prime medicine

Delivering on the promise
of Prime Editing

LNP delivered Prime Editors restore glycemic control in humanized rodent models of Glycogen Storage Disease Type 1b (GSD1b)

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American Association for the Study of Liver Diseases
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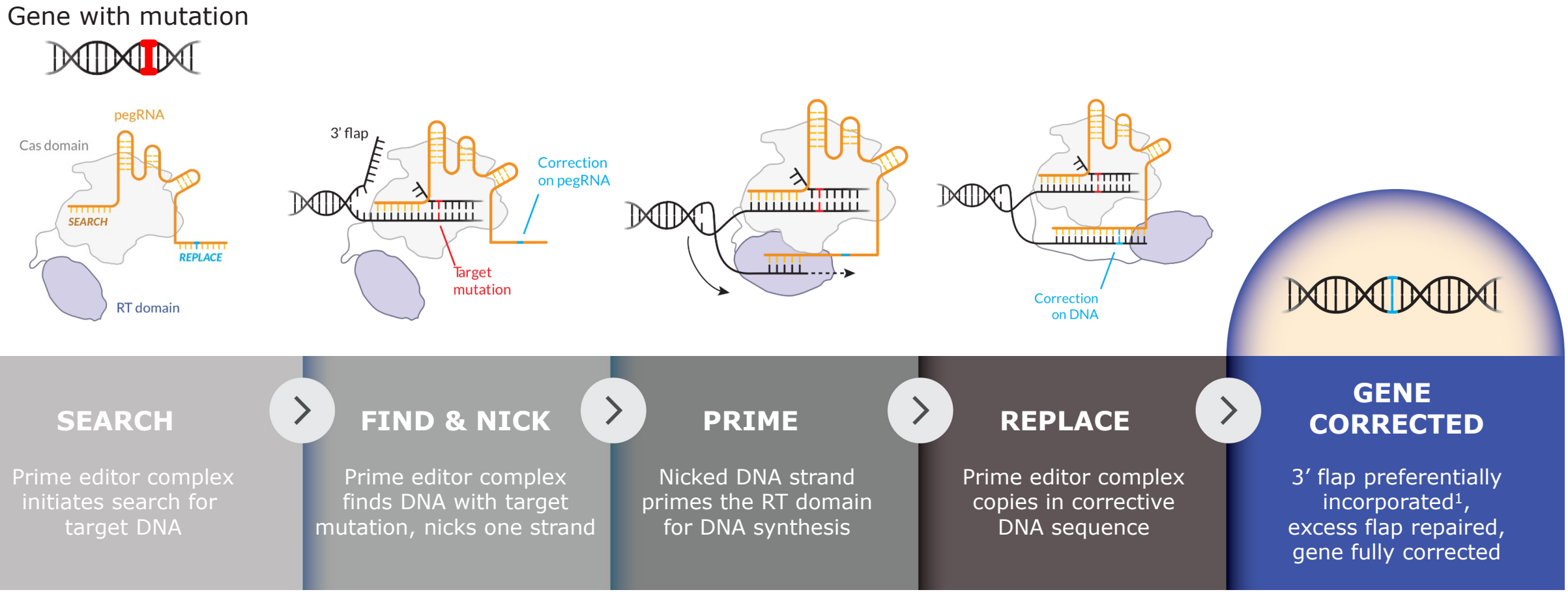
On behalf of the team at Prime Medicine

Disclosures

Vivian Choi declares she is currently an employee of Prime Medicine, Inc. and owns equity in Prime Medicine.

Prime Editing is programmable for both search and replace

The PE technology utilizes a Prime Editor protein and a Prime Editing guide RNA (pegRNA) to directly write new genetic information into a targeted DNA site without requiring a DSB



[1] Completion of an edit requires 3 'edit checks"; pegRNA = Prime Editing guide RNA; RT = reverse transcriptase; Cas = CRISPR associated protein; DSB = Double-stranded break

We believe Prime Editing is the only gene editing technology that can edit, correct, insert and delete DNA sequences in any target tissue

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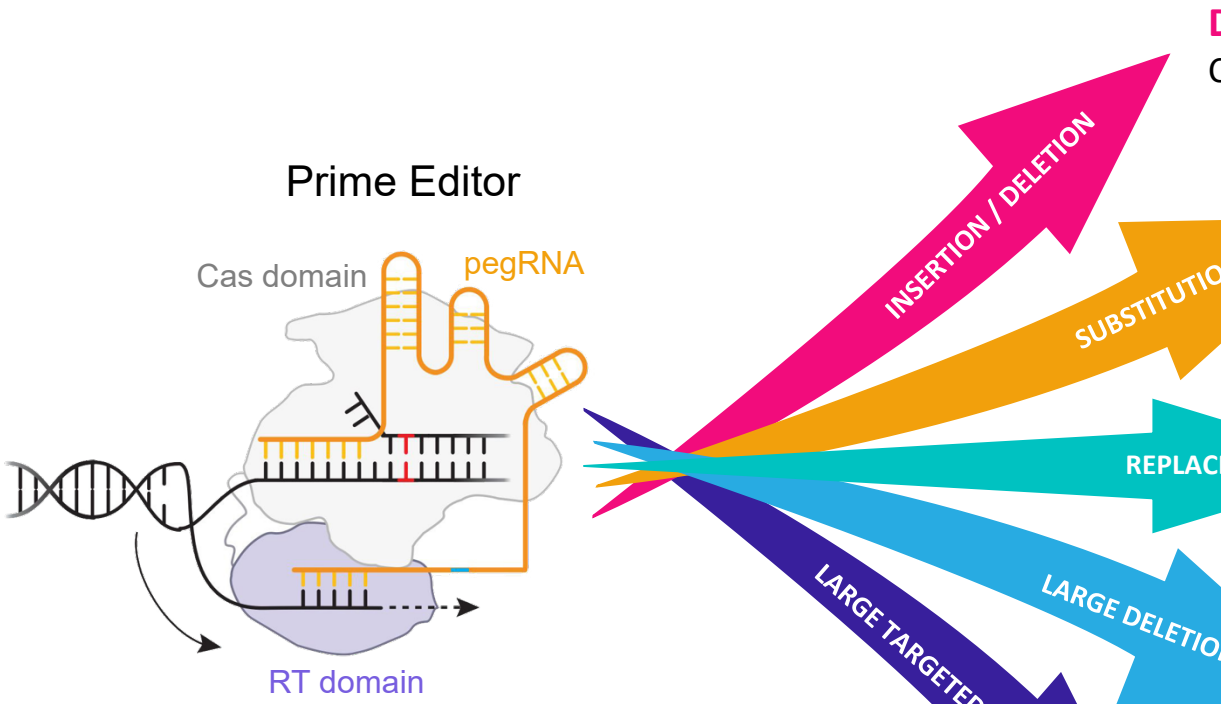
Corrects mutations across many tissues, organs and cell types, in dividing and non-dividing human cells



Deletion / Insertion Correction
Chronic Granulomatous Disease (CGD)

Point Mutation Correction
Wilson's Disease
Glycogen Storage Disease

Hotspot Correction
Retinitis Pigmentosa / Rhodopsin (RHO adRP)

Repeat Excision
Friedreich's Ataxia

Targeted Gene Insertion (PASSIGE™)
CAR-T

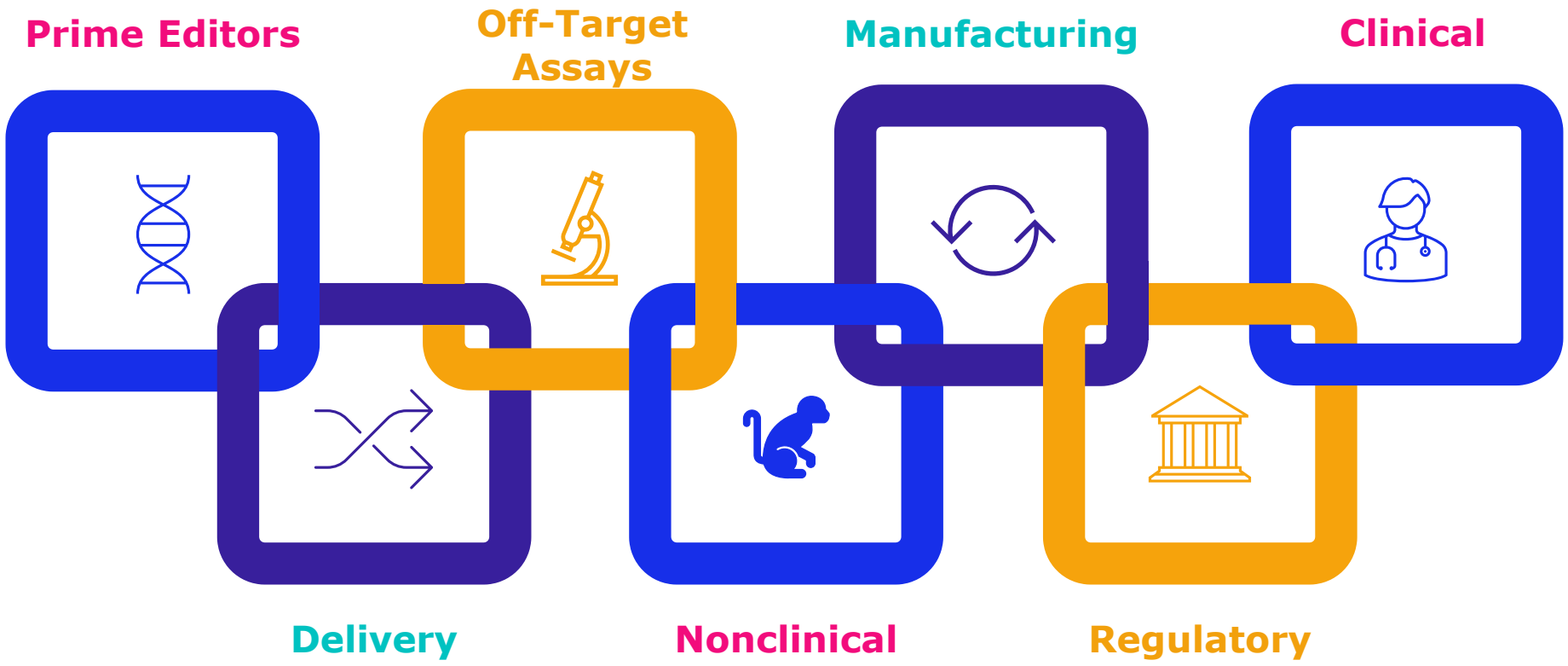
Broad and versatile editing capabilities unlock opportunities across **thousands of indications,** including genetic diseases, infectious diseases, cancers and immunological diseases

Detailed movie of how Prime Editing works: www.primemedicine.com
https://primemedicine.com/science/#how-prime-editing-works

CAR-T = chimeric antigen receptor (CAR)-T cell therapy

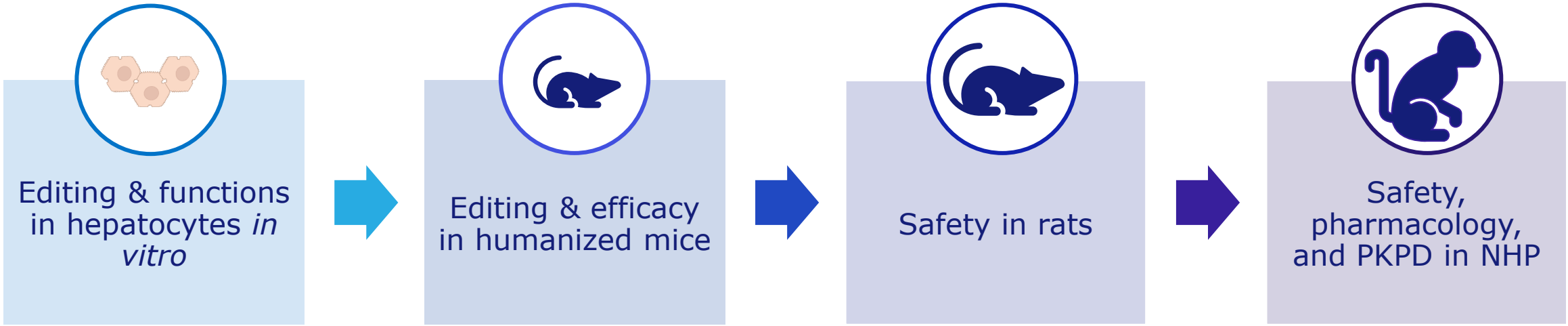
Prime Editing platform modularity accelerates and de-risks ongoing efforts, enabling rapid generation of new product candidates

Core components can be readily leveraged to accelerate pipeline growth, efficiency and execution



Prime Medicine's approach to developing Prime Editors to treat liver and metabolic diseases

Prime Editors are specific to *human* patient DNA sequence and designed for the correction of *human* mutations

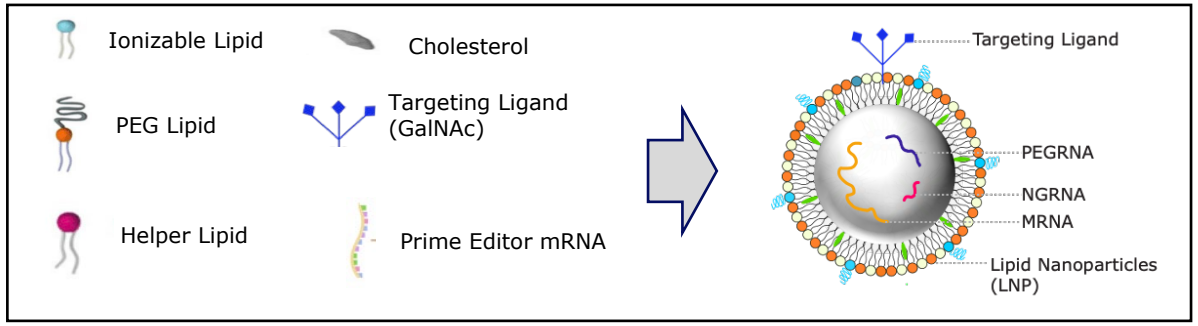


- Establish potency of lead Prime Editor drug candidates
- Establish genotype to phenotype correlation and off-target profile
- Establish pharmacology, safety, tolerability
- Determine biodistribution, drug pharmacokinetics
- Determine PK/PD relationships, human dose projections

Prime has developed a universal LNP for our liver & metabolic programs

Prime Medicine's Universal LNP contains a novel GalNAc targeting ligand

Shared LNP/PE components



- ✓ Delivery to the liver via the ASGPR is a validated delivery mechanism

Compared to LNPs without a targeting ligand, Prime's Universal LNP*:

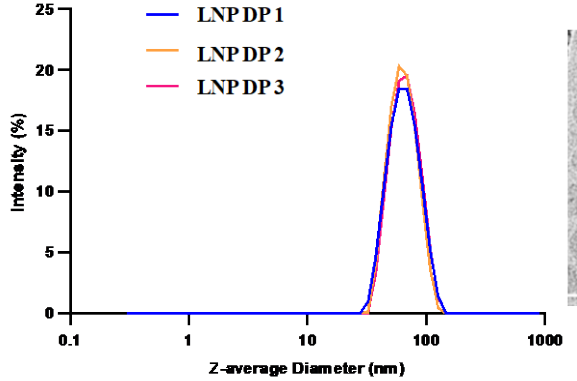
- ✓ Increases potency
- ✓ Improves safety profile
- ✓ Improves biodistribution

Prime Medicine's modular LNP can be used to generate multiple different drug product candidates (DP)

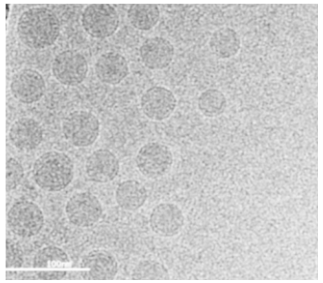
A

	Avg. Size (nm)	PDI	%EE
LNP DP 1	60	0.056	99.3
LNP DP 2	59	0.045	97.9
LNP DP 3	60	0.052	98.8

B



C



By swapping only the guide RNAs while keeping the other components constant, we have a new product with the potential for the same critical quality attributes

DP = drug product; PDI = polydispersity index; EE = encapsulation efficiency; PEG = polyethylene glycol; GalNAc = N-acetyl galactosamine; ASGPR = Asialoglycoprotein receptor
* Tested in rodent and large animal studies

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

One-time delivery of LNP Prime Editor cargo with potential to correct pathogenic mutations in the liver

Prime Editor Cargo Components

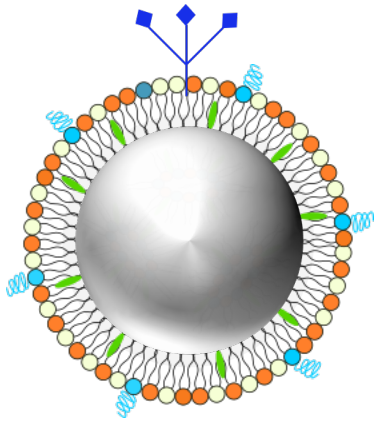
mRNA

pegRNA

ngRNA

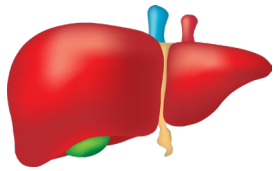
LNP formulation

LNP-encapsulated Prime Editor Cargo



Single IV infusion

Liver Delivery



Prime Editing in hepatocytes

Precise Correction of Mutation in Hepatocytes

***SLC37A4* p.G339C**

G T A T T T **T** G T T T C T

G/T substitution

Prime Editing

G T A T T T **G** G T T T C T

T/G correction

Precise Correction of Mutation in Hepatocytes

***SLC37A4* p.L348fs**

C C C C A T T G C C **--** G T T

CT deletion

Prime Editing

C C C C A T T G C C **C T** G T T

CT Insertion

Prime Editors to correct pathogenic mutations causing von Gierke disease or Glycogen Storage Disease Type 1b (GSD1b)

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:

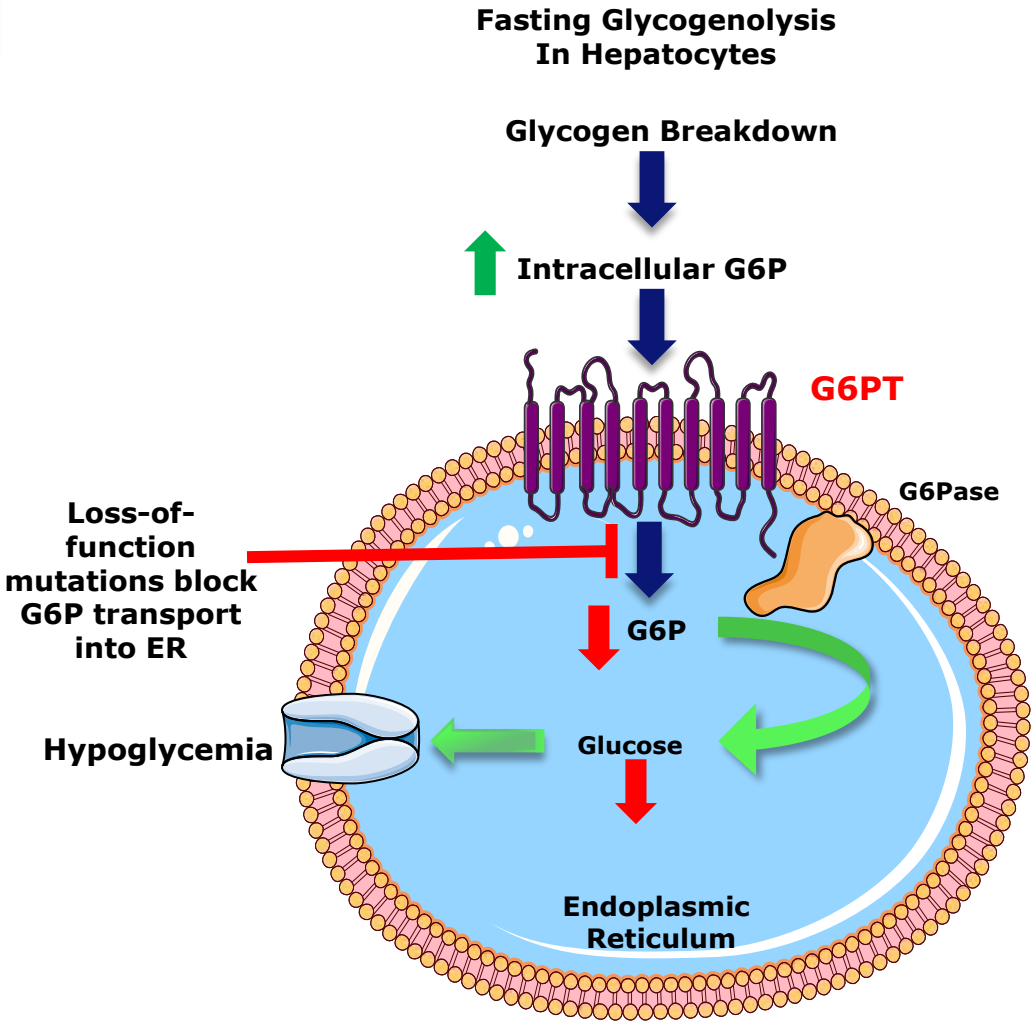
- Autosomal recessive, caused by mutations in the *SLC37A4* gene that encodes G6PT, a glucose-6-phosphate transporter
- ***SLC37A4* p.L348fs and p.G339C mutations** found in ~50% of GSD1b patient population

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 the p.L348fs or p.G339C mutations to restore glucose homeostasis in patients with GSD1b



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

Identification of lead Prime Editors for correction of the p.G339C and p.L348fs mutations

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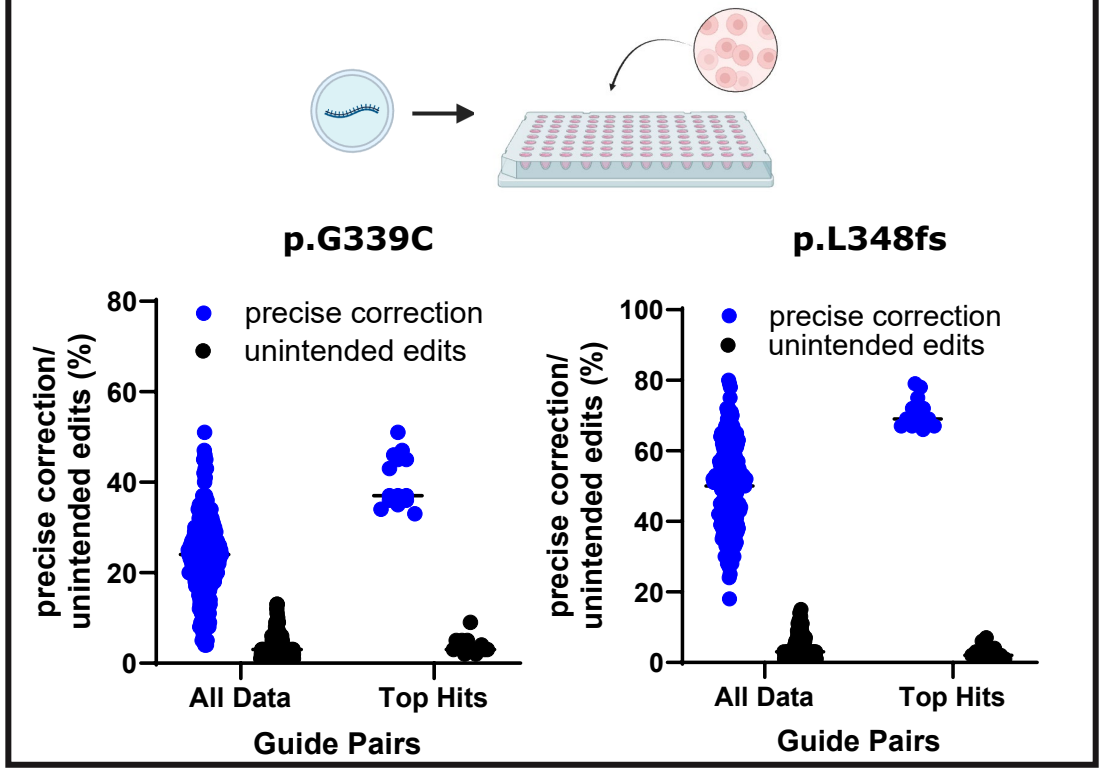
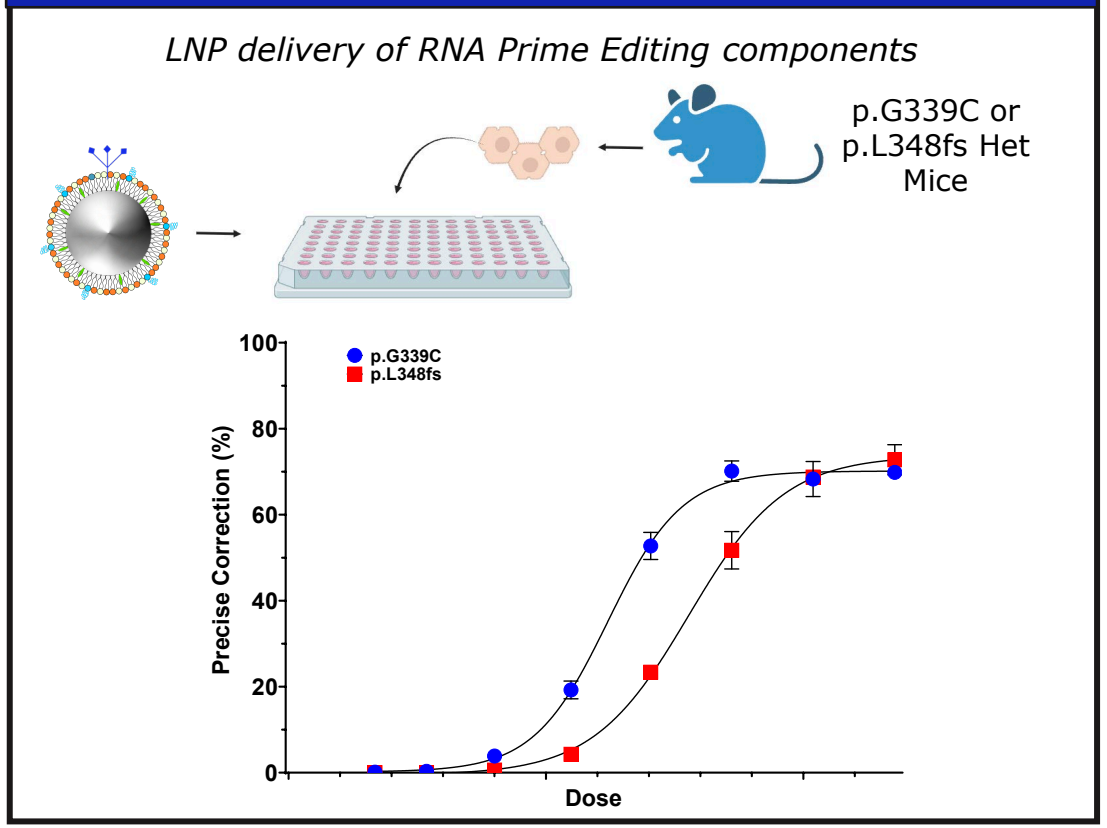
High throughput screening in mutant cell lines



High throughput screening in primary fully humanized hepatocytes



Prime Medicine's high throughput screening platform identifies multiple Prime Editors capable of efficient correction of p.G339C and p.L348fs in primary mouse hepatocytes isolated from GSD1b humanized mice

Optimization of components improves precise correction of the p.L348fs mutation *in vivo*

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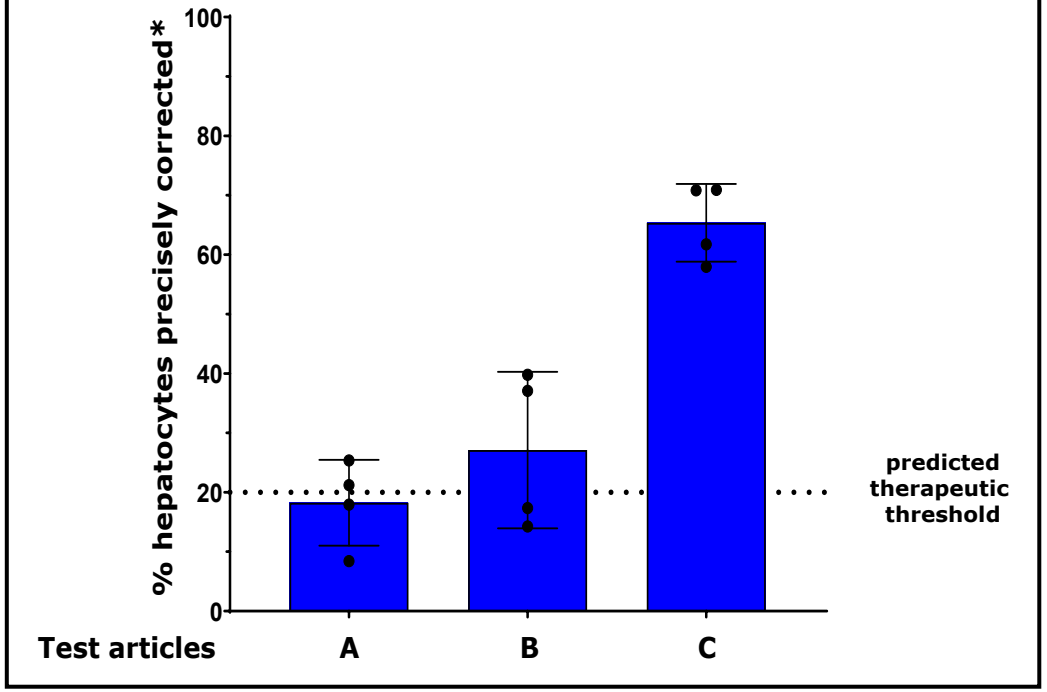
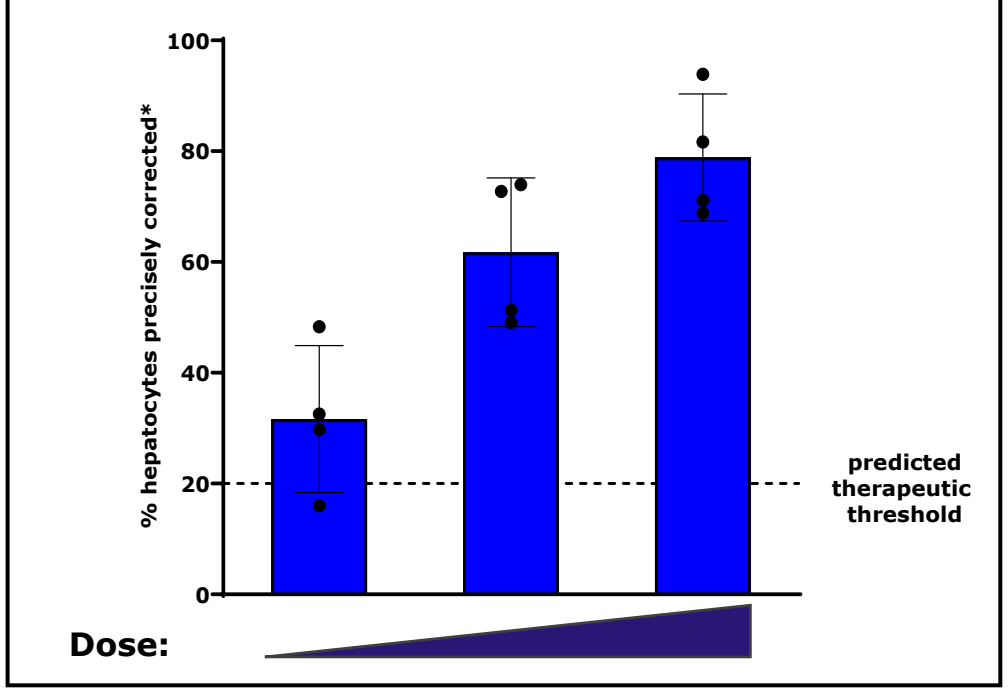
Improved ***in vivo*** correction observed following optimization of Prime Editors components

Component optimization improves precise correction of the p.L348fs mutation *in vivo*



***In vivo* dose response demonstrates efficient correction of the p.L348fs mutation in fully humanized mice**



Unintended editing rates are below 0.2% at every dose tested

*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. ** Calculation based on 60% of cells in whole liver are hepatocytes; # Unintended edits = any SNVs or indels within 300bp either side of the edit site.

Optimization of components improves precise correction of the p.G339C mutation *in vivo*

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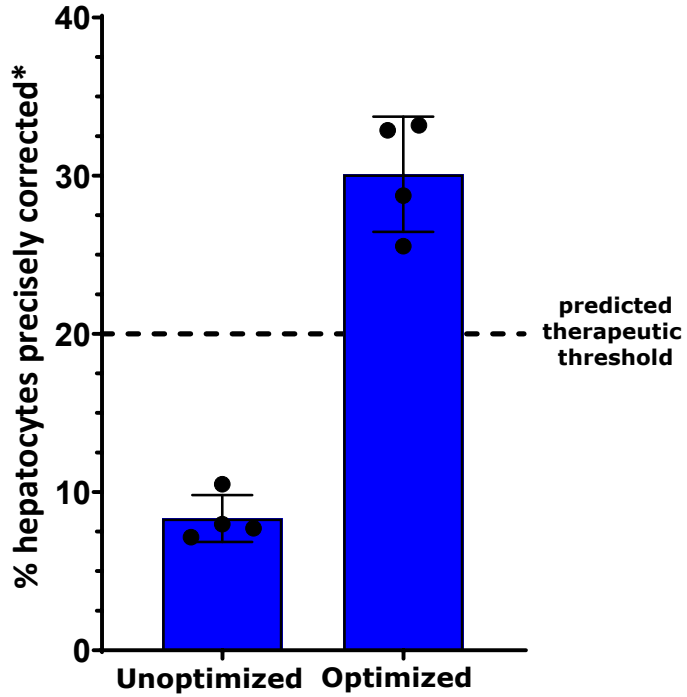
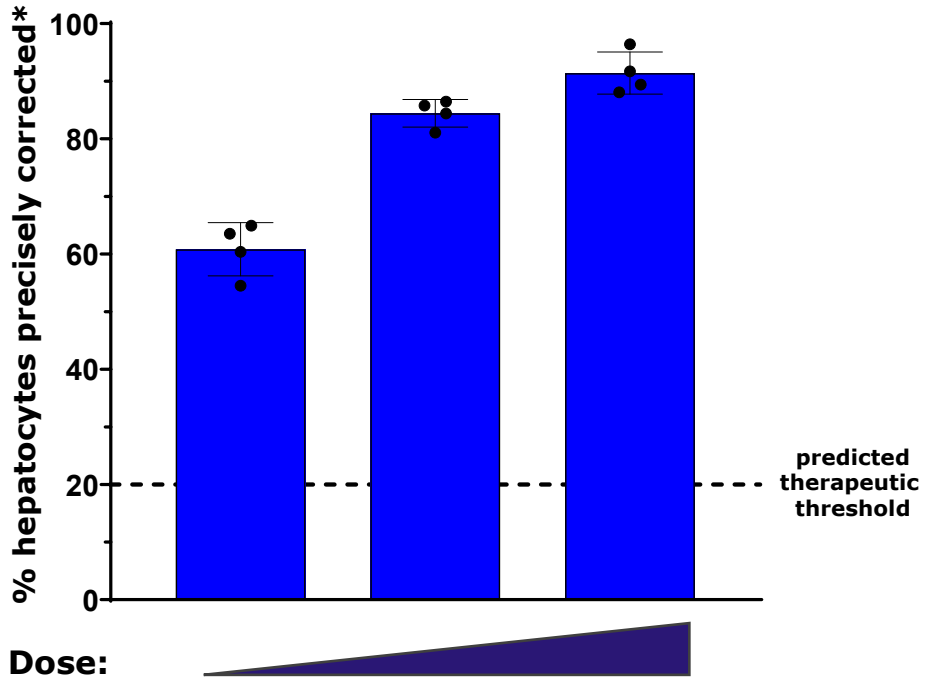
Improved ***in vivo*** correction observed following optimization of Prime Editors components

Improved correction of the p.G339C mutation in fully humanized mice using an optimized Prime Editor



Improved **correction of the p.G339C mutation in fully humanized mice following further optimization**



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

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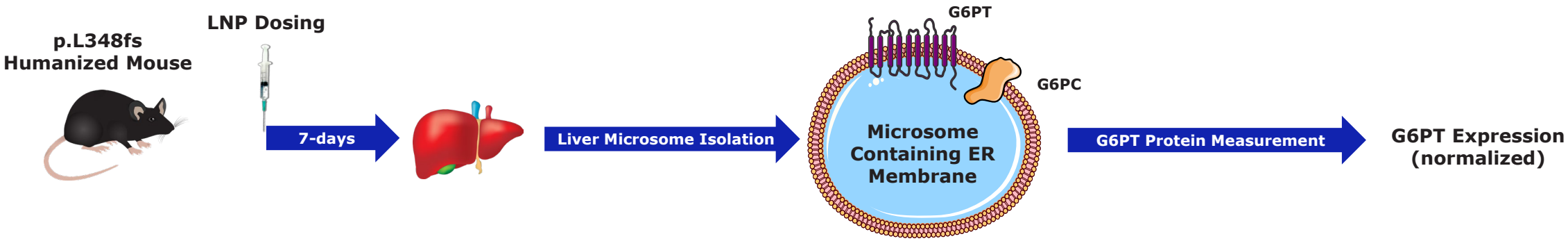
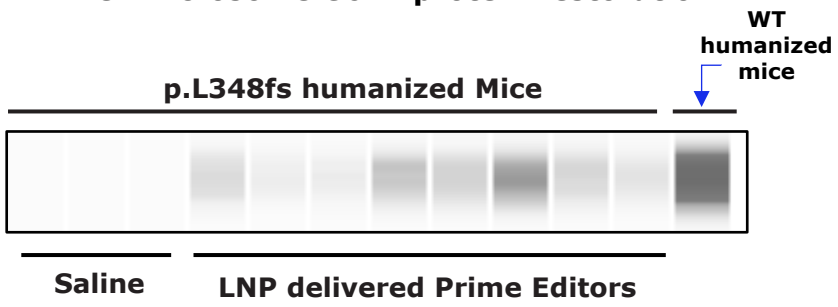
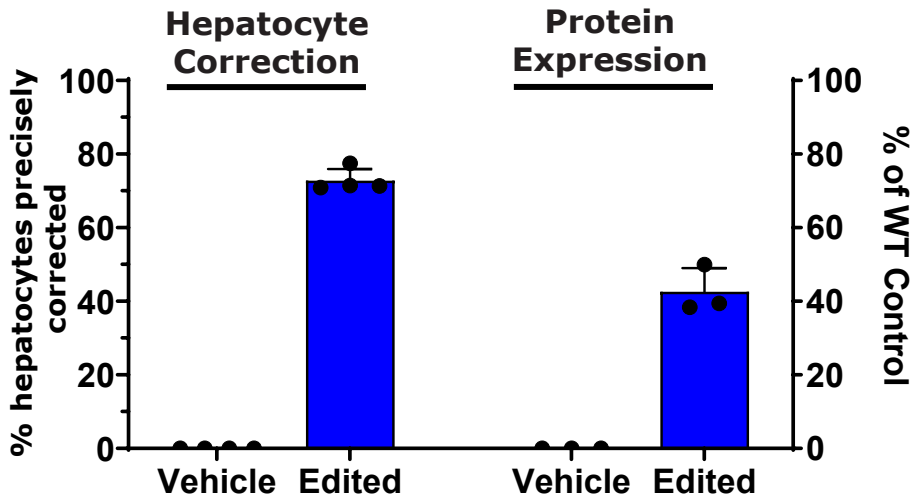
Precise editing of the G6PT gene restores G6PT protein expression



Liver microsome G6PT protein restoration



Anti-human G6PT specific antibody



Key Takeaways:

- **Prime Editing restores expression of G6PT protein**
- **Extent of *SLC37A4* mutation correction correlates with G6PT protein restoration**

Prime Editing restores glucagon-dependent glucose production in a mouse model of GSD1b

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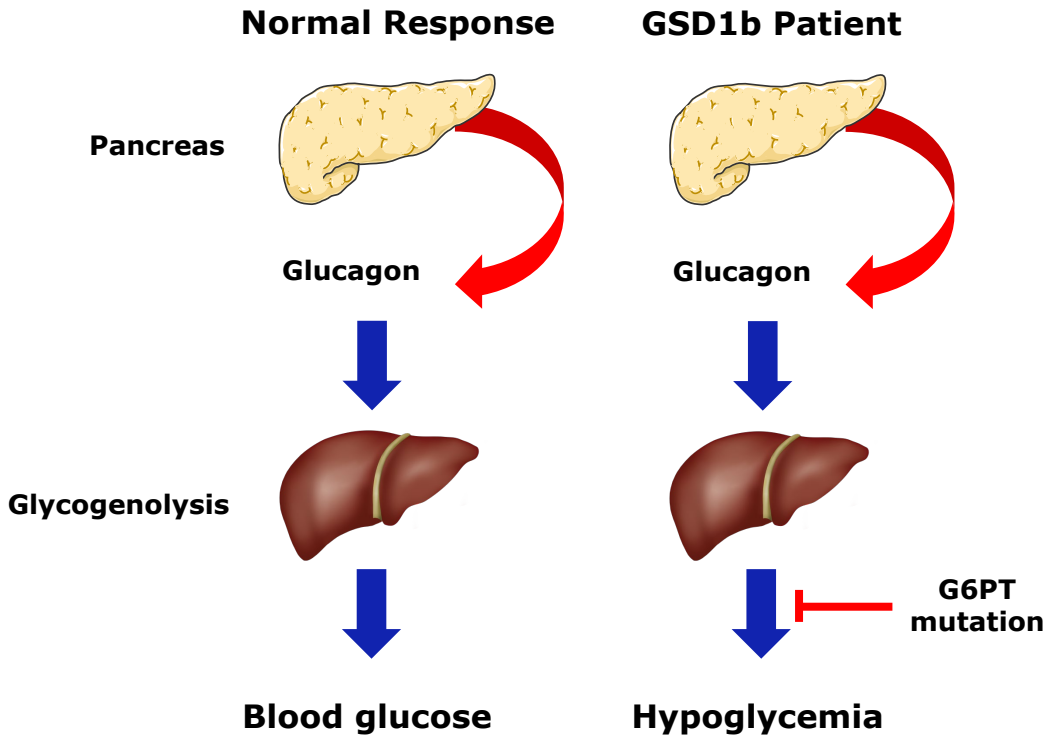
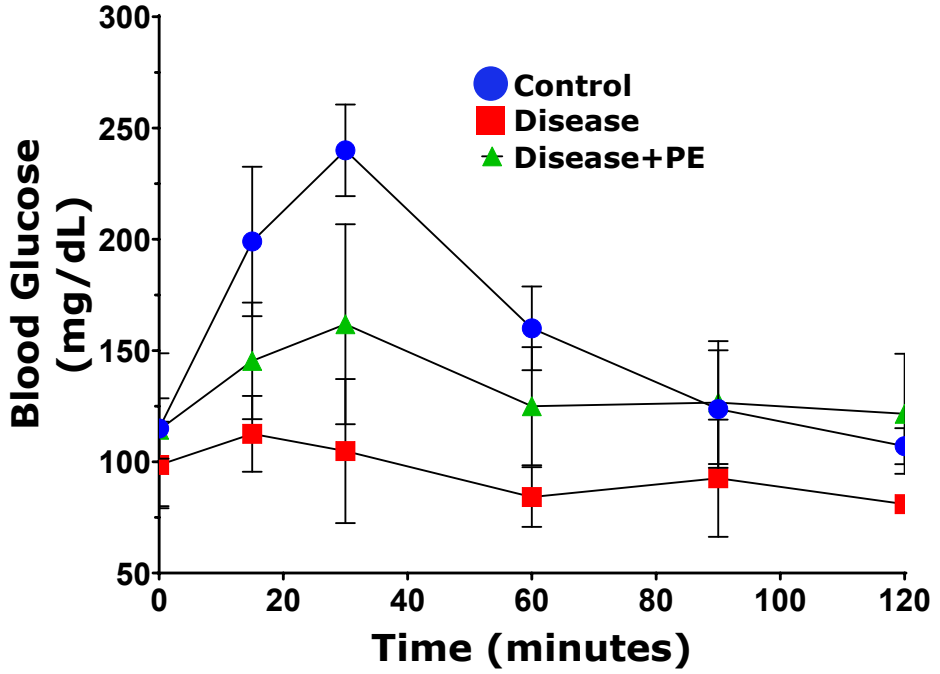
Pathophysiological response to glucagon in GSD1b patients results in hypoglycemia



Prime editing improves glucose production following a glucagon challenge



Prime editing improves metabolic dysfunction observed in a mouse model of GSD1b

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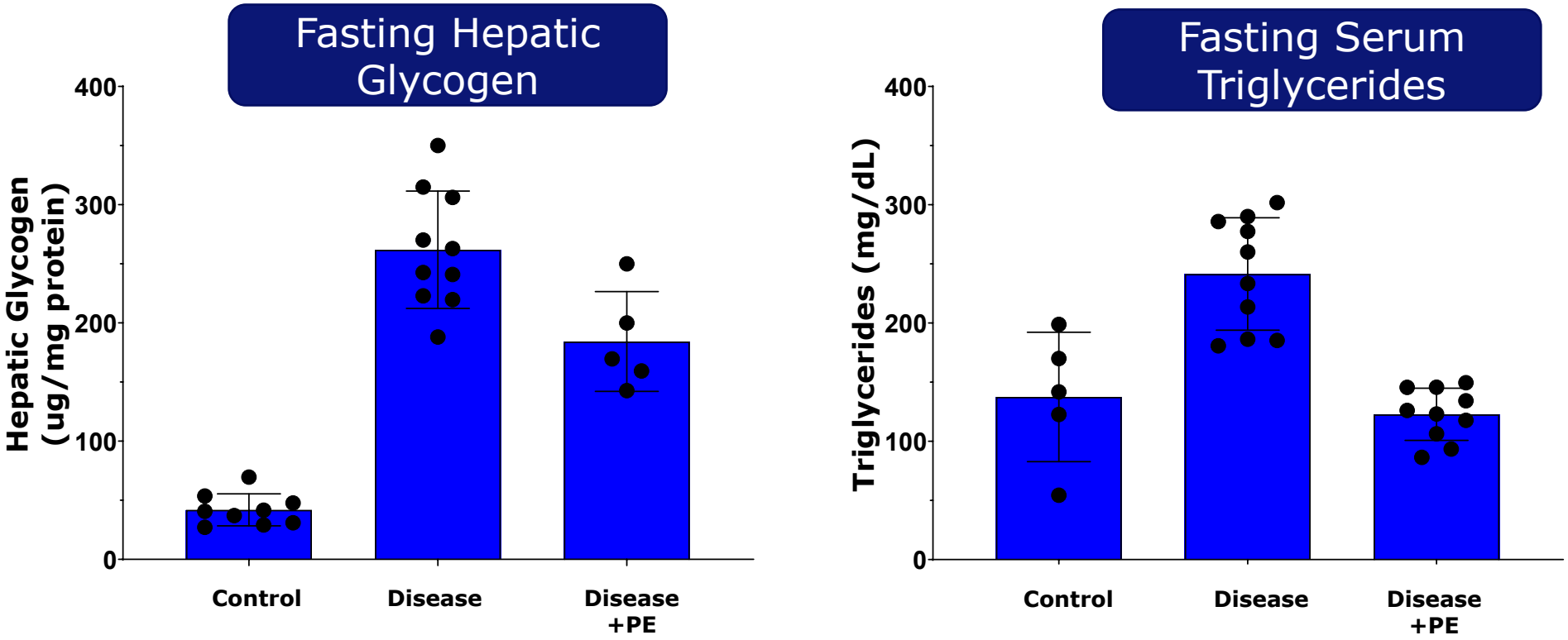

Successful Prime Editing with precise correction leads to **reduction of fasting hypoglycemia, triglycerides, liver glycogen & restores glucagon responsiveness** in humanized mice at a dose predicted to be clinically relevant for human disease

Precise editing of p.L348 in *SLC37A4* (G6PT) gene using a surrogate Prime Editor is durable up to 44 weeks in NHP

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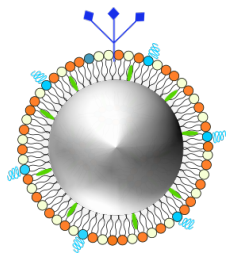
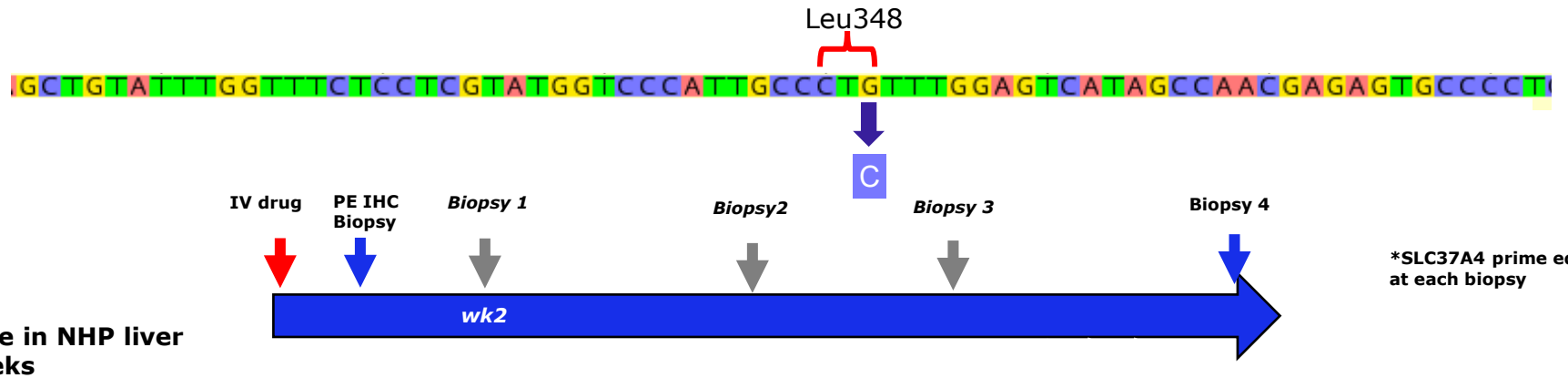
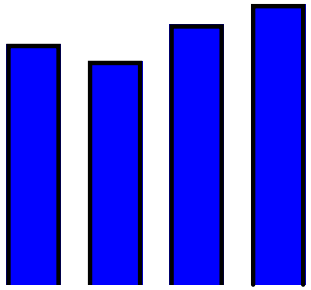


LNP-formulated PE



*SLC37A4 prime editing at each biopsy

Prime Editing is durable in NHP liver over 44 weeks

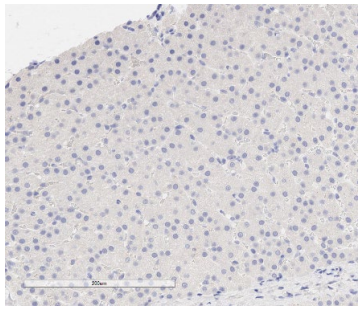


Biopsy

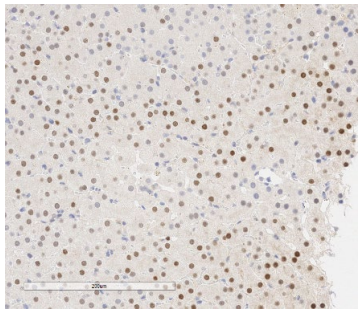
Week

Prime Editor IHC shows robust nuclear localization

6.5 hr biopsy



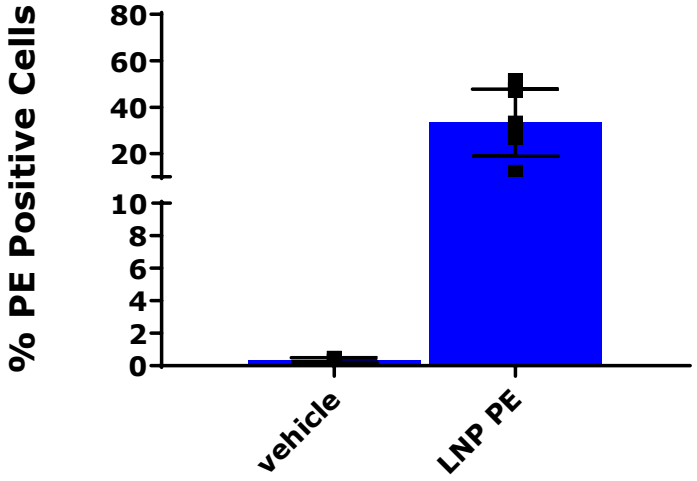
Vehicle (0.38%)



IV LNP PE (35.6%)

Brown nuclei indicate +Prime Editor immunoreactivity

Nuclear Prime Editor detection correlates closely with editing outcomes



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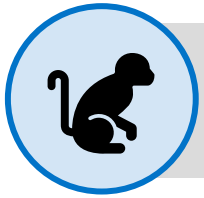
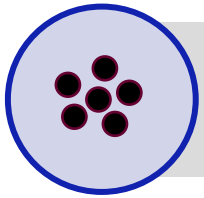
Prime Medicine's Universal LNP exhibits an excellent safety profile in cynomolgus monkey (NHP)

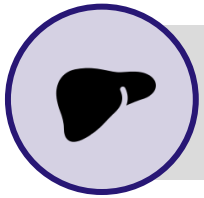


- Well-tolerated with no acute reactions, clinical observations, or body weight changes
- Animals healthy at 54 weeks

- No observed change in platelets, coagulation time or blood count
- No observed change in blood biochemistry panel



- Minimal changes in serum IL6 levels
- No other observed cytokine changes



- No changes observed in liver histopathology (H&E)
- Minimal transient LFT elevations

Benchmarked against other LNPs in clinical development

LFT = liver function tests; NHP = non-human primate; IL-6 = interleukin 6; H&E = hematoxylin & eosin

Preliminary analysis: no detectable off-target editing in patient cells treated with Prime Editor

"IND ready" set of comprehensive off-target assays

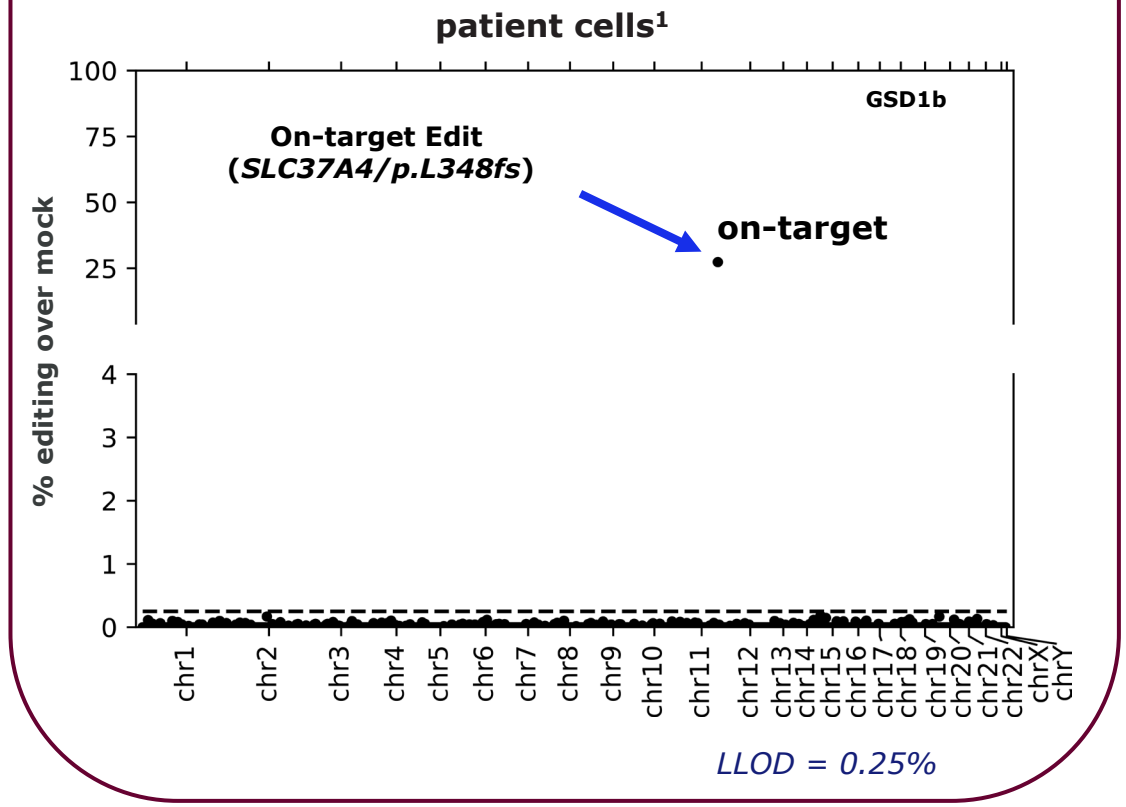
Genome wide identification using in silico and biochemical methods

Potential off-target editing sites

Targeted sequencing

Identified off-targets

Genome-wide Off-target Editing



[1]Targeted Analysis of potential off-target sites using targeted deep sequencing in Prime Edited human patient iPSC cells. GSD1b: glycogen storage disease type 1b, ATP7B: ATPase copper transporting beta, G6PT: glucose-6-phosphate translocase, Indels: insertions/deletions

Summary

Modular LNP platform

Prime has developed a universal liver targeted LNP-PE platform with the potential to deliver Prime Editors to precisely correct disease-causing mutations

- GalNAc targeting ligand improves dose potency, editing, and biodistribution compared to LNPs without targeting ligand
- Excellent and differentiated safety profile in large animal & rodent studies

Glycogen Storage Disease type Ib

LNP-RNA Prime Editor candidates achieve 80-90% precise hepatocyte correction of the *SLC37A4* (G6PT) gene mutations p.L348fs and p.G339C in humanized mice at clinically relevant doses

- IV delivery Prime Editor restores hepatic glycogen metabolism in a humanized mouse model of GSD1b
- Large animal cynomolgus monkey studies demonstrate up to 83% precise hepatocyte editing of G6PT gene at p.L348 using a NHP surrogate pegRNA at a dose that was safe, well tolerated and durable

Off-target editing

- No off-target editing was detected in human cells derived from either Wilson's Disease or GSD1b patients

Prime Medicine's presentation on Prime Editors development for Wilson's Disease
Monday Nov 18th at 8:45am (Location: 6C – Session Genetic & Metabolic Liver Diseases)

NHP: nonhuman primate; pegRNA = Prime Editor guide RNA

THANK YOU!

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