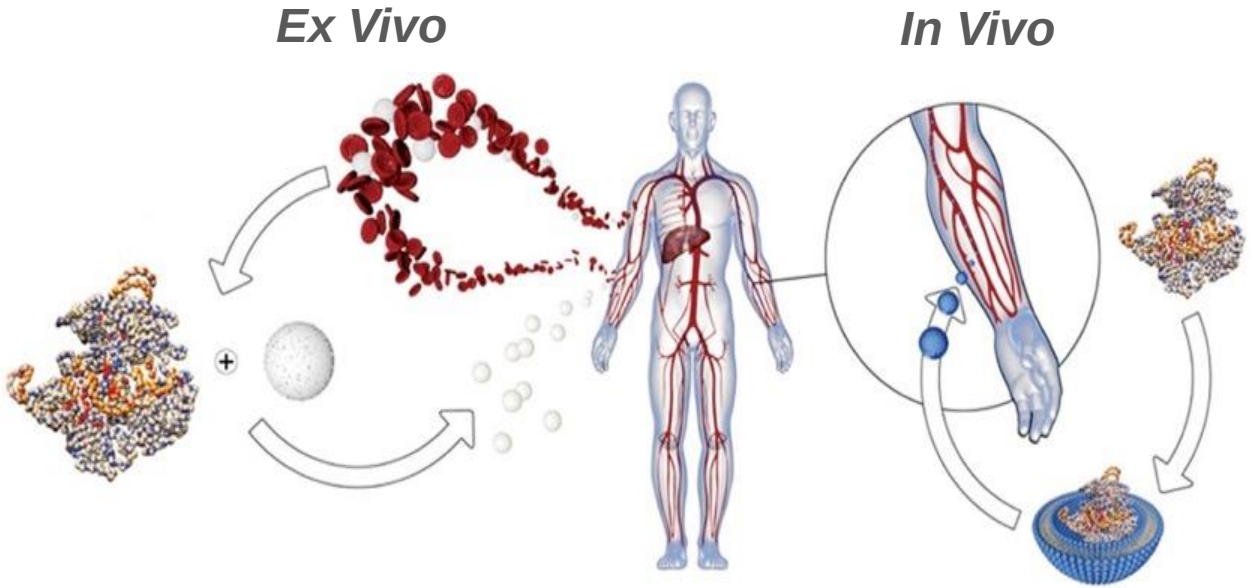
crRNA and trRNA
connected by tetraloop

Multiple Genome Editing Approaches

Addressing the Underlying Genetic Driver of Disease



A Delivery Focused Genome Editing Approach



- Electroporation
- Microfluidics

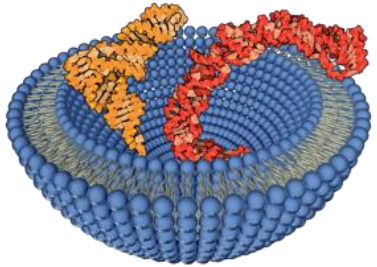
- Viral vectors
- Nanoparticles

The Challenge of In vivo Delivery

- **Focus of gene therapy has been on stable expression**
  - lentivirus/retrovirus
  - AAV
- **Genome editing only requires transient expression**
  - no need for nuclease after edit has been made
  - reduce potential for off-target and immunogenicity
- **Characteristics of ideal delivery system for genome editing:**
  - transient
  - clinically viable therapeutic index
  - non-immunogenic- potential for re-administration
  - targeted
  - large capacity, mixed cargo
  - scalable manufacturing

Lipid Nanoparticle (LNP) as Delivery Vehicle

- **Transient delivery of nucleic acid**
  - plasmid
  - siRNA
  - mRNA



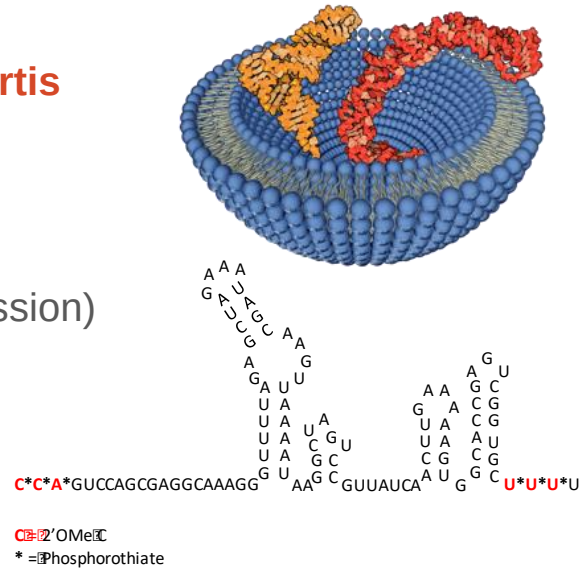
- **Liver delivery**
  - Endogenous ApoE specific targeting of hepatocytes via LDL receptor

- **Demonstrated clinical safety/efficacy**
  - e.g. Patisiran : TTR-siRNA
  - Phase III ATTR
  - re-administration every 3 weeks

- **Scalable GMP manufacturing**

LNP Technology for Delivery of Cas9 mRNA/gRNA

- **LNP formulation licensed from Novartis**
  - Novel, degradable ionizable lipid
  - Multi-component, self-assembling particle
  - Chemically defined, scalable process
  - Mixed cargo, >10 kb capacity
- **Cas9 mRNA** (for transient Cas9 expression)
  - *S.py* Cas9; codon optimized
  - 1:1 ratio with gRNA by weight
- **gRNAs:** all chemically synthesized
  - crRNA: 42 nt
  - tracrRNA: 74 nt
  - sgRNA: 100 nt
  - Initial chemical modifications:
    - 2’-O-methyl and PS linkages on three terminal positions

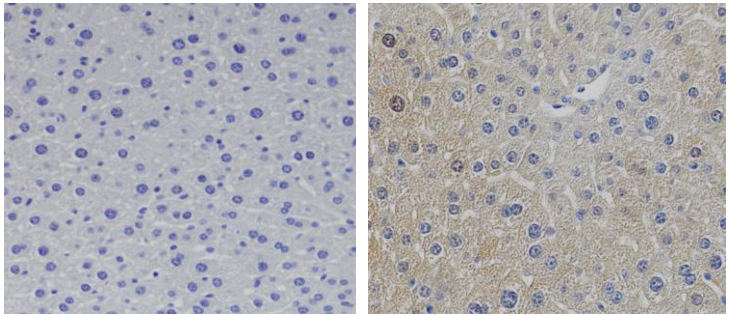


Consistent Formulation of Cas9 mRNA and sgRNA

LNP mediated delivery throughout liver

LNP	Size (nm)	PDI	EE%	gRNA:mRNA weight ratio
LNP1	77.2	0.161	95	1.02
LNP2	69.2	0.168	96	1.15
LNP3	74.3	0.161	96	1.04
LNP4	69.1	0.149	96	1.03
LNP5	72.7	0.185	97	1.15
LNP6	75.1	0.169	96	1.1

- LNP formulations produced with consistent particle size and mRNA:gRNA ratio (by weight)



Untreated GFP mRNA

- LNP/GFP mRNA: expression in liver by IHC

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Hereditary Transthyretin Amyloidosis (ATTR)

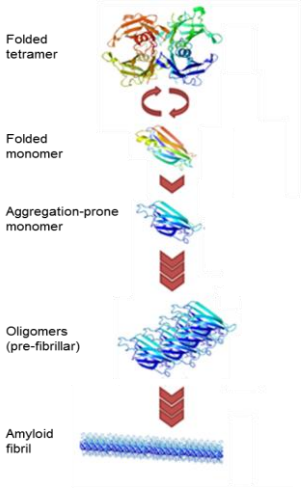
Liver-directed gene knockout program

CLEAR UNMET MEDICAL NEED

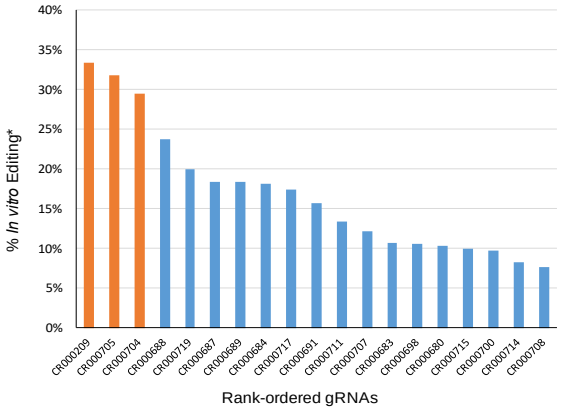
- Orphan disease affecting approximately ~50,000 patients worldwide
- Autosomal dominant; >100 known mutations
- Misfolded mutant protein aggregates in nerves, heart, gastrointestinal tract, etc. leading to loss of function

OUR APPROACH

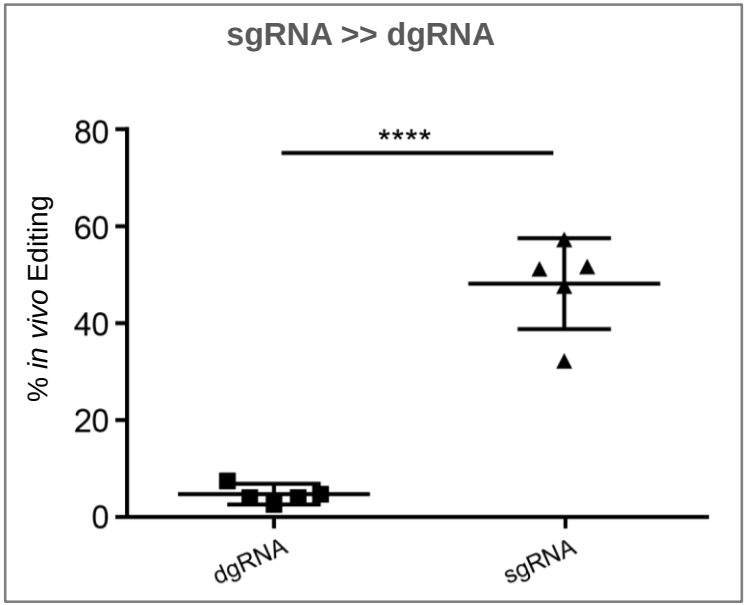
- Knock-down of disease causing protein is a clinically validated strategy
- Deletion of disease gene (mut TTR) in hepatocytes reduces supply of misfolded protein – halts disease progression and may enable regression
- Potential for curative treatment



Screening of mouse TTR gRNAs:

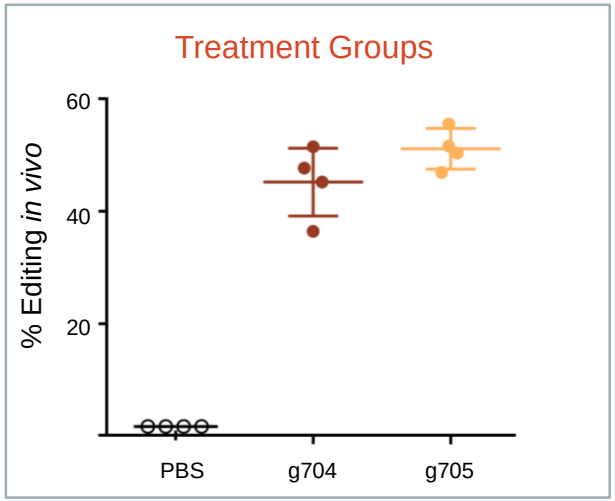


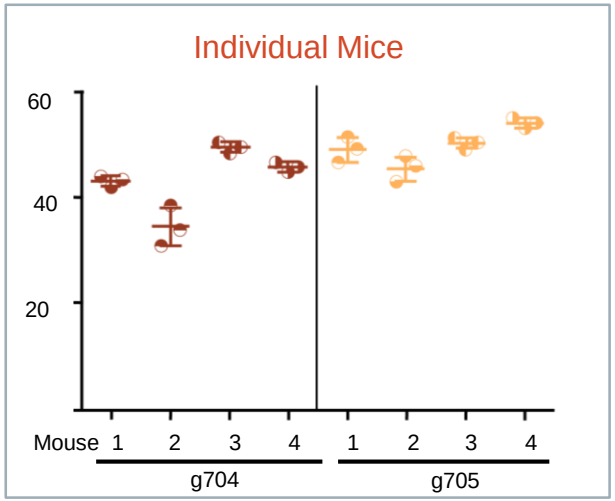
Greater Editing in Liver with sgRNA vs. dgRNA



- Single i.v. administration (CD-1 mice)
- 2 mg/kg
- Livers harvested 7 days post administration (NGS)
  - dgRNA: 3 terminal bases PS modified
  - sgRNA 3 terminal bases PS-O methyl modified

LNPs Mediate Consistent Editing across Liver

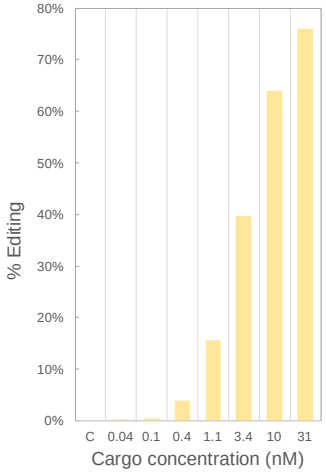




- Two i.v. administrations on consecutive days, 2 mg/kg total RNA payload
- Endpoint is 7 days post dosing
- Biopsy from right median, left median and left lateral lobes
- Data points for individual liver lobes plotted for each animal

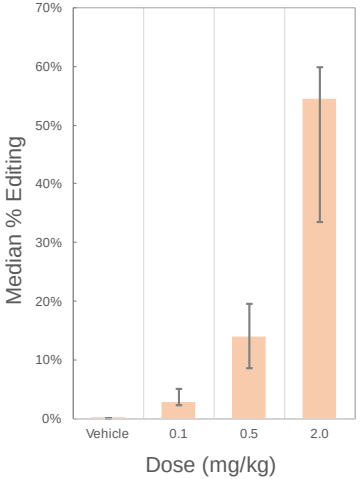
Dose-Dependent Editing of mTTR

In vitro editing



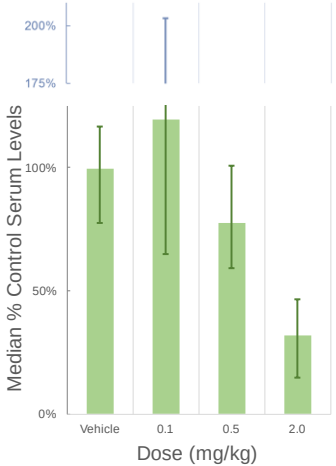
Mouse liver cells LNP directly administered to cells (n=3)

In vivo editing



Single administration in mice
Median ± range (n=5)

In vivo serum TTR levels



Assay for serum protein
Median
% of mean control ± range (n=5)

- Co-formulation of Cas9 mRNA and sgRNA
- i.v. administration at 2 mg/kg total RNA payload with sg209
- 9 day time point for liver editing and TTR serum levels

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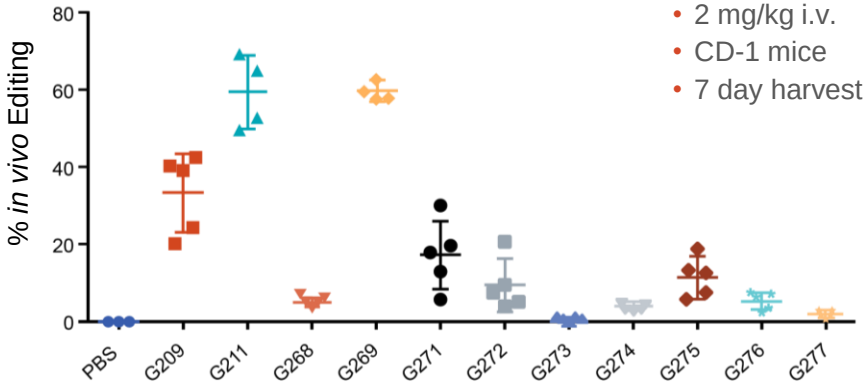
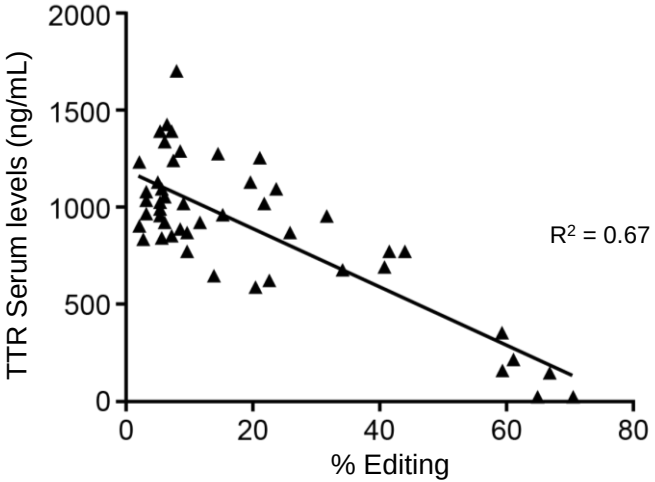
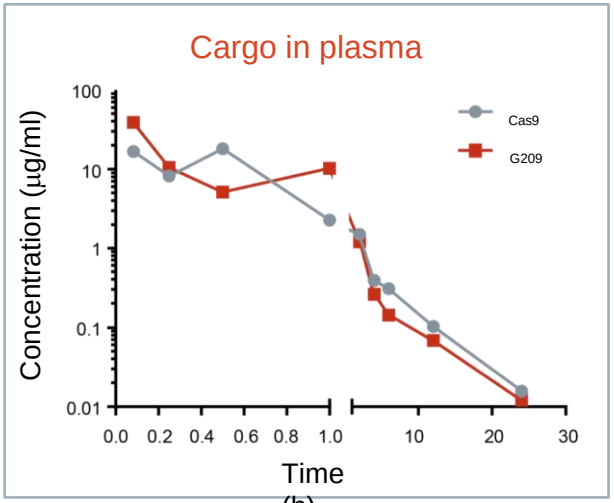
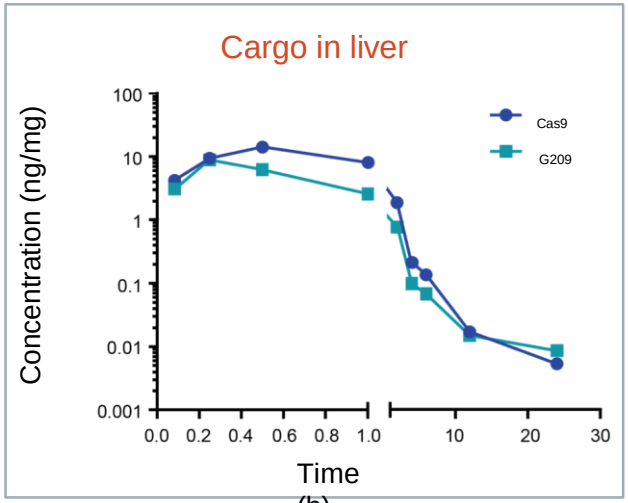
Guide Sequence Affects *in vivo* Activity





Mouse PK of LNP Encapsulated Cas9 mRNA and sgRNA





- qRT-PCR based analytical method
- Coordinated loss of Cas9 mRNA and sgRNA implies LNP structural stability during circulation
- Significant uptake of LNPs in liver
- Cas9 mRNA and sgRNA undetectable at 72 hours post dose

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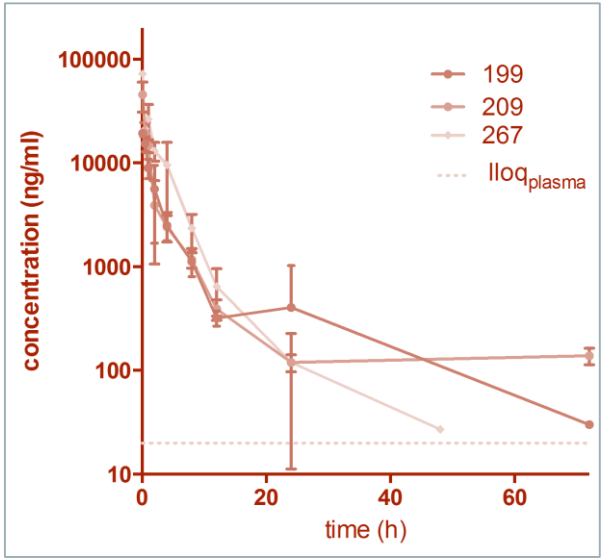
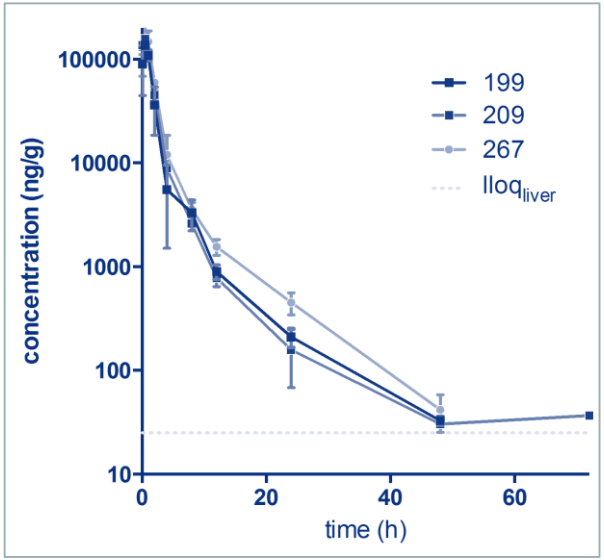
Ionizable Lipid Clears Rapidly in Mouse

Plasma



Liver



- LC/MS based method
- Significant uptake of lipid in liver
- Lipid cleared from liver with half-life of ~6 hrs

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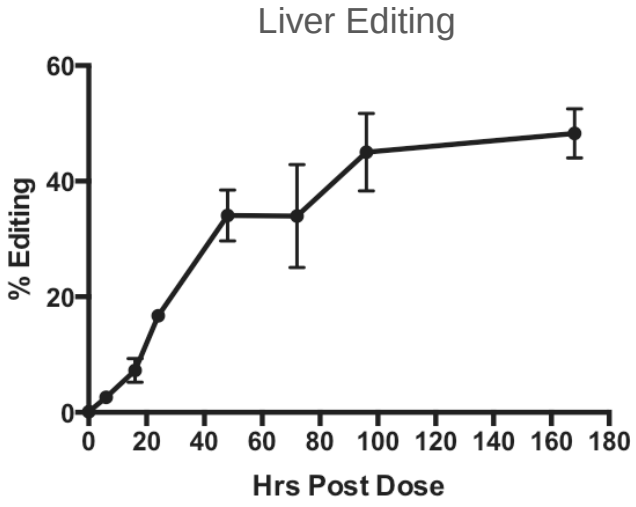
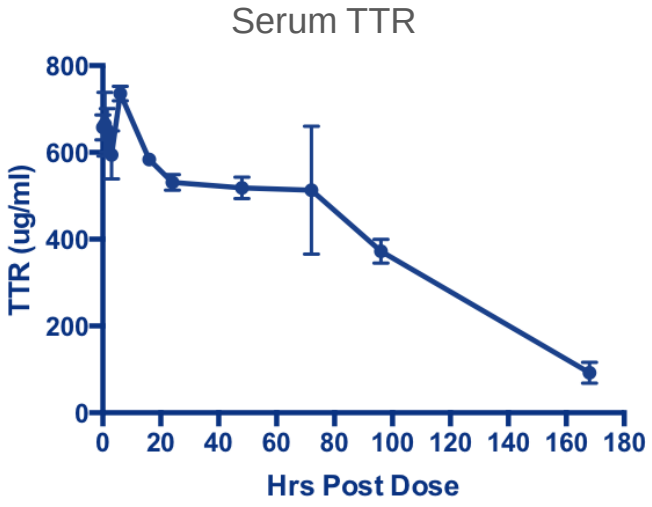
Time Course of TTR Editing in the Mouse

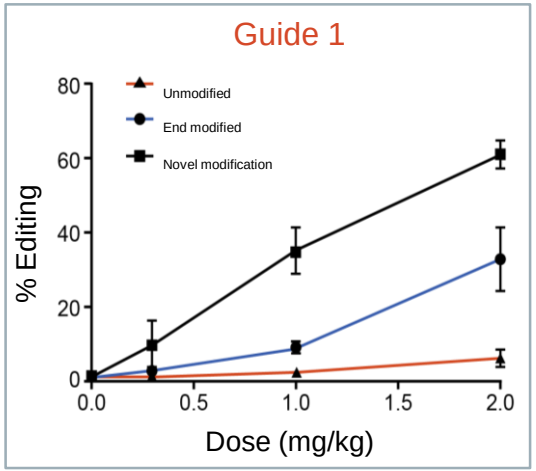


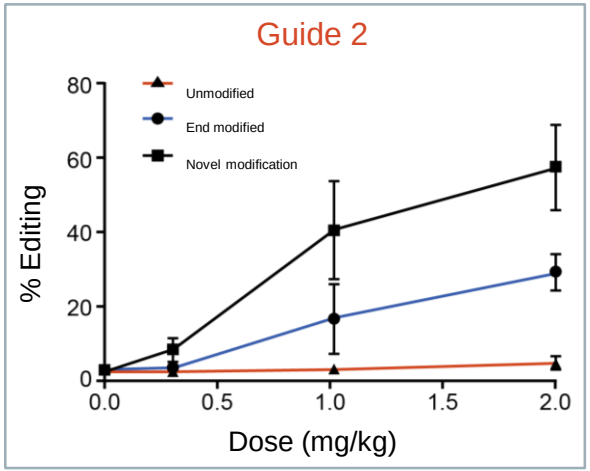


- CD-1 mice
- 2 mg/kg dose

Guide Chemical Modifications are Critical for Activity

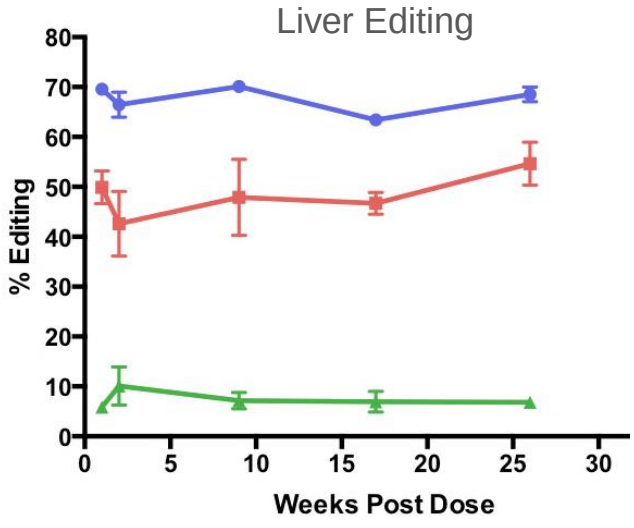
- sgRNA chemical modification campaign identified novel modification pattern
- Increased potency relative to standard end-modified sgRNA
- Guide sequence independent

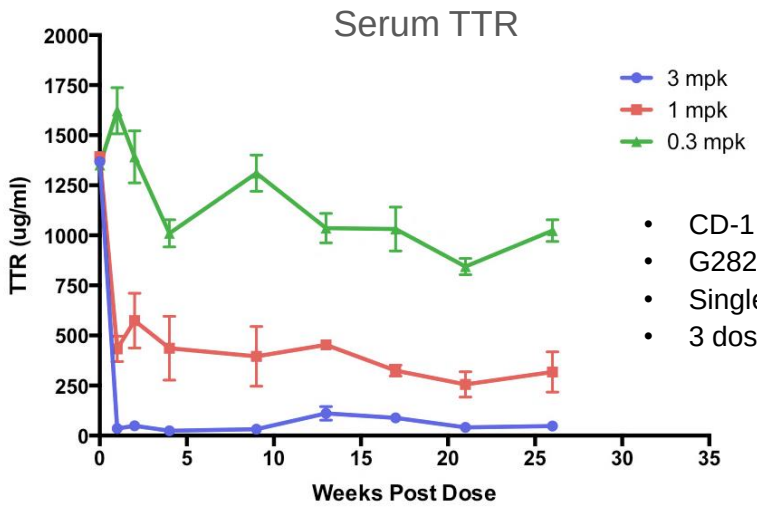




LNP Mediated Editing is Durable for >6 Months

>97% reduction in serum TTR levels, 70% liver editing





- CD-1 mice
- G282 (heavily modified)
- Single administration given at Day 0
- 3 doses (0.3, 1.0, 3.0 mg/kg)

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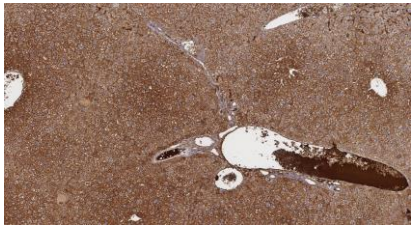
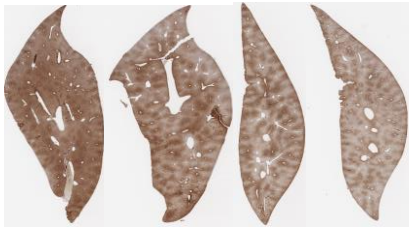
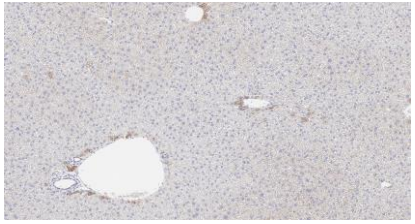
Duration Study: TTR IHC in Liver Sections

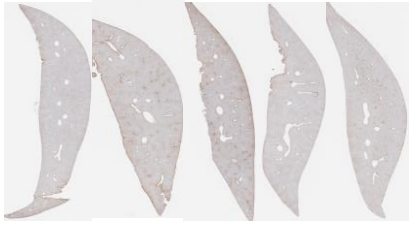
Vehicle
1 week



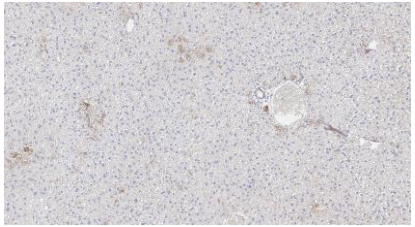


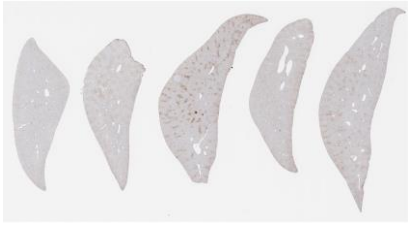
3 mg/kg
1 week





3 mg/kg
6 months

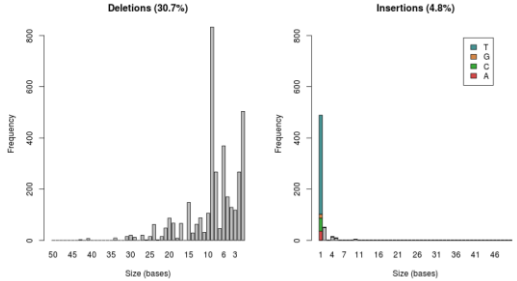




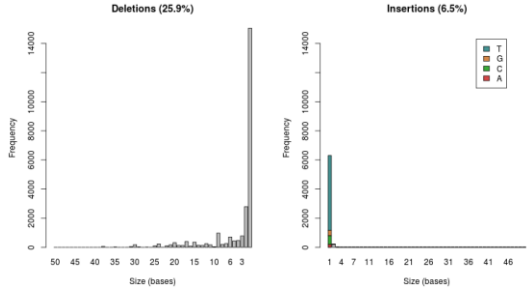
Importance of Repair Spectra Characterization

Cell line vs. primary hepatocytes vs. hepatocytes in vivo

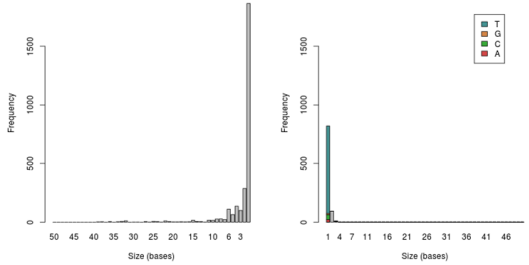
Neuro 2A (mouse)



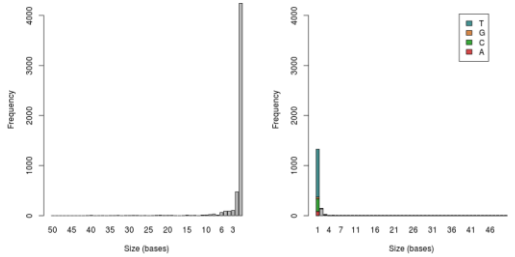
Hepa1-6 cells (mouse)



Primary mouse hepatocytes



Mouse liver (total) *in vivo*



- Not all cell lines and cell types *in vitro* mirror the repair events seen *in vivo*
- Evaluation of gRNA activity in primary hepatocytes will be important for selection of therapeutic leads

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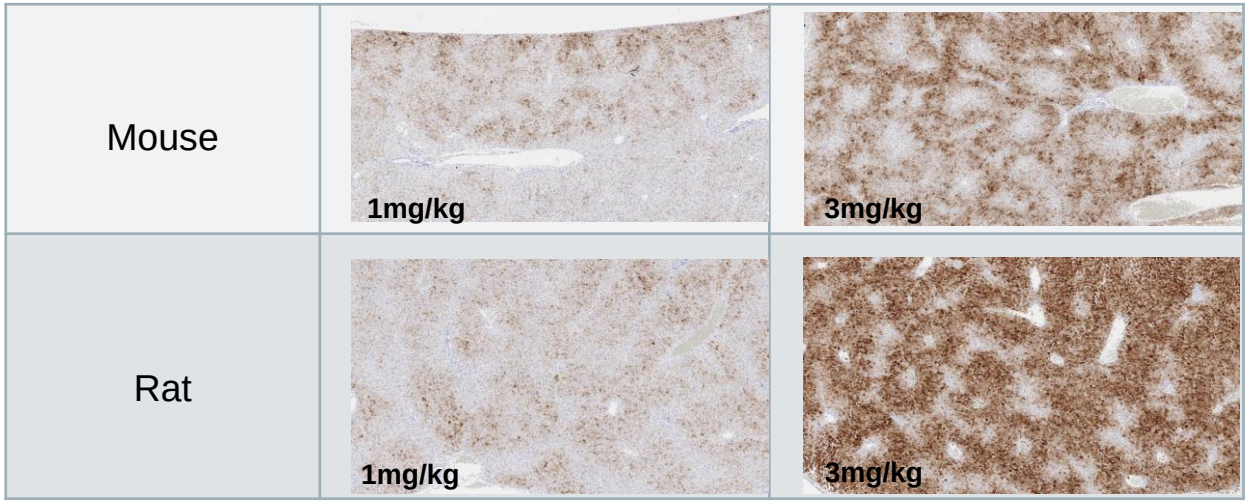
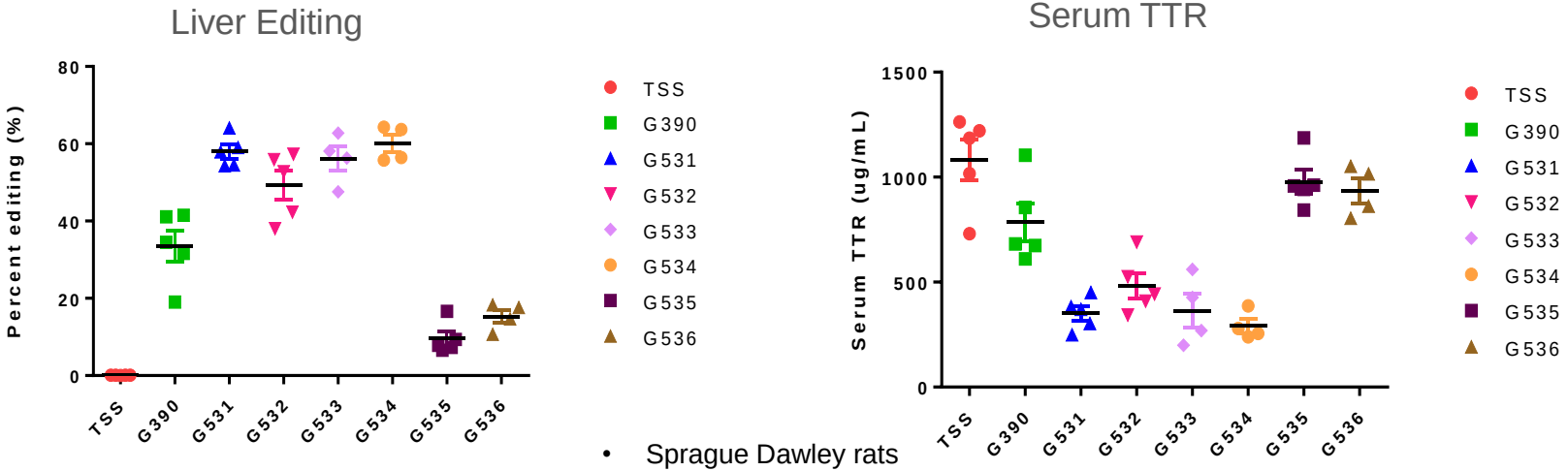
LNPs Enable Broad, Robust GFP Expression in Rat Liver



- LNP delivery of GFP mRNA
- 24 hour time point

LNP Mediated Editing of Rat TTR

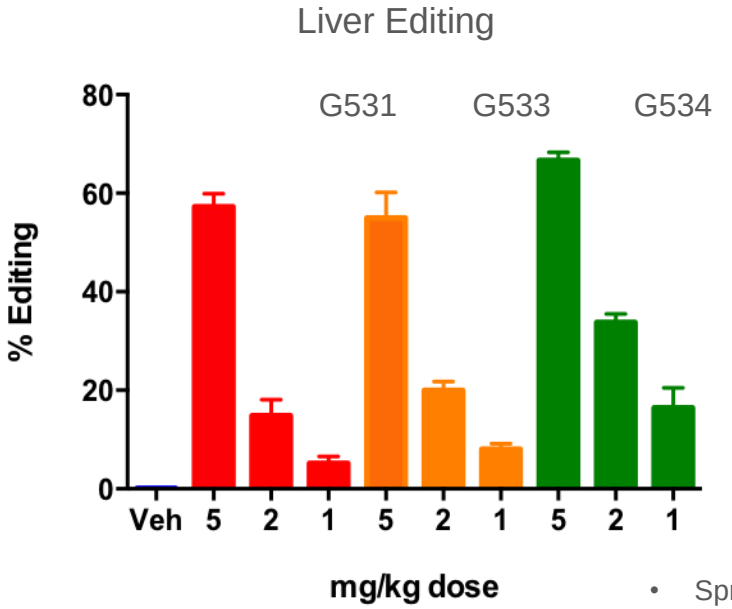
Up to 60% liver editing, 70% reduction serum TTR

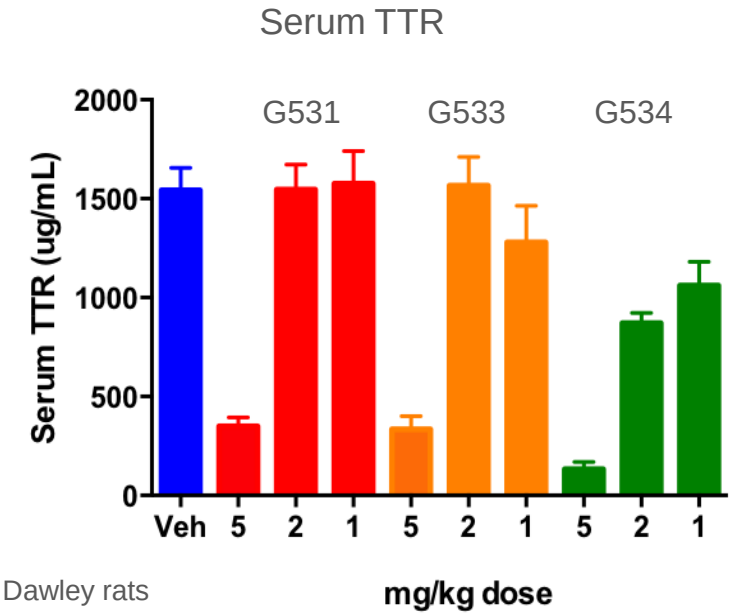


- Sprague Dawley rats
- Single 5 mg/kg dose
- 7 day time point

Dose Responsive Editing in Rat Liver

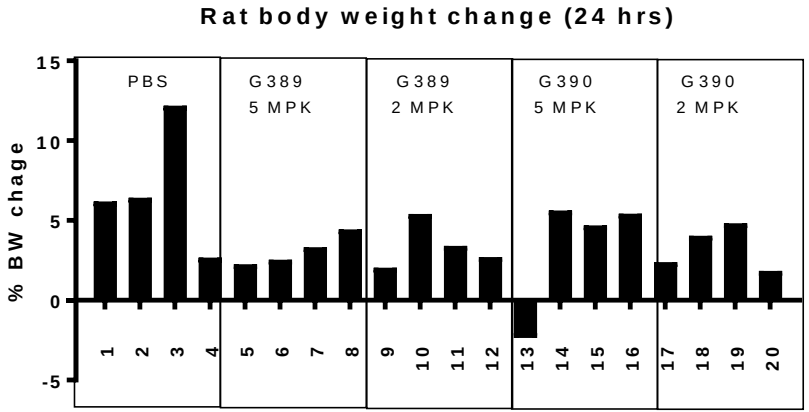
Up to 66% liver editing and 91% reduction serum TTR

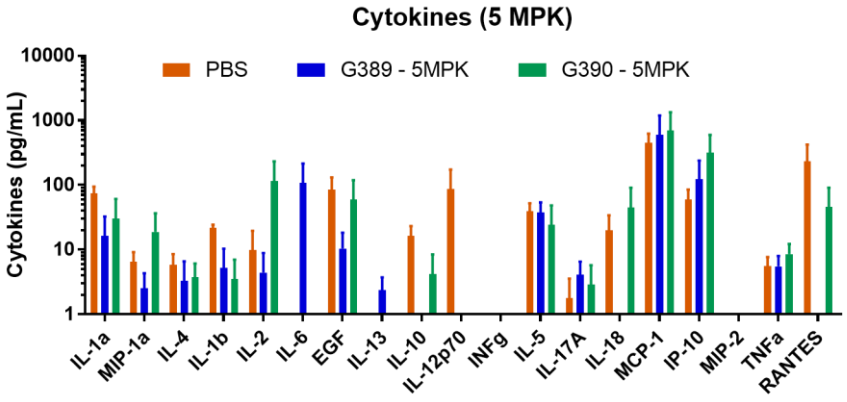




- Sprague Dawley rats
- Single administration
- 7 day time point

Tolerability of LNP/Cas9 mRNA/sgRNA in Rat





4 hrs post dose

Summary

- LNPs: a clinically viable delivery vehicle for *in vivo* CRISPR/Cas9 editing
  - High level of LNP-delivered, Cas9-mediated editing observed in rodent liver after a single administration, resulting in significant decrease in circulating levels of target protein
- Pharmacokinetics of LNP delivered Cas9 mRNA and sgRNA
  - mRNA and gRNA were undetectable in the liver at 72 hours post administration
  - Ionizable lipid is biodegradable and cleared with ~6 hr half-life
- Chemical modification of sgRNA is critical for *in vivo* activity
  - Identified novel modification pattern that enhances activity
- Single LNP administration yields robust, durable liver editing >6 months

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Thank You!

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