prime medicine

Delivering on the promise
of Prime Editing

OR077-LNP delivered Prime Editors restore glycemic control in humanized rodent models of Glycogen Storage Disease Type 1b (GSD1b)

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European Society of Gene and Cell Therapy, Rome, Italy
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On behalf of the team at Prime Medicine

Disclosures

Jeremy Duffield declares he is currently an employee of Prime Medicine, Inc. and owns equity in Prime Medicine.

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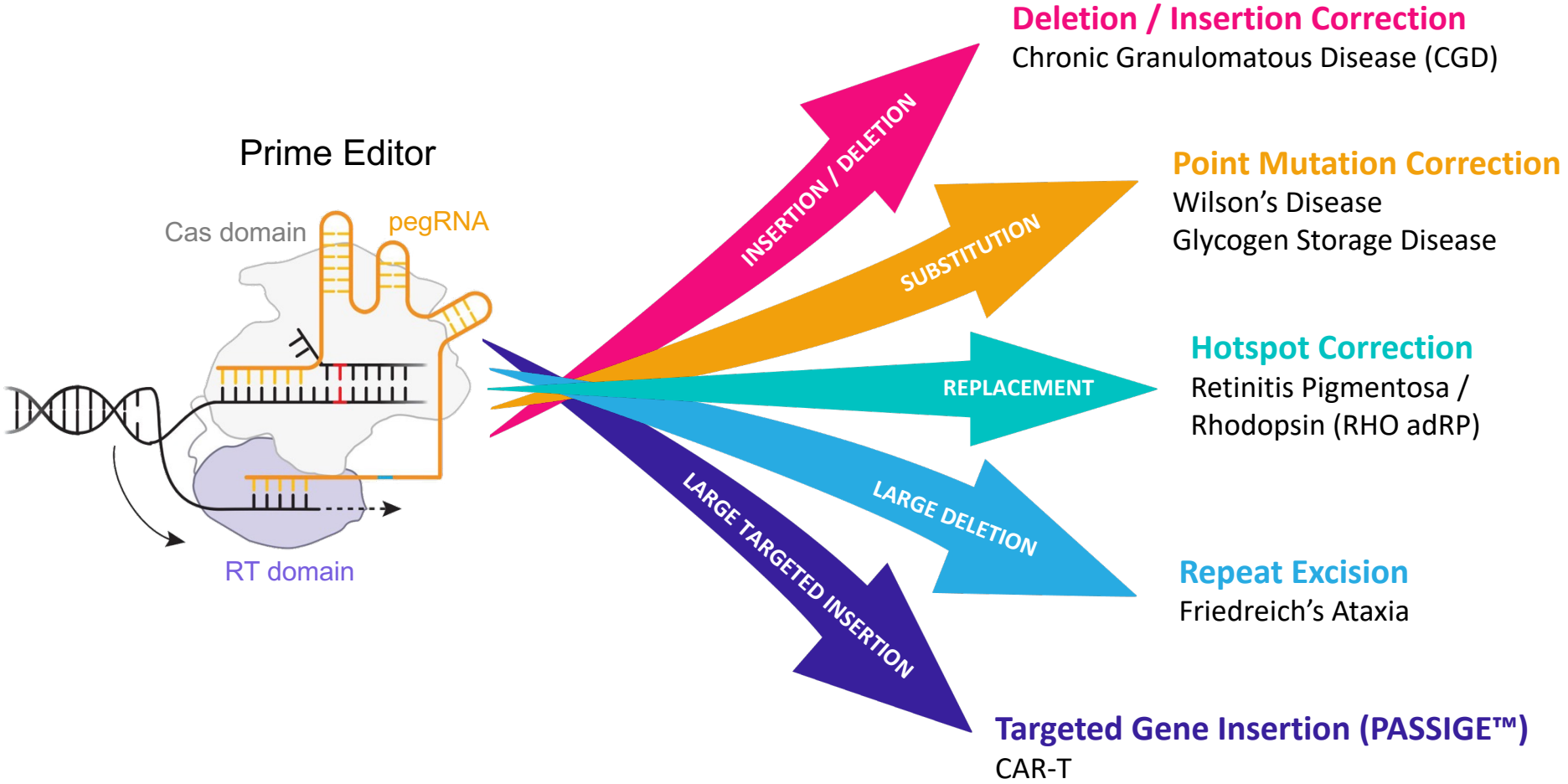
Corrects mutations across many organisms, organs and cell types, in dividing and non-dividing human cells



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

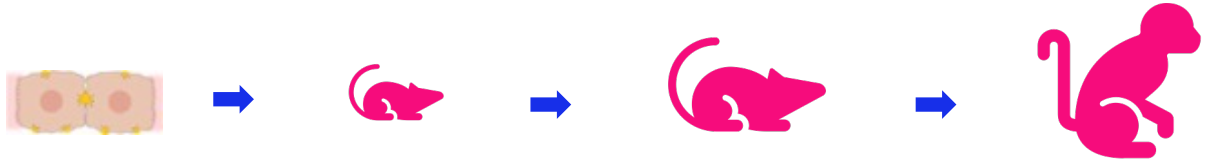
Detailed movie of how Prime Editing works: www.primemedicine.com
https://primemedicine.com/science/#how-prime-editing-works

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

Prime 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

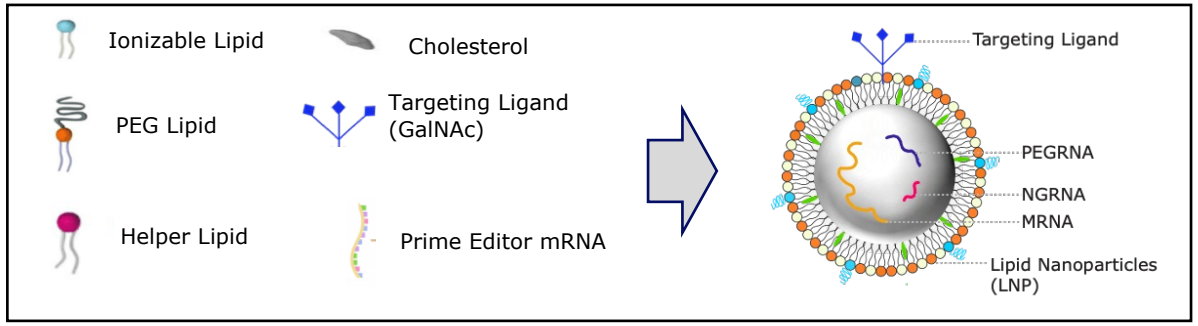
- Establish potency and activity of lead Prime Editor drug candidates
- Establish genotype to phenotype correlation and off-target profile
- Establish pharmacology, safety, tolerability
- Determine biodistribution, drug pharmacokinetics
- Determine PK/PD relationships, human dose projections



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*:

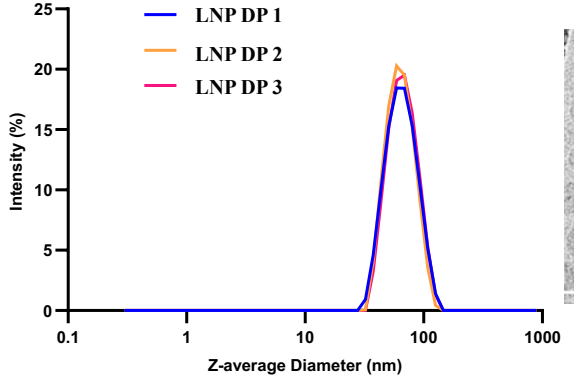
- ✓ Increases potency
- ✓ Improves safety profile
- ✓ Improves biodistribution

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



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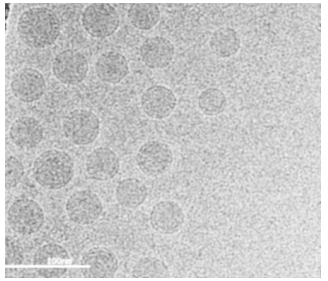
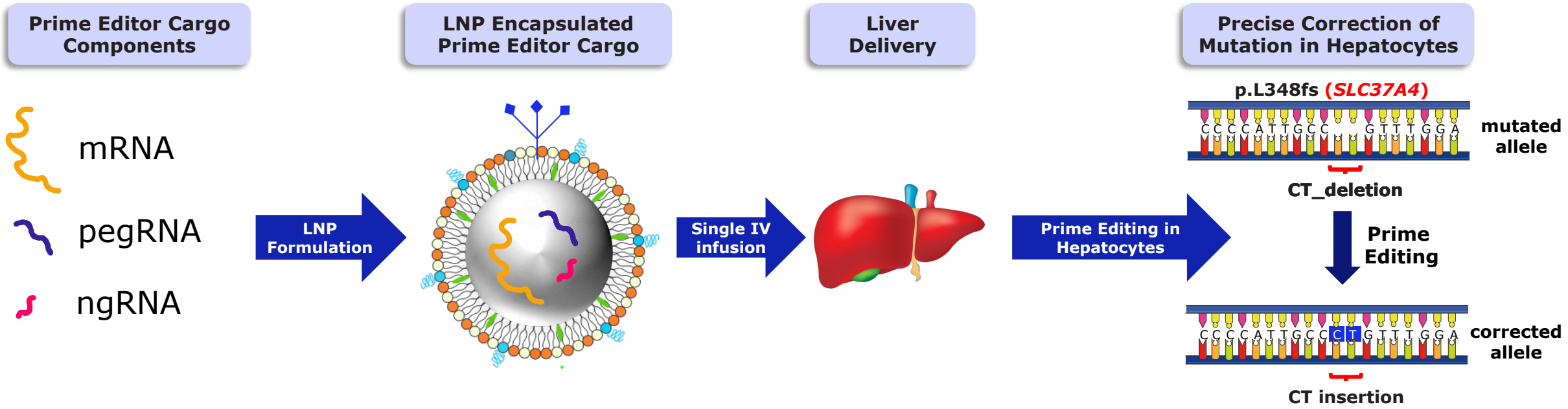


By swapping only the guide RNAs while keeping the other components constant, we have a new product with the potential for the same critical quality attributes

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 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:

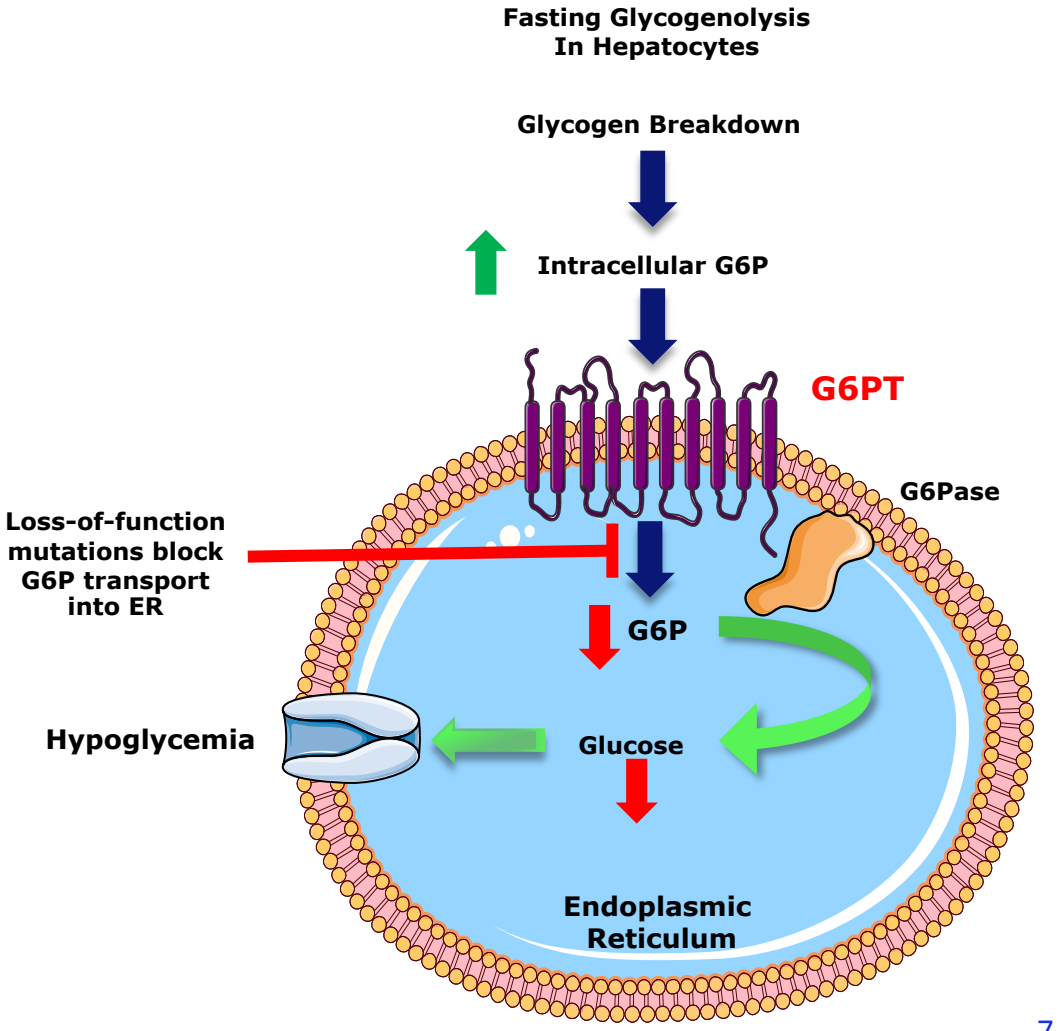
- Autosomal recessive, caused by mutations in the *SLC37A4* gene that encodes G6PT, a glucose-6-phosphate transporter
- ***SLC37A4* p.L348fs and p.G339C mutations** found in ~50% of GSD1b patient population

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

GSD1b program established a roadmap for liver-based Prime Editor Drug Development

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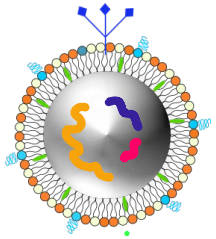
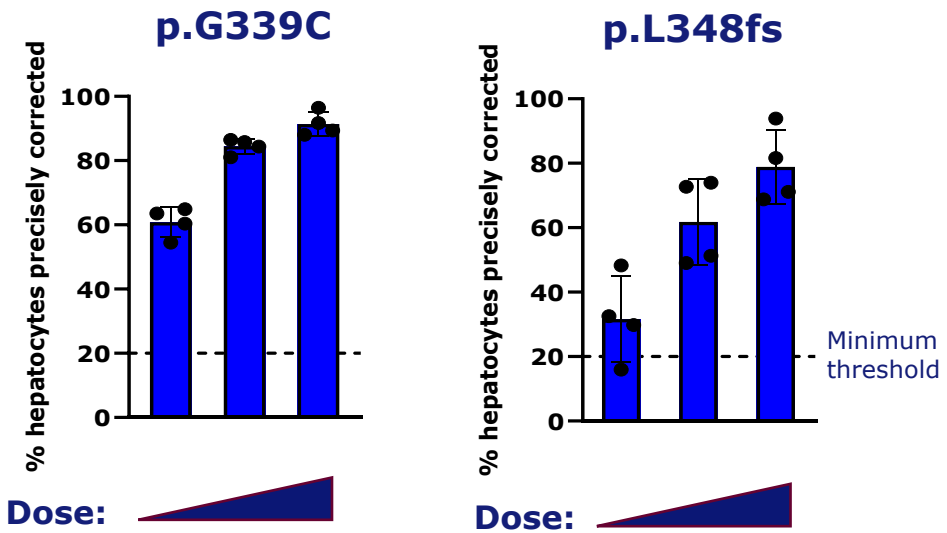
LNP-formulated PE

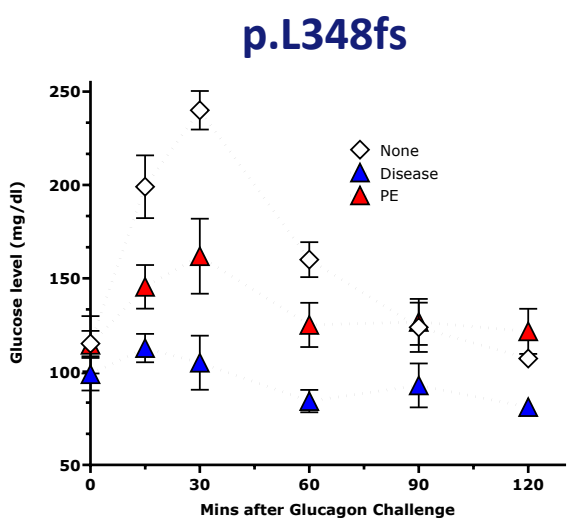


Demonstrated *in vivo* proof of concept for the two most prevalent GSD1b mutations

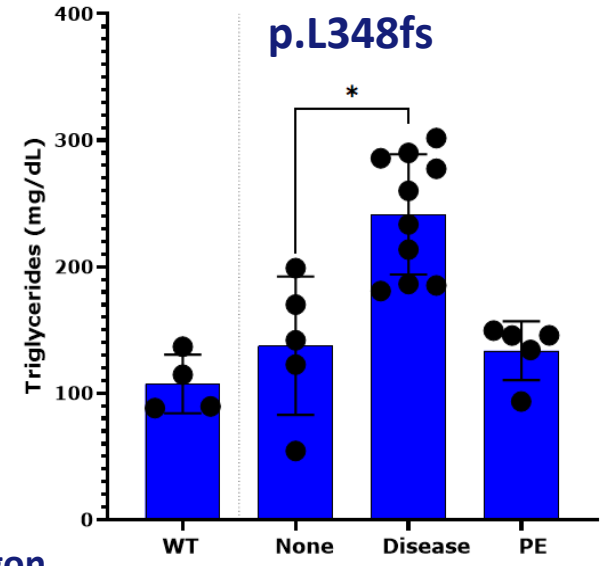
In vivo dose response with lead Prime Editors efficiently edited *SLC37A4* mutations in fully humanized mice



Successful Prime Editing in humanized GSD1b mice resulted in phenotypic rescue



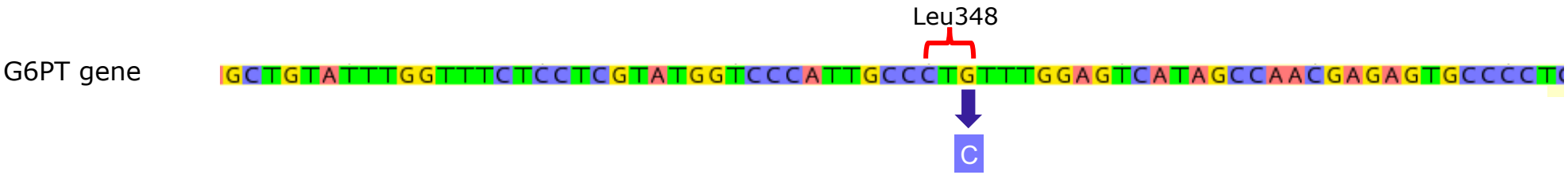
plasma glucose response to glucagon

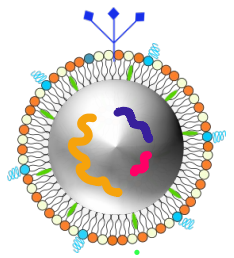


12-hr fasting liver triglycerides

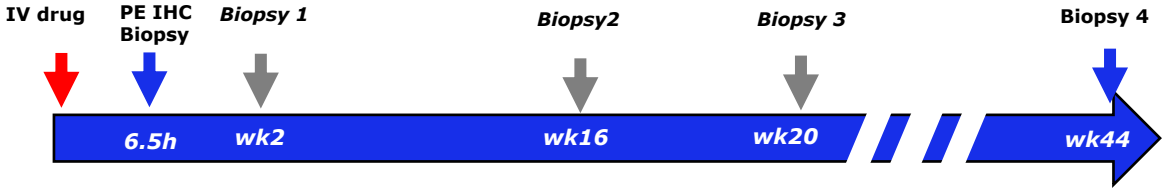
- **Precise *in vivo* correction in humanized** mice for both p.L348fs and p.G339C mutations
- Editing exceeded threshold levels and led to **reduction of fasting hypoglycemia, triglycerides, liver glycogen & restores glucagon responsiveness** in humanized mice at a dose predicted to be clinically relevant for human disease
- **Totality of preclinical data suggests GSD1b editing leads to precise correction and phenotypic rescue of disease at acceptable dose levels**
- **No detectable off-target** activity in preliminary studies

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



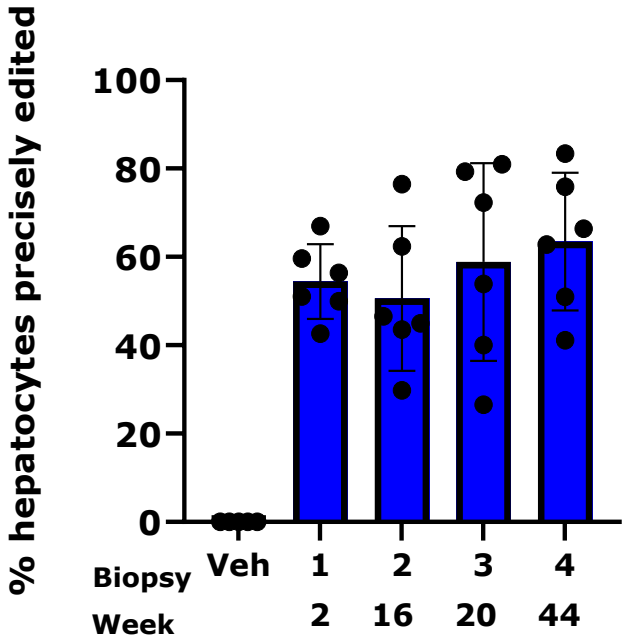


LNP-formulated PE



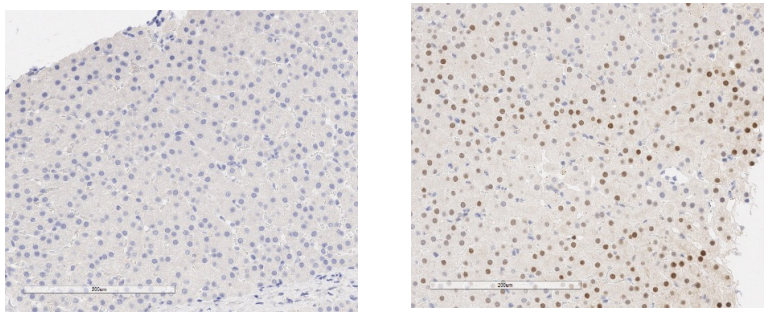
*SLC37A4 prime editing at each biopsy

Prime Editing is durable in NHP liver over 44 weeks



Prime Editor IHC shows robust nuclear localization

6.5 hr biopsy

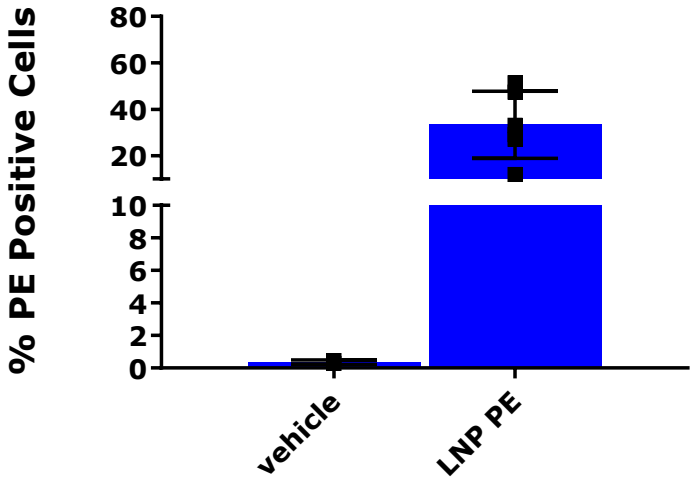


Vehicle (0.38%)

IV LNP PE (35.6%)

Brown nuclei indicate +Prime Editor immunoreactivity

Nuclear Prime Editor detection correlates closely with editing outcomes



*Calculation based on 60% of cells in whole liver are hepatocytes: 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) 9:438. Toxicol (2014) 62:14.12.1; Kmiec, Adv Anat Embryol Cell Biol. (2001) 161:III–XIII. 1–151.

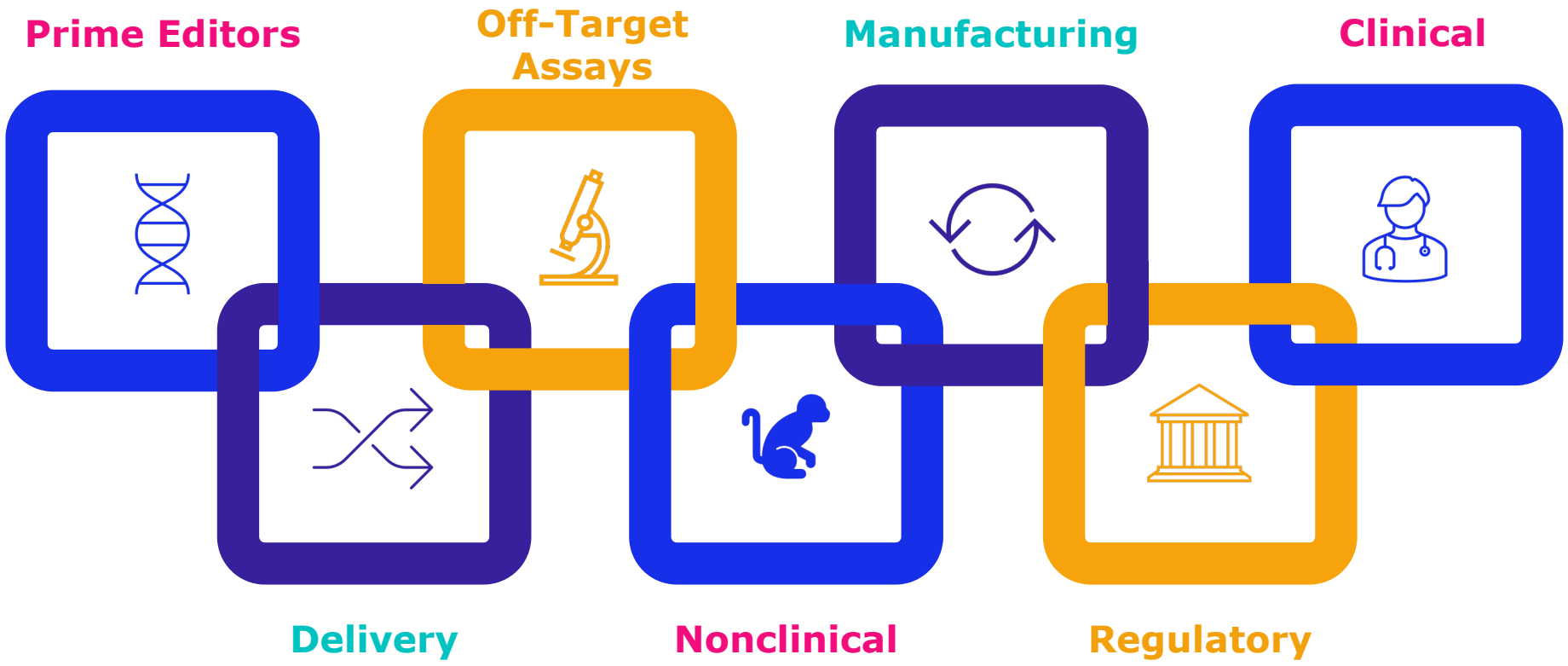
Prime's LNP exhibited an excellent safety profile in cynomolgus monkey (NHP)

- Well-tolerated with no acute reactions, clinical observations, or body weight changes
- Minimal transient LFT elevations
- 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)
- Animals healthy at 44 weeks
- Benchmarked against other LNPs in clinical development

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

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



LNP-RNA Prime Editors to correct common pathogenic mutations causing Wilson's Disease (WD)

Wilson's Disease

Disease severity

- Common liver and systemic disease presenting in teens to 20's (prevalence approx. 1:30,000)
- Leads to liver failure, neurocognitive decline and premature death

Unmet need

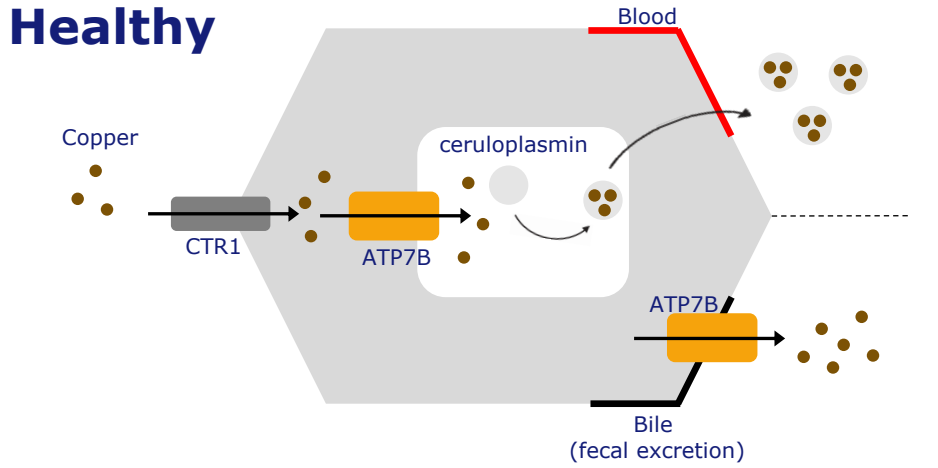
- Many patients die without liver transplant. No approved disease-modifying therapies

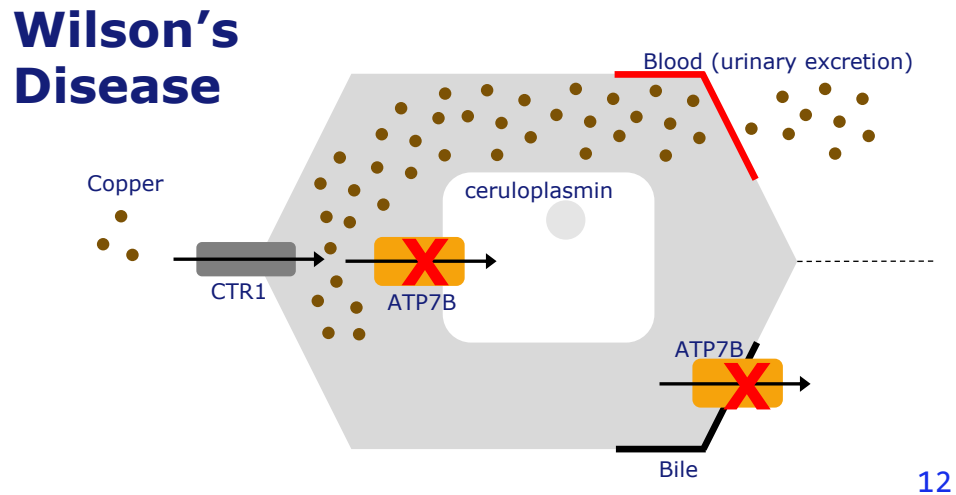
Human biology

- Autosomal recessive due to loss of function mutations in *ATP7B*
- Affects copper homeostasis, leading to toxic accumulation of copper in liver and brain
- H1069Q and R778L are two prevalent mutations found in up to 50% of patients
- Correction of 20-30% of hepatocytes may be curative

Prime Medicine's therapeutic approach

- Prime's universal liver-targeted LNP to deliver RNA Prime Editors to patient liver to correct mutations in ATP7B to restore copper metabolism





Prime Editors demonstrated efficient editing, reached therapeutic levels of mRNA correction and reduced liver copper in humanized Wilson's Disease mouse model

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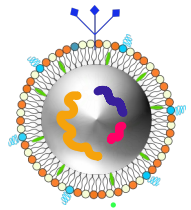
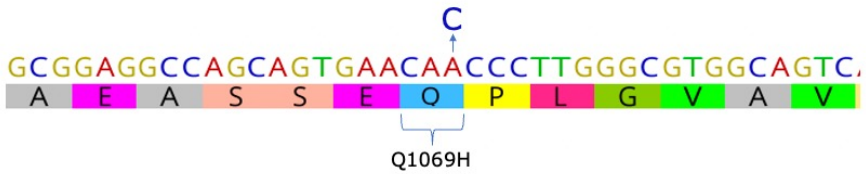
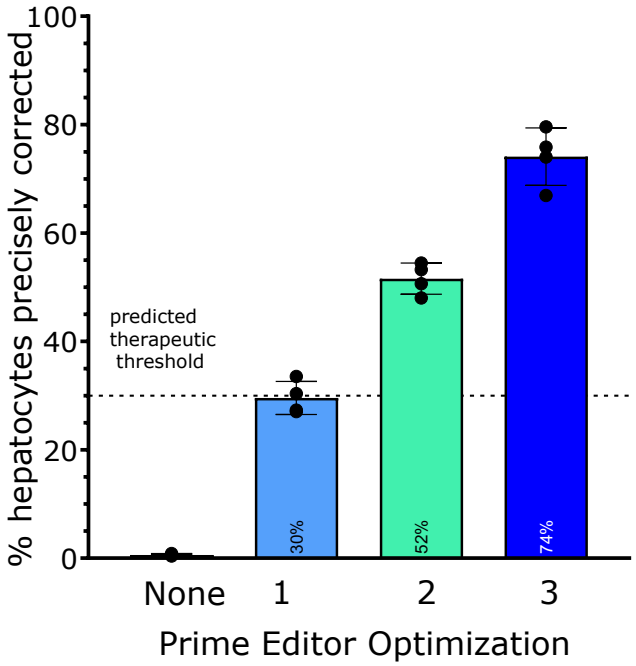


Fully humanized homozygous p.H1069Q *ATP7B* mouse model

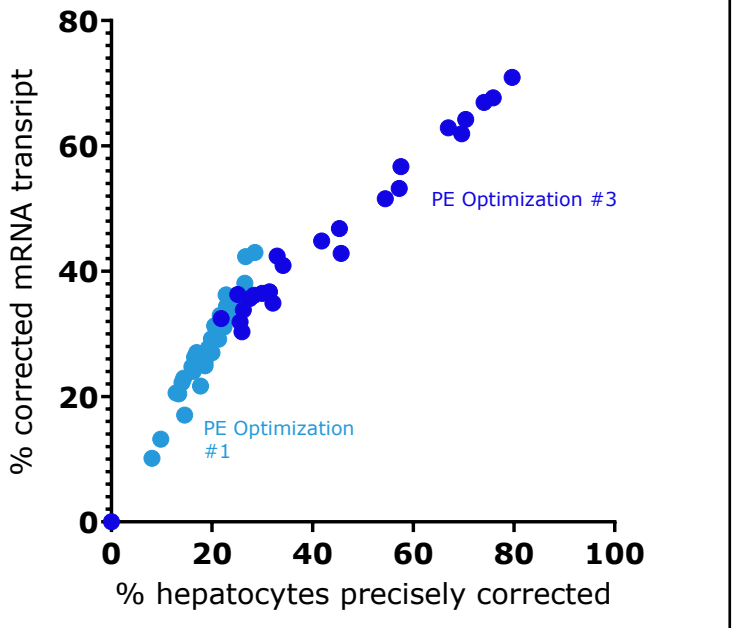


Prime Editors correct the **H1069Q transversion** mutation in humanized mice *in vivo*



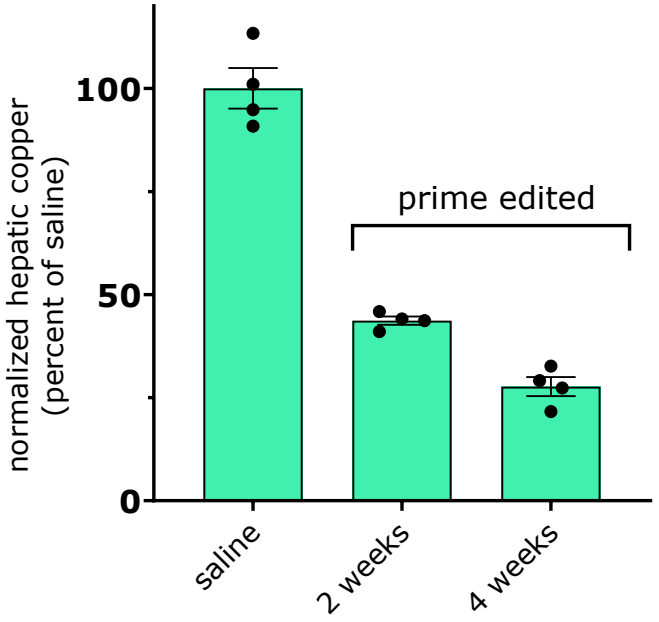
Optimizations to the PE enzyme, gRNAs and mRNA improved Prime Editor editing efficiency

Series of Prime Editors yielded high efficiency of transcript and genome correction in humanized mice *in vivo*



Data generated from series of Prime Editors Optimizations #1 and #3

Prime Editor Optimization #2 yielded a time-dependent 75% reduction in copper accumulation in the liver in humanized mice *in vivo*



Further liver copper studies ongoing with Prime Editor Optimization #3

*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; LNP: lipid nanoparticle, WD: Wilson's Disease, RNA: ribonucleic acid, pegRNA: Prime Editor guide RNA, mRNA: messenger RNA.

Proof of concept for Wilson's Disease H1069 surrogate Prime Editor in NHP using Prime Medicine's Universal LNP

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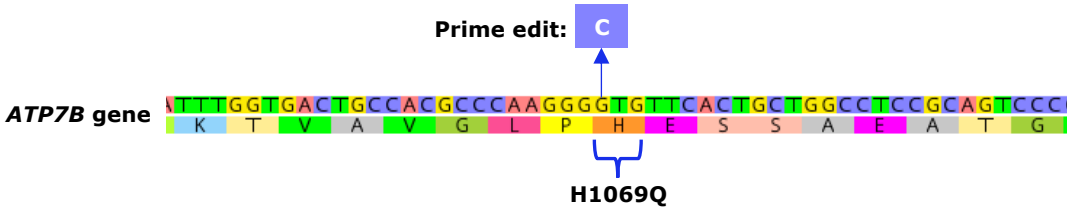
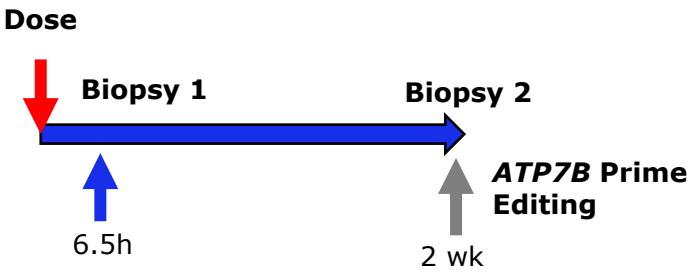
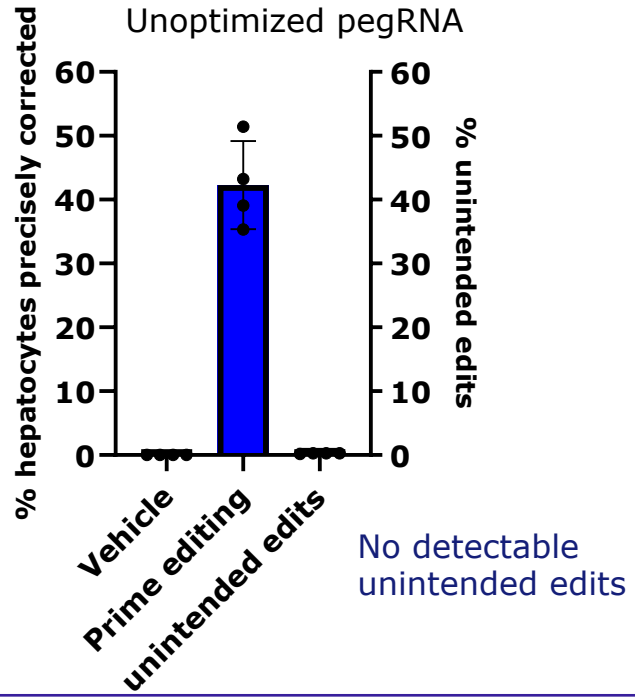
Initial *in vivo* WD NHP studies show up to 51% *ATP7B* p.H1069 precise hepatocyte editing (interim data)



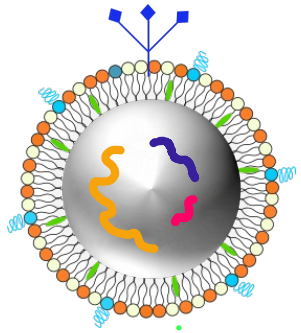


- Excellent *in vitro* to *in vivo* translation
- Further surrogate Prime Editor optimizations ongoing

ATP7B in vivo* Prime Editing in NHP Precise Editing at H1069 with no detectable unintended edits



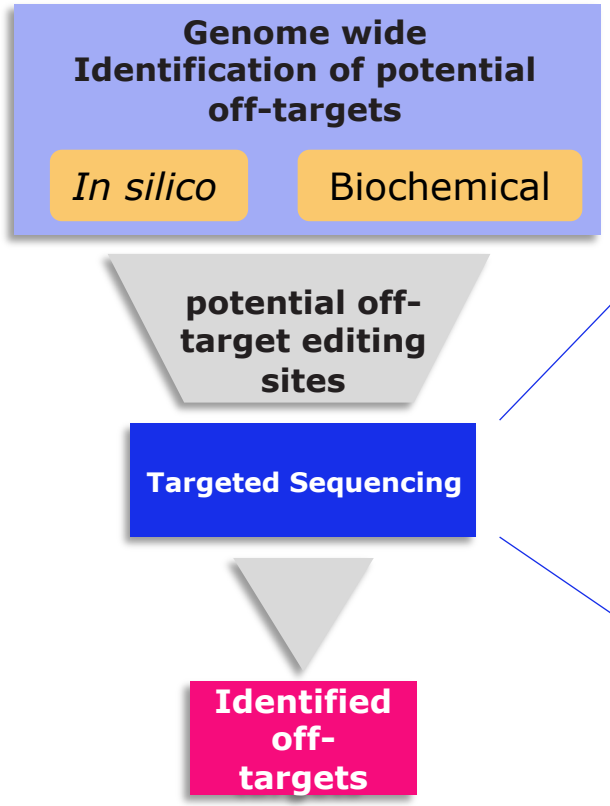
mRNA, pegRNA & ngRNA Formulated in Prime Universal liver LNP

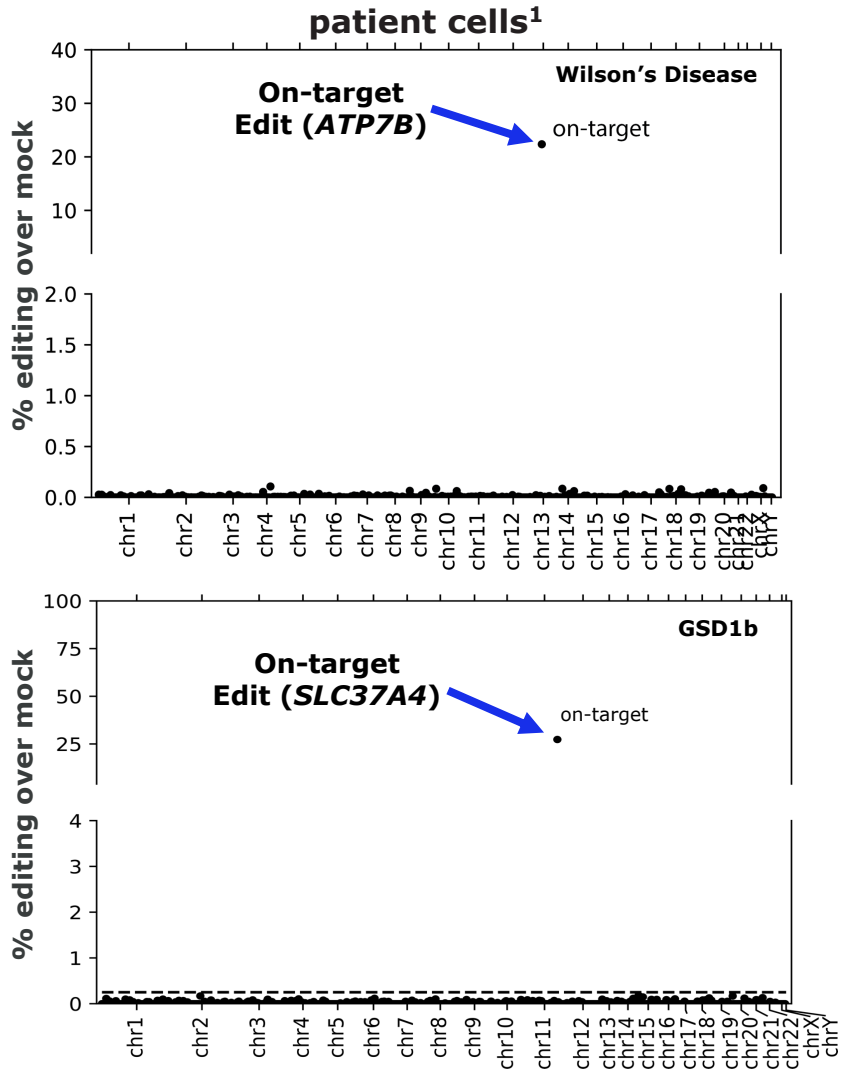


*Based on PK/PD relationships and quantification of cell types in liver: Wang et al Sci. Rep. (2021) 11:19396; MacParland et al Nat Commun. (2018) 9:4383; Hansel et al, Curr Protoc Toxicol (2014) 62:14.12.1; Kmiec, Adv Anat Embryol Cell Biol. (2001) 161:III–XIII. 1–151. ** Calculation based on 60% of cells in whole liver are hepatocytes; # Unintended edits = any SNVs or indels within 300bp either side of the edit site. LNP: lipid nanoparticle, WD: Wilson's Disease, NHP: non-human primate, PE: Prime Editor

Preliminary analysis: no detectable off-target editing in patient cells treated with Wilson's Disease or GSD1b Prime Editors

"IND ready" set of comprehensive off-target assays





[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

Wilson's Disease

- Prime Medicine's Universal LNP-formulated Prime Editors for Wilson's Disease precisely corrects the p.H1069Q mutation, with up to 80% precise correction *in vivo*, restores wild-type mRNA expression and reduces hepatic copper levels in p.H1069Q Wilson's Disease humanized mice at clinically relevant doses
- Results from the initial NHP study demonstrated up to 51% precise hepatocyte editing of *ATP7B* at p.H1069 using an unoptimized surrogate NHP Prime Editor at a dose that was safe and well tolerated

Off-target editing

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

Additional Presentations by Prime Medicine

Poster Topic	ID
Wilson's Disease	P0568
Retinitis Pigmentosa	P0610
Chronic Granulomatous Disease (CGD)	P0575
CAR-T	P0581
Off-target (PEG-seq)	P0617
Platform (knock-knock)	P0578

NHP: nonhuman primate; pegRNA = Prime Editor guide RNA

THANK YOU!

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