Recent Advances in Non-Viral Delivery of Prime Editors Toward the Treatment of Patients with Glycogen Storage Disease Type 1b (GSD1b)

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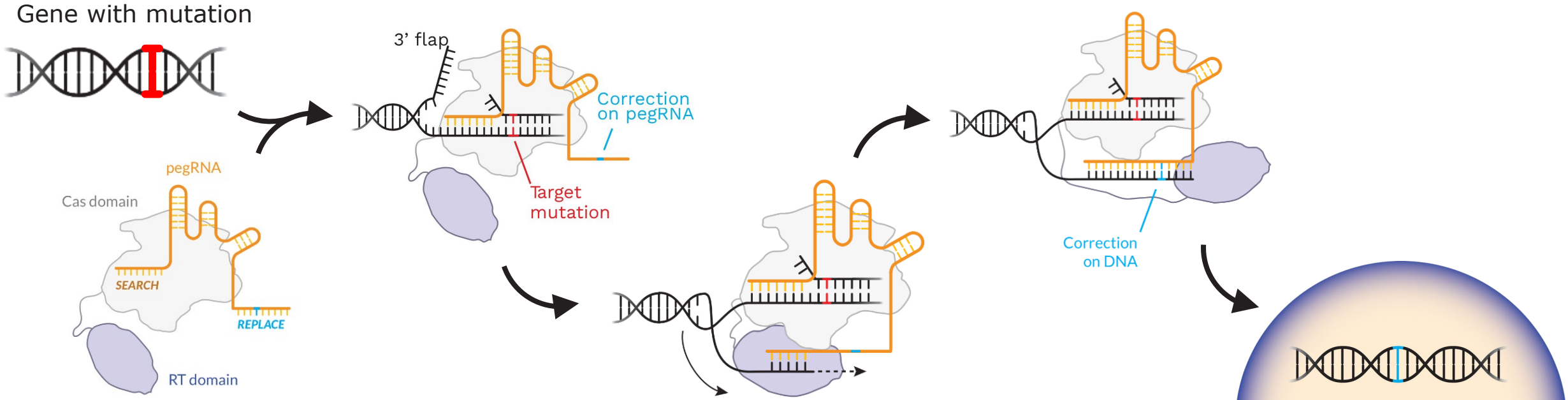
Prime Medicine

On behalf of the team at Prime Medicine

Disclosures

Seth Alexander declares he is a current employee of Prime Medicine, Inc. and owns equity in Prime Medicine.

Prime Editing is programmable for both *Search* and *Replace*



SEARCH

Prime editor complex initiates search for target DNA

FIND & NICK

Prime editor complex finds DNA with target mutation, nicks one strand

PRIME

Nicked DNA strand primes the RT domain for DNA synthesis

REPLACE

Prime editor complex copies in corrective DNA sequence

GENE CORRECTED

3' flap preferentially incorporated[1], excess flap repaired, gene fully corrected

[1]*Completion of an edit requires 3 'edit checks,' or places where there has to be a match between the editor and the target DNA*

Note: pegRNA = prime editing guide RNA; RT = reverse transcriptase; Cas = CRISPR associated protein

Therapeutic approach: LNP-mediated delivery of Prime Editor components to liver

A universal LNP-RNA platform has potential to deliver Prime Editors to correct pathogenic mutations in the liver

Prime Editor Cargo Components

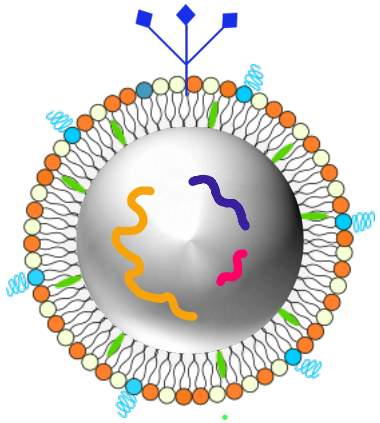
PE mRNA

pegRNA

ngRNA

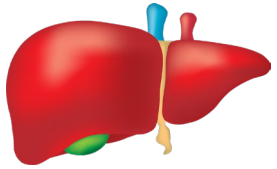
LNP Formulation

LNP Encapsulated Prime Editor Cargo



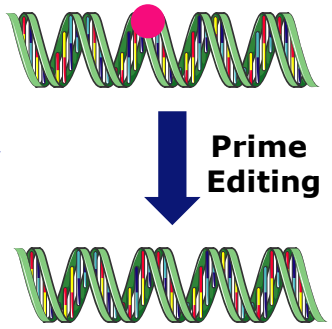
Single IV Dose

Liver Delivery



Prime Editing in Hepatocytes

Precise Correction of Mutation in Hepatocytes



Liver-specific **Universal LNP** contains active targeting ligand that **reduces biodistribution to secondary organs**

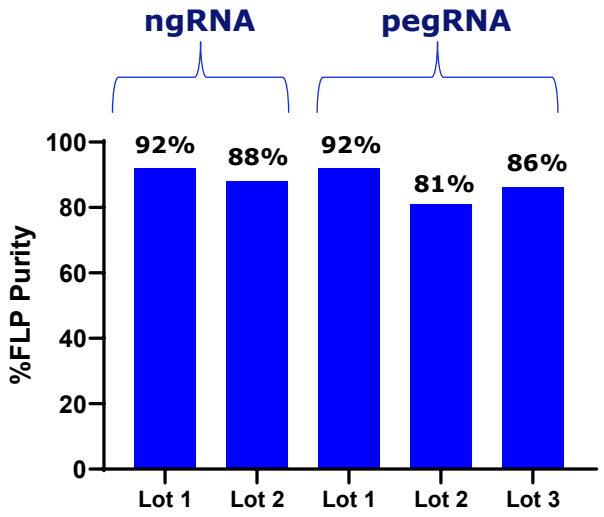
Prime's internal guide RNA capabilities provide high-quality materials supporting multiple programs

pegRNA

ngRNA

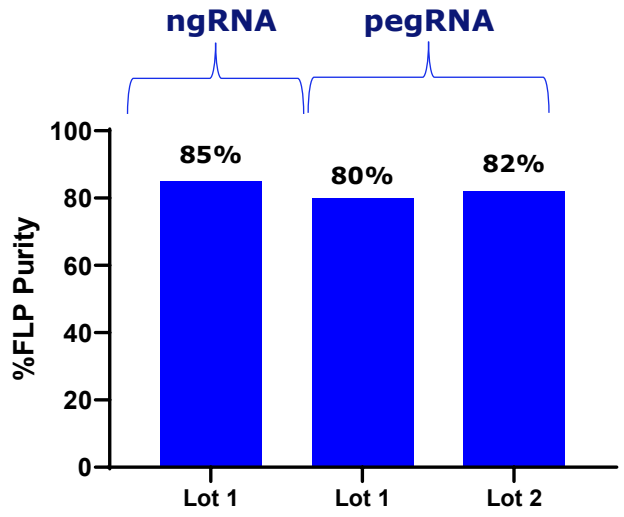
- ✓ High purity pegRNA produced internally with consistently >80% purity
- ✓ Scalable from a few mg to multi-hundred mgs
- ✓ Robust production across gRNA sequence diversity, lengths, and chemical modifications

Program 1



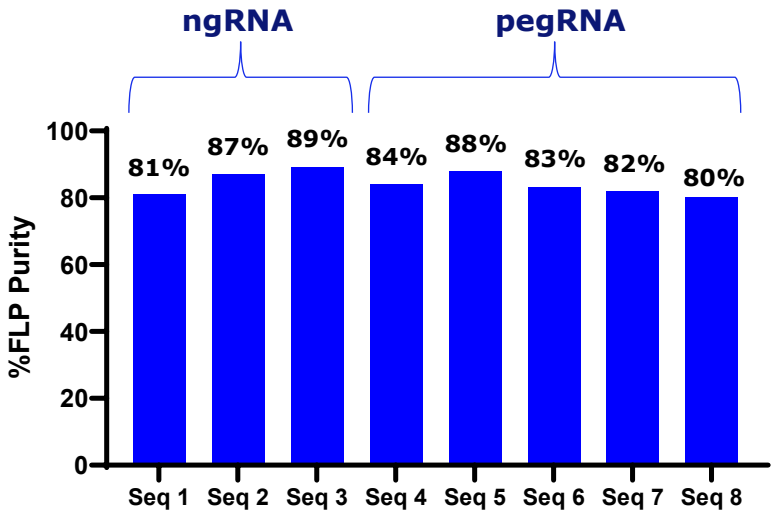
Mass (mg)	224	326	171	64	170

Program 2



Mass (mg)	30	7	34

Program 3



Mass (mg)	54	82	294	58	81	472	114	101

Optimization of mRNA increases Prime Editing efficiency and leads to reduction of PCSK9 protein in serum

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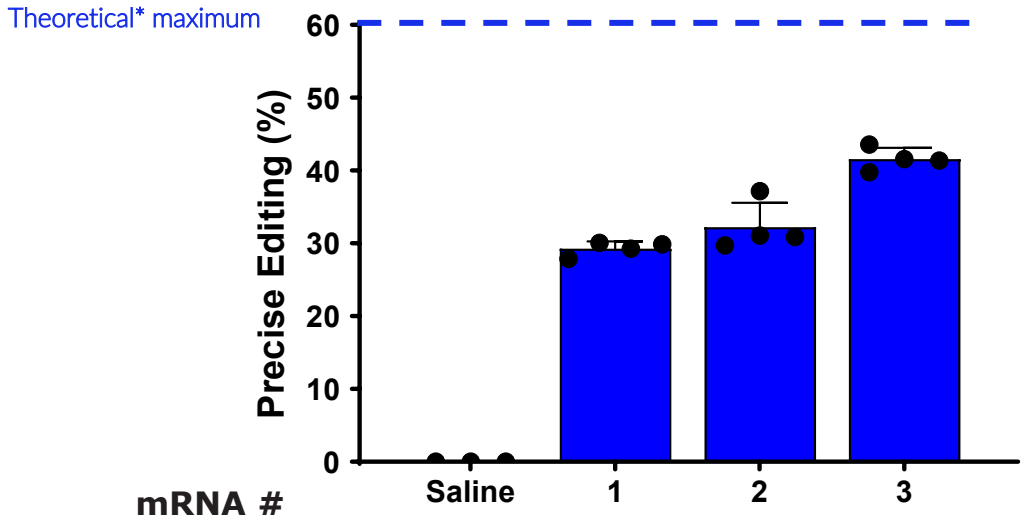
Optimized mRNA leads to improved Prime Editor performance

PE mRNA

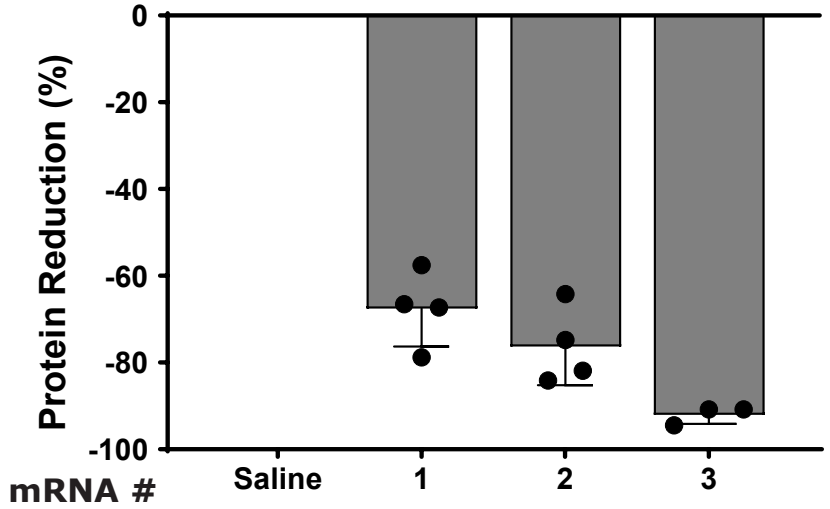
PCSK9 Prime Editor LNP delivered systemically

- Prime Editor mRNA
- Prime editor guide RNA coding for PCSK9 premature stop codon

Optimized mRNA increases Prime Editing



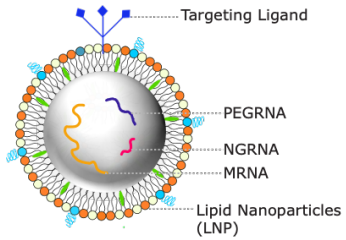
Optimized mRNA decreases PCSK9 protein



LNP delivery to mice results in 42% PCSK9 Prime Editing and 92% serum protein reduction

*Based on PK/PD relationships and quantification of cell types in liver:
Wang et al Sci. Rep. (2021) 11:19396; MacParland et al Nat Commun (2018) 9:4383; Hansel et al, Curr Protoc Toxicol (2014) 62:14.12.1; Kmiec, Adv Anat Embryol Cell Biol. (2001) 161:III–XIII. 1–151

Optimized Prime LNPs provide robust whole liver precise editing in mouse PCSK9 model



PCSK9 Prime Editor LNP delivered systemically

- Prime Editor mRNA
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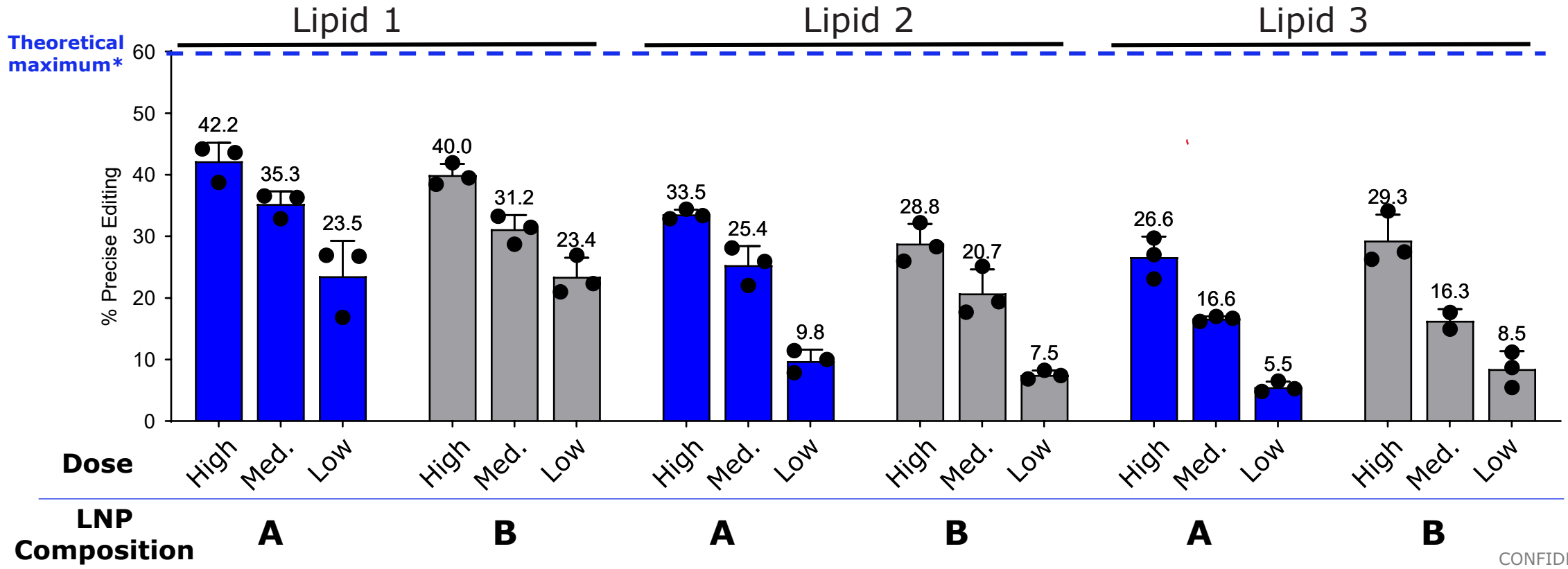

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Prime Editing to correct pathogenic mutations causing von Gierke disease or Glycogen Storage Disease Type 1b

Initially correct the two most prevalent mutations that cause GSD1b, carried by ~ 50% of patients

Glycogen Storage Disease Type 1b (GSD1b)

Description:

- Rare disease preventing production of glucose during fasting state, causing hypoglycemic episodes and associated seizures

Human genetics and biology:

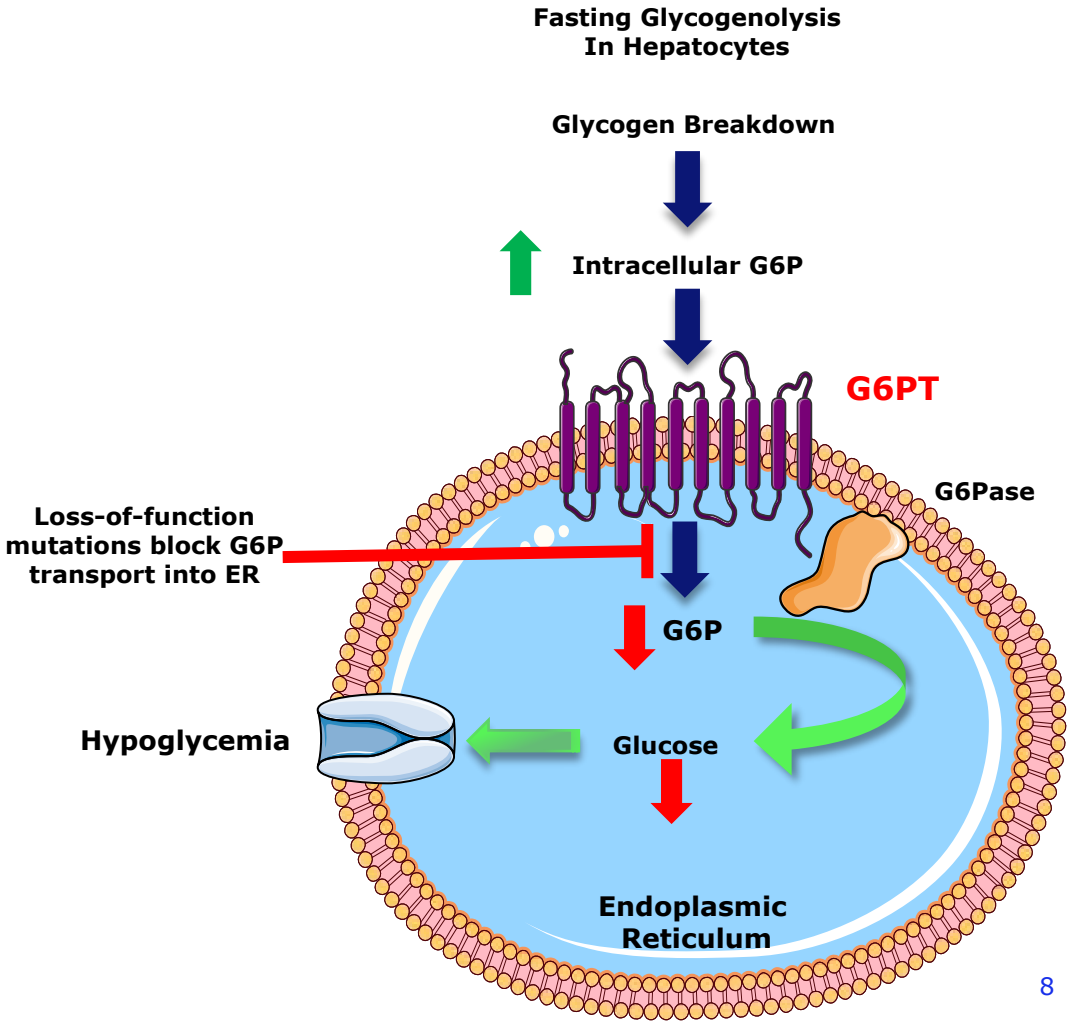
- Autosomal recessive, caused by mutations in the *SLC37A4* gene that encodes G6PT, a glucose-6-phosphate transporter
- **p.L348fs and p.G339C mutations** found in ~50% of GSD1b patient population

Unmet need:

- Standard of care focuses on reducing symptom severity rather than treating the underlying cause of the disease
- No disease modifying therapies approved

Prime Medicine's approach:

- IV administration of liver targeted LNP Prime Editors to correct either p.L348fs mutation or the p.G339C mutation to restore glucose homeostasis in patients with GSD1b



G6PT: glucose-6-phosphate transporter (SLC37A4), G6Pase: glucose-6-phosphate phosphatase, Pi: inorganic phosphate; G6P: glucose-6-phosphate

Identification of pegRNAs that precisely correct the p.L348fs and p.G339C mutations *in vitro*

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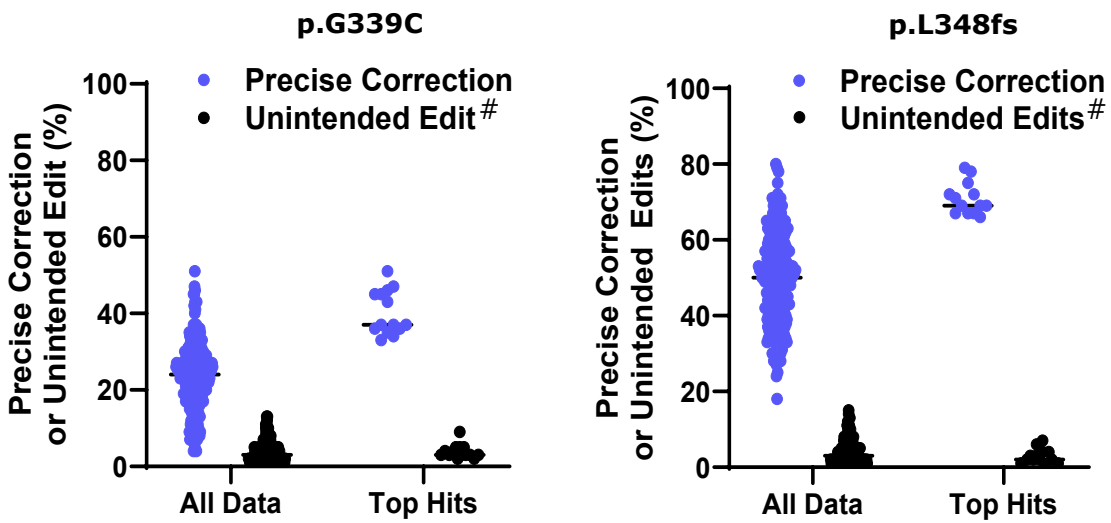
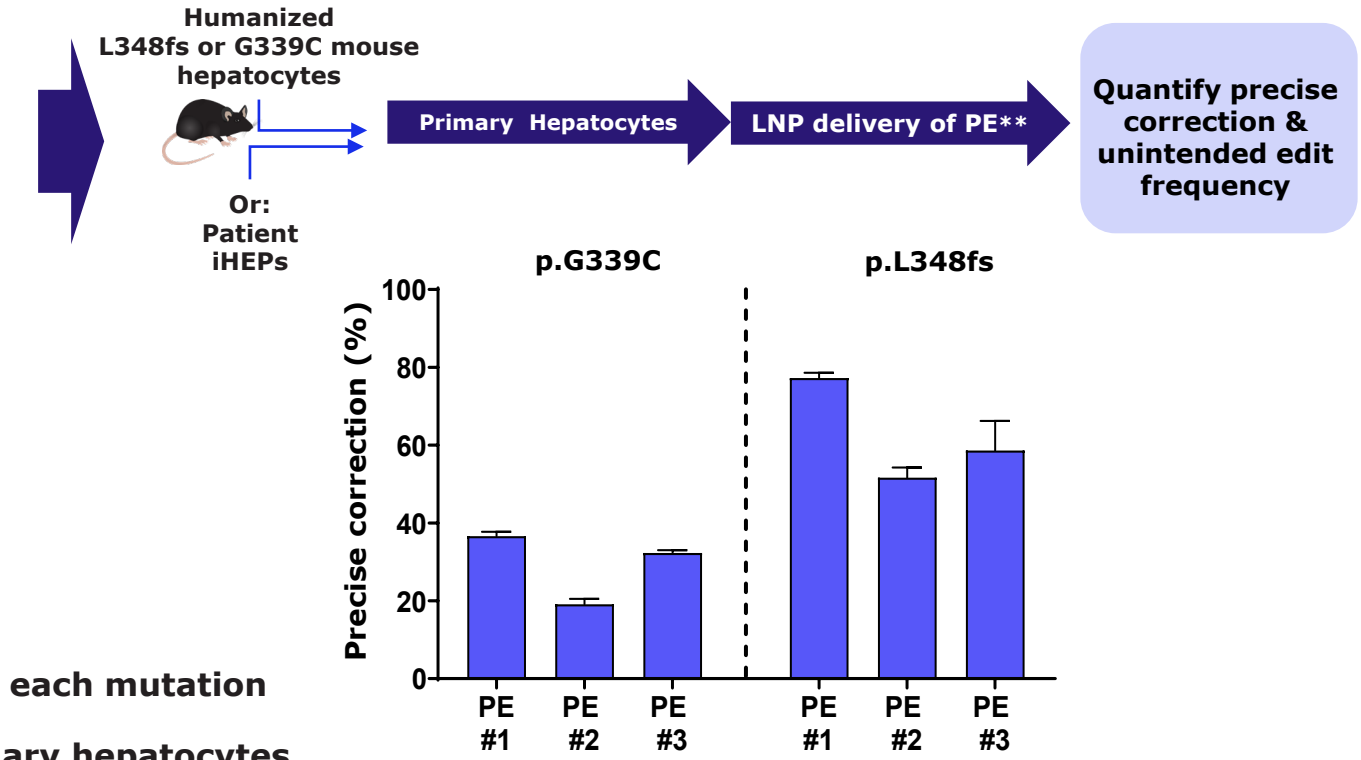
High throughput screening (HTS) identifies pegRNAs that precisely correct the p.L348fs and p.G339C mutations in hepatocytes

HTS in cells harboring the p.G339C or p.L348fs mutation



Evaluation of pegRNA potency in primary humanized hepatocytes* or patient iHEPs



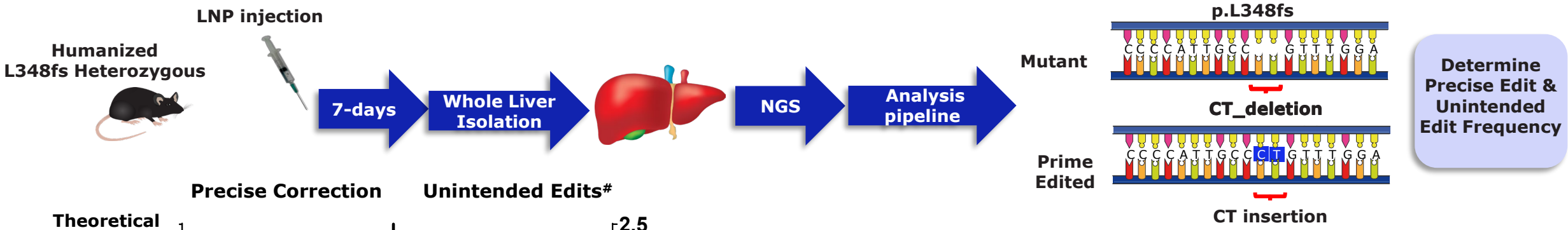
Key Takeaways:

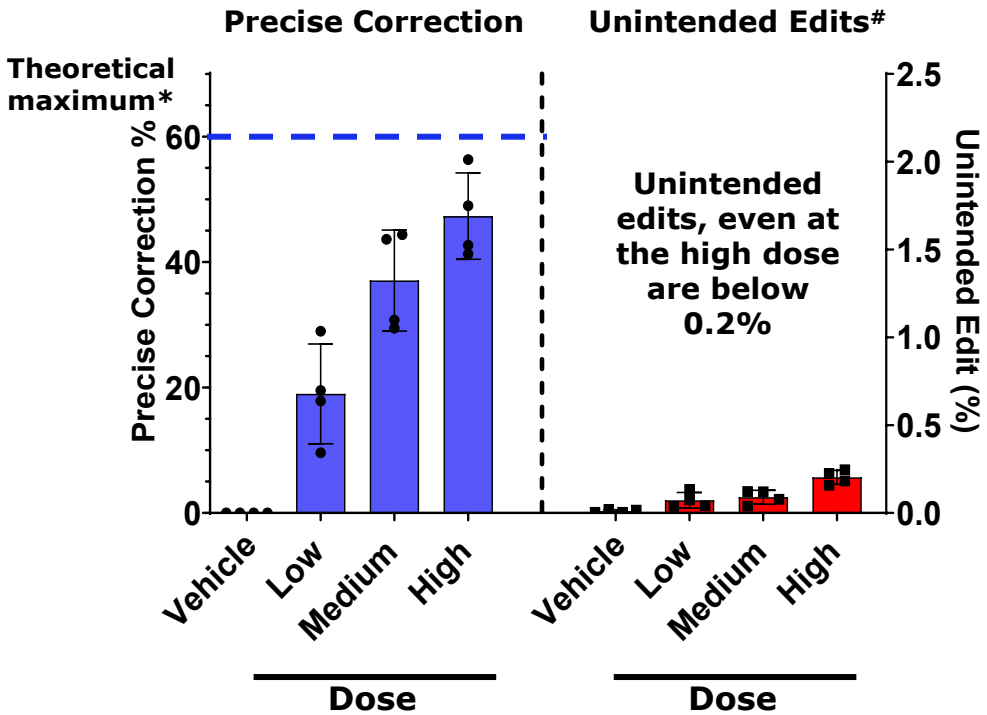
- ***In vitro* screening identified highly active candidates for each mutation**
- **77% precise correction of the p.L348fs mutation in primary hepatocytes**
- **52% precise correction of the p.G339C mutation following HTS, and 37% in primary hepatocytes – further optimizations underway**

#Unintended edits = any SNVs or indels within 300bp either side of the edit site; *Data shown using humanized primary mouse hepatocytes; **PE = Prime Editor

LNP delivery of Prime Editor components to the liver precisely corrects the p.L348fs mutation in humanized mice

Up to 56% whole liver precise correction in GSD1b humanized mouse





Key Takeaways:

- **A dose-dependent increase in precise correction of the p.L348fs mutation is observed following LNP-mediated delivery of Prime Editor components to the liver**
- **Unintended edit rates are below 0.2% at every dose tested**

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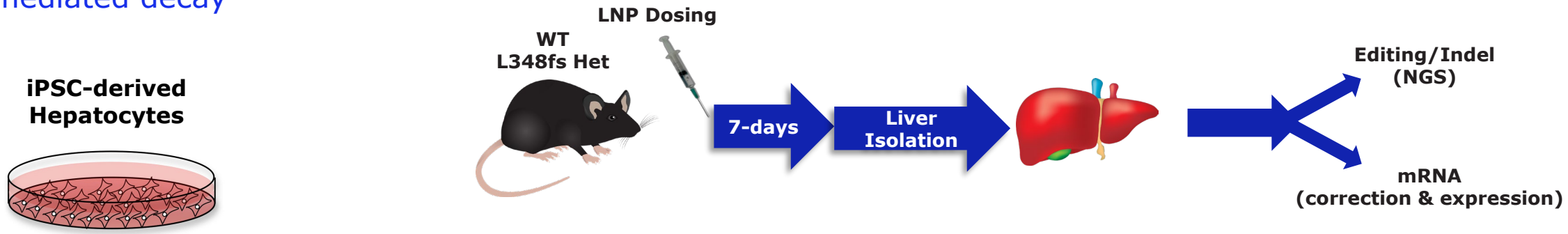
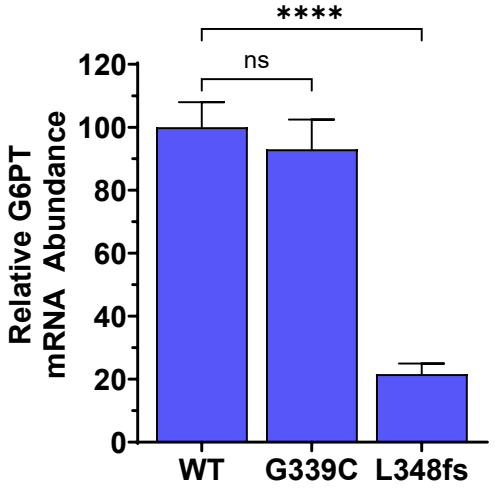
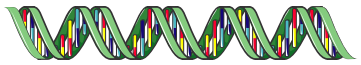
Precise editing of the G6PT gene in liver corrects mRNA transcript and restores mRNA expression

Precise correction of the L348fs mutation corrects transcripts and reverses G6PT mRNA non-mediated decay

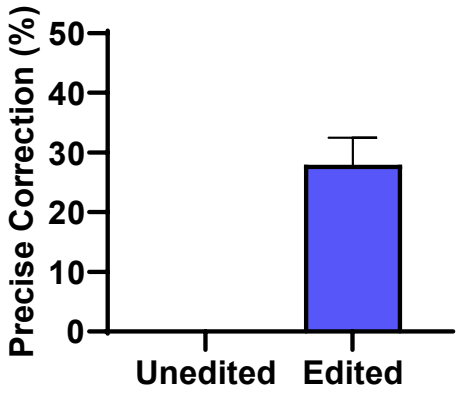


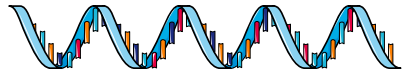
NMD lowers the prevalence of L348fs mRNA transcripts



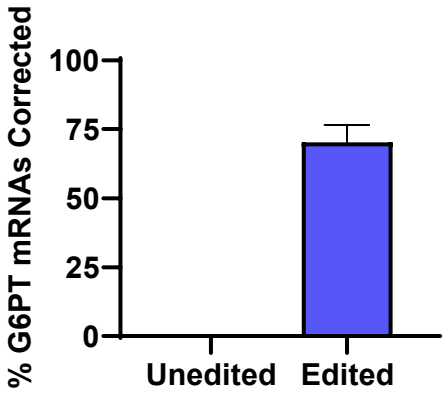


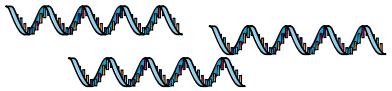
~30% G6PT alleles Corrected in Whole Liver



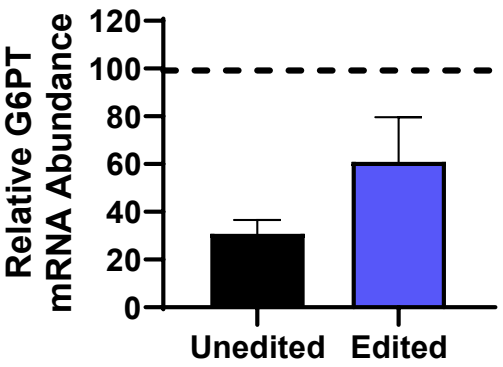


75% of G6PT mRNA Transcripts Corrected





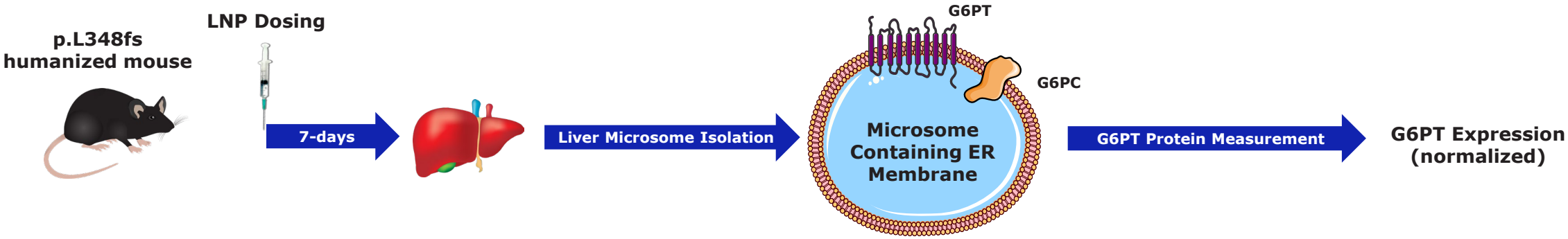
NMD Reversed by Prime Editing



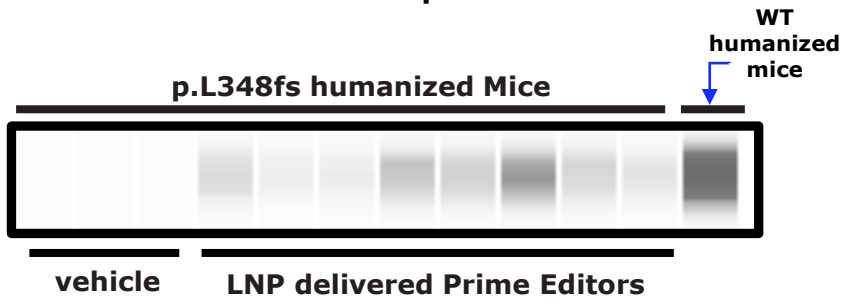
NMD: Nonsense-Mediated Decay; G6PT: glucose-6-phosphate transporter

G6PT transcripts and protein are restored in humanized p.L348fs mouse liver following delivery of Prime Editors

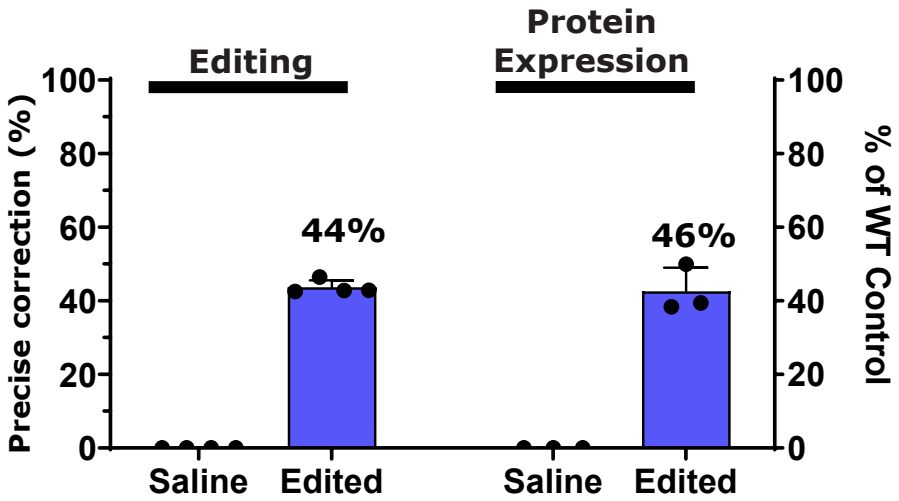
Precise editing of the G6PT gene restores G6PT protein expression



Liver microsome G6PT protein restoration



Anti-human G6PT specific antibody



Key Takeaways:

- **Prime Editing restores expression of G6PT protein**
- **Extent of G6PT mutation correction correlates with extent of G6PT protein restoration**

G6PT: glucose-6-phosphate translocase, het: heterozygous, WT: wild-type, ER: endoplasmic reticulum

Preliminary analysis showed no detectable off-target editing in patient cells

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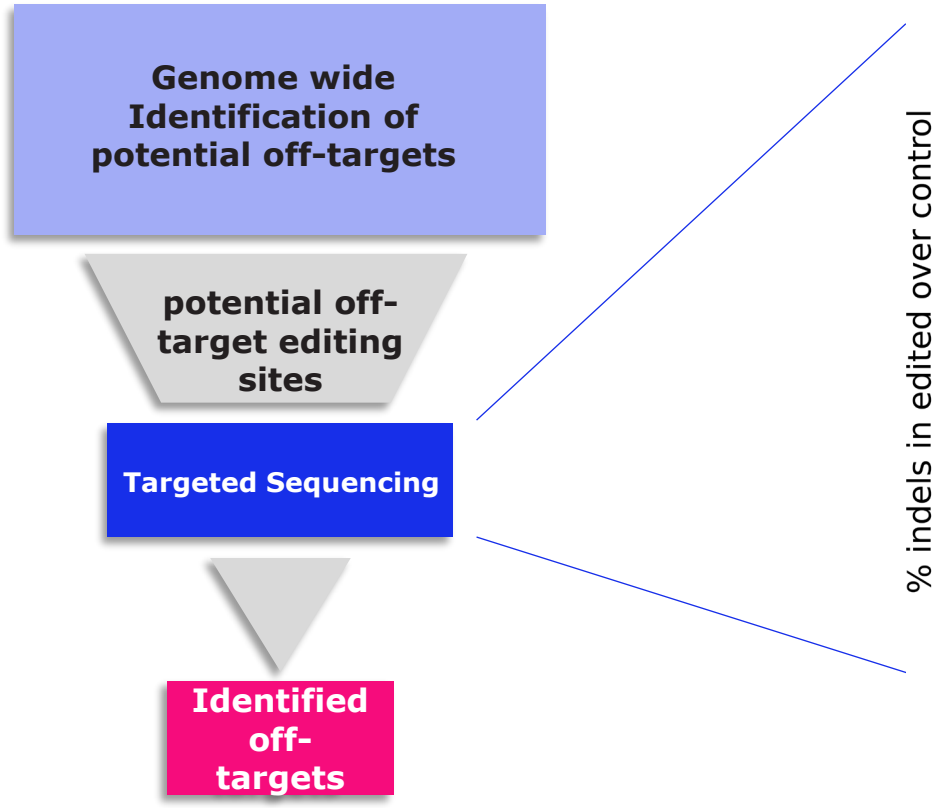
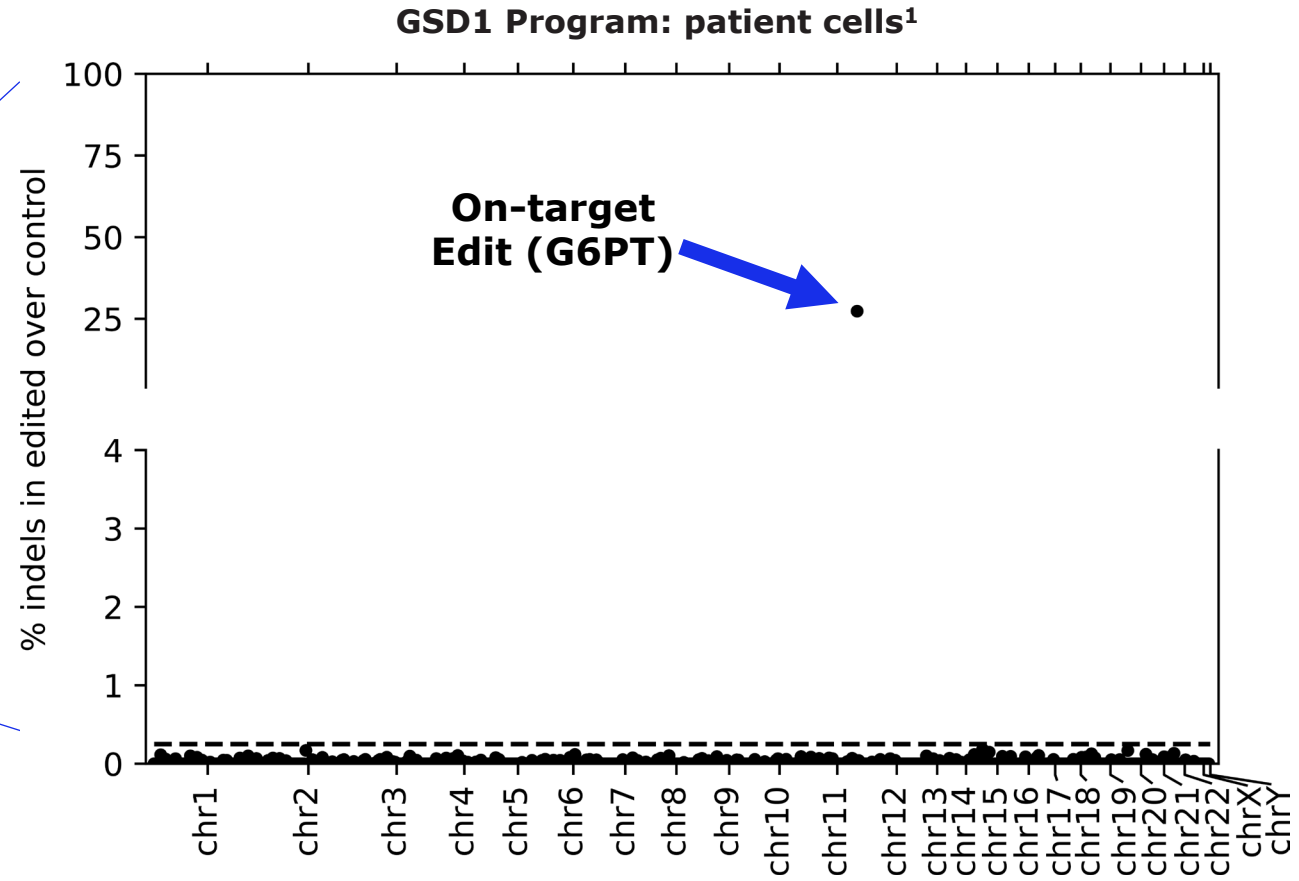
Performed using a lead p.L348fs pegRNA and lead LNP delivery system





[1]Targeted Analysis of potential off-target sites using deep sequencing in Prime Edited human iPSC cells carrying the p.L348fs

Summary

- Prime is developing a universal LNP-RNA platform to deliver Prime Editors to treat liver and metabolic diseases
- Potent Prime Editors correcting the two most prevalent GSD1b mutations were identified and engineered, resulting in high levels of allele correction [up to 56% correction of the p.L348fs mutation] in humanized mouse whole liver
- GSD1b LNP-RNA Prime Editors restore G6PT mRNA and protein expression consistent with allele correction
- Initial studies from Prime's off-target pipeline have not detected any off-targets genome-wide

THANK YOU!

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