prime medicine

Delivering on the promise
of Prime Editing

Abstract 102
LNP-formulated Prime Editor enables *in vivo* therapeutic precise editing of the *ATP7B* p.H1069Q and p.R778L mutations causing Wilson disease

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American Society of Gene & Cell Therapy
May 14th, 2025

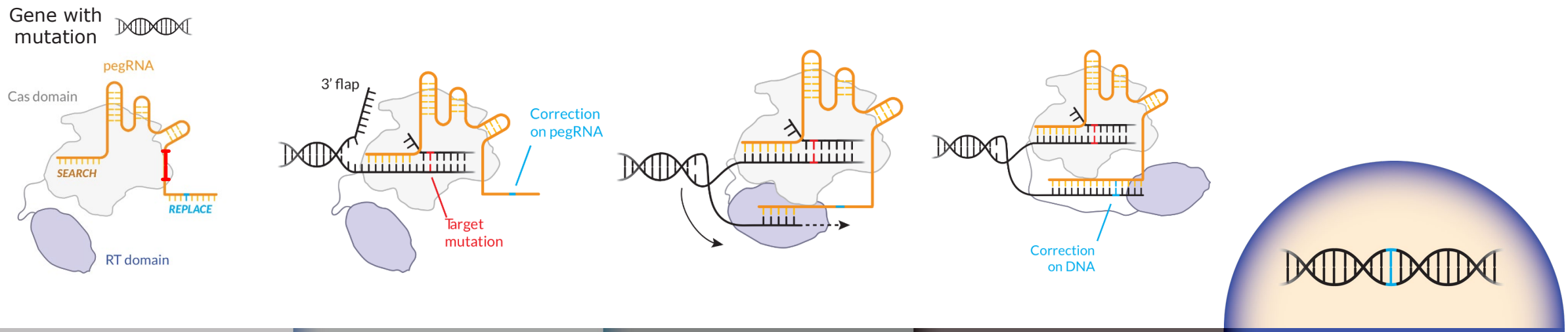
On behalf of Wilson Disease Project Team

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



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

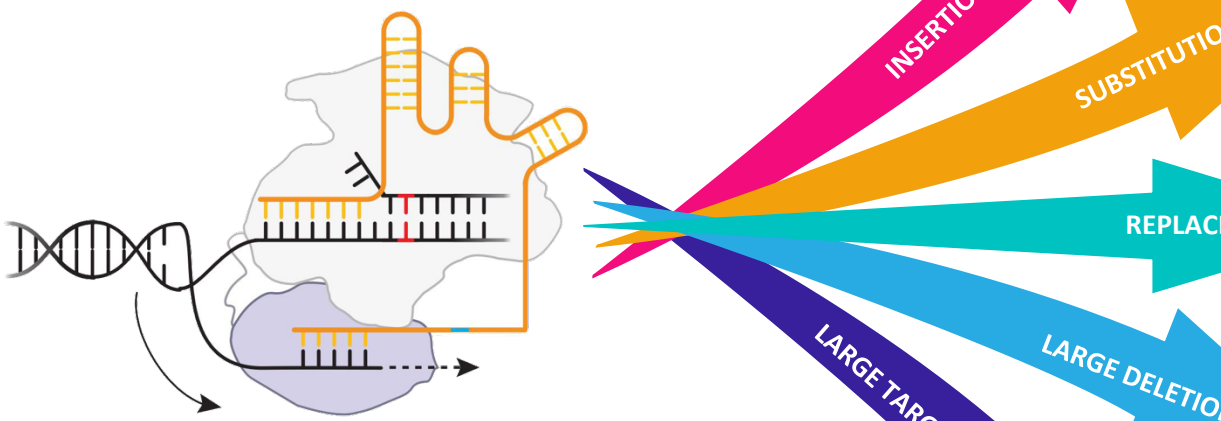
GENE CORRECTED

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

Detailed movie of how Prime Editing works: www.primemedicine.com

[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



Deletion / Insertion Correction
Chronic Granulomatous Disease (CGD)

Point Mutation Correction
Wilson Disease, AATD

Hotspot Correction
Cystic Fibrosis, Retinitis Pigmentosa (RHO adRP)

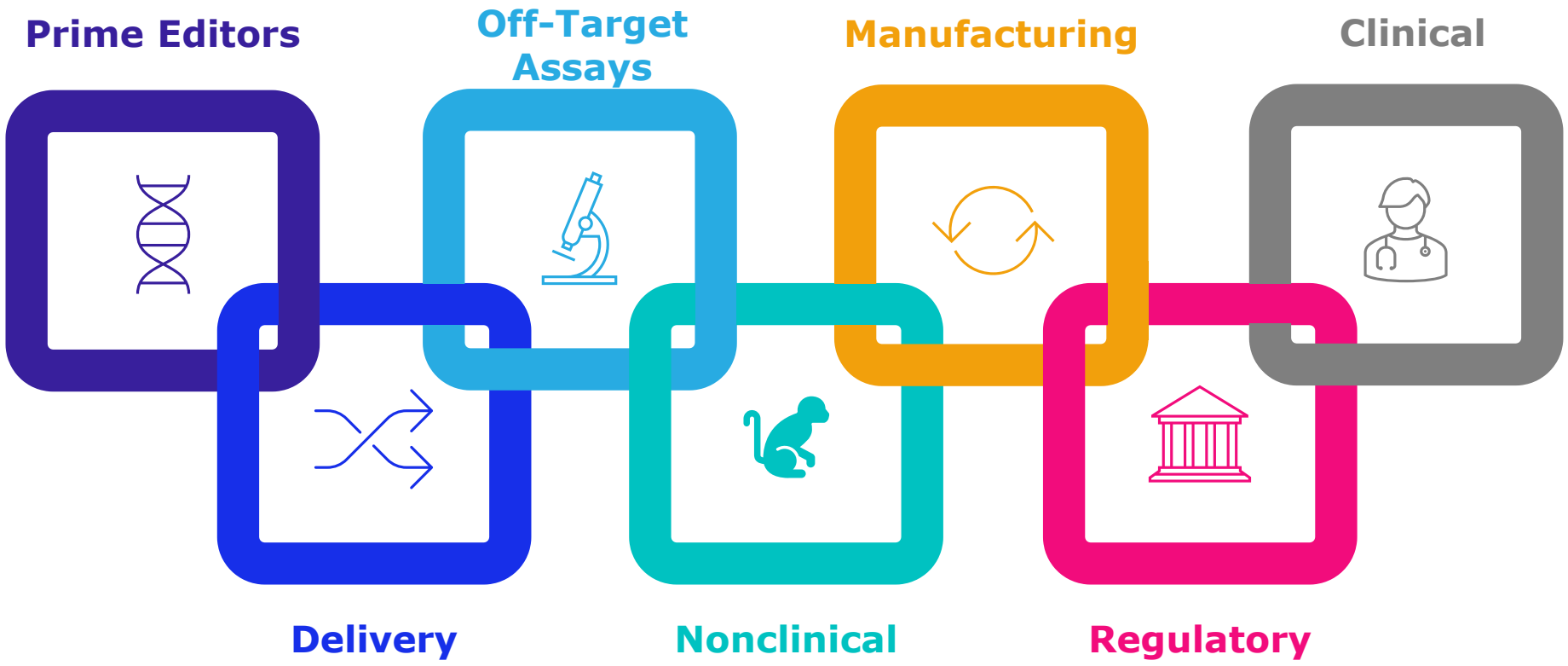
Repeat Excision
Repeat expansion diseases

Targeted Gene Insertion (PASSIGE™)
XCGD, CAR-T, Cystic Fibrosis

Prime Editing is designed with a **wide range of genome editing capabilities** and the **ability to make edits of any size**, from small base pair swaps to large, multi-kilobase inversions or insertions. This provides tremendous flexibility to select the right approach for each indication and editing need.

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

Prime Editing Platform Modularity Accelerates and De-Risks Ongoing Efforts, Enables Rapid Generation of New Product Candidates

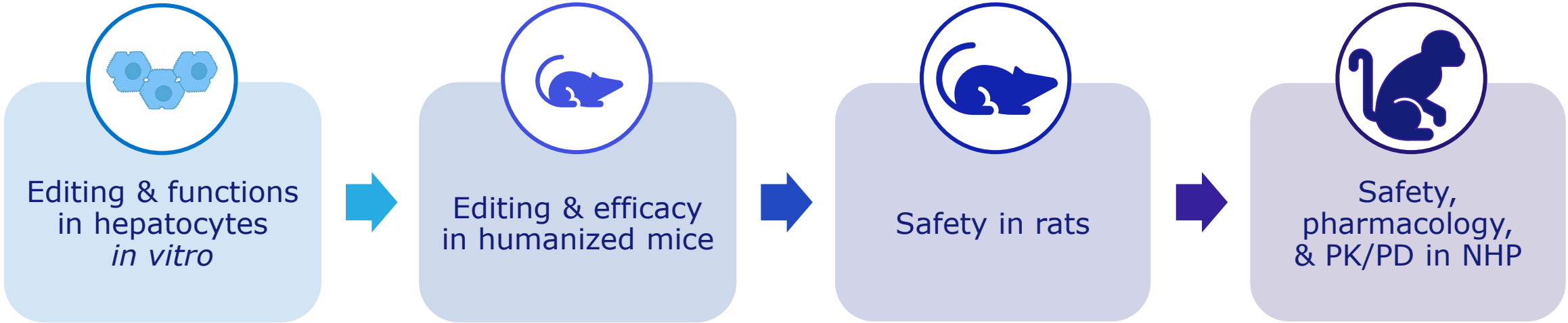


Prime Medicine's approach to developing Prime Editors to treat liver and metabolic diseases

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

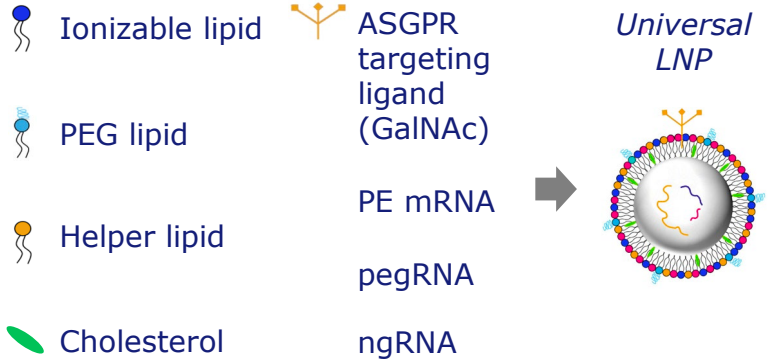
Prime Medicine's approach:

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



Prime Medicine has developed a universal LNP for our liver and metabolic programs

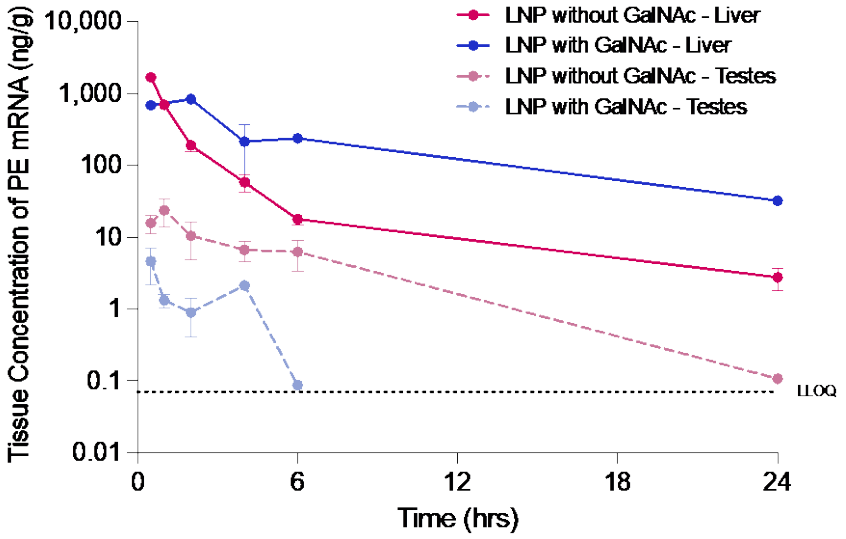
Universal LNP contains a novel GalNAc ligand



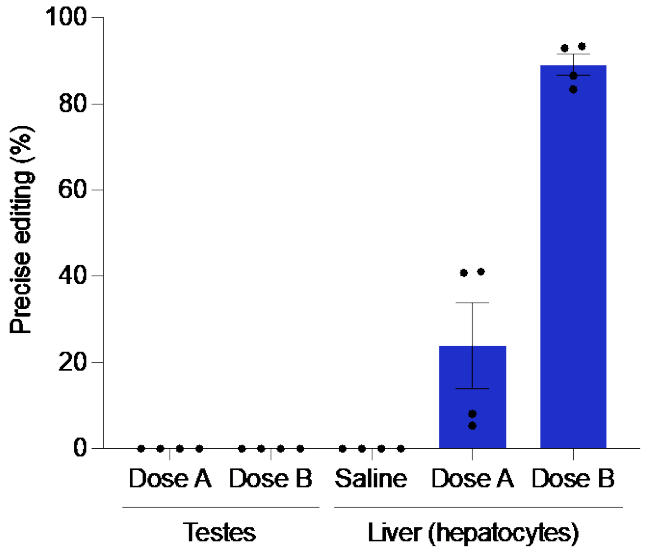
Swapping only the guide RNAs while keeping the other components constant yields a new drug product with the potential for the same critical quality attributes

Universal LNP leads to minimal off-target tissue biodistribution and no detectable editing

mRNA biodistribution in rat



Tissue editing in humanized mice



Prime Medicine's Universal LNP utilizes a liver-targeting ligand to increase dose potency, improve safety, and improve biodistribution*

PEG = polyethylene glycol; GalNAc = N-acetyl galactosamine; ASGPR = Asialoglycoprotein receptor
* Tested in rodent and large animal studies

Hepatocyte correction based on 60% hepatocytes in whole 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, AdvAnat Embryol Cell Biol. (2001) 161:III–XIII. 1–151

LNP-Formulated Prime Editors to correct common pathogenic mutations causing Wilson Disease (WD)

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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

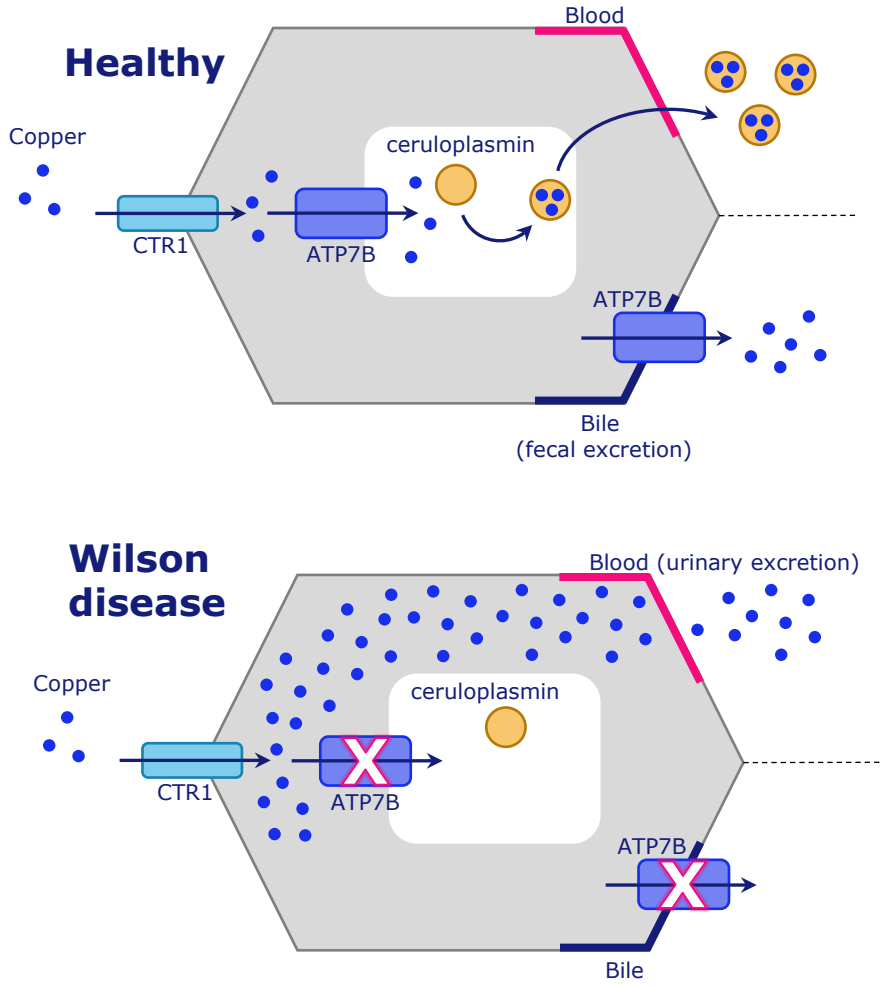
- Many patients die without liver transplant
- No approved disease-modifying therapies

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

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



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

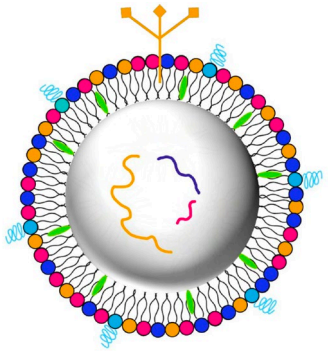
PE mRNA

pegRNA

ngRNA

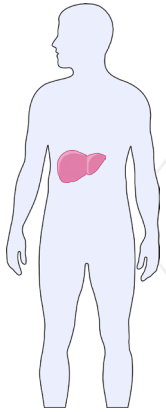
LNP formulation

LNP-encapsulated Prime Editor Cargo



Single IV infusion

Liver Delivery



Precise Correction of Wilson disease Mutation in Hepatocytes

p.H1069Q

p.R778L

Prime Editing

Prime Editing

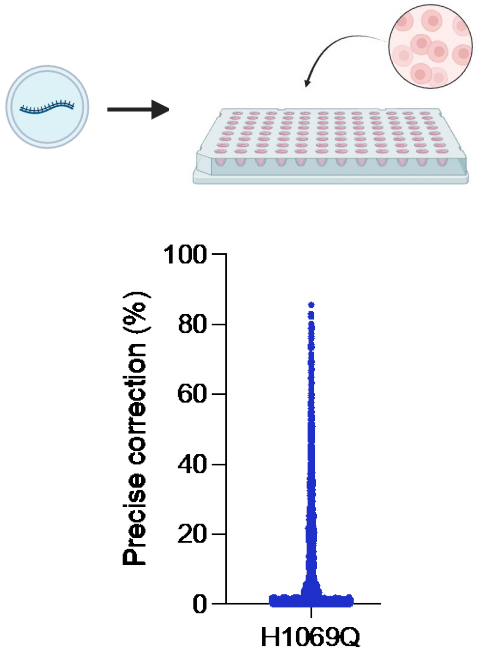
p.H1069

p.R778

Identification of lead Prime Editors for correction of *ATP7B* p.H1069Q mutation

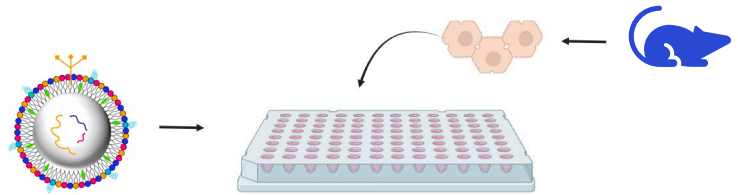
High throughput screening in hepatocyte cell line

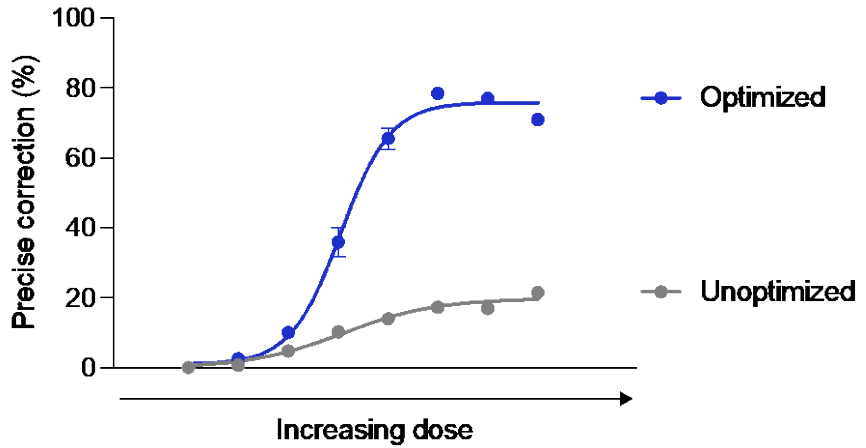
Delivery of RNA Prime Editing components

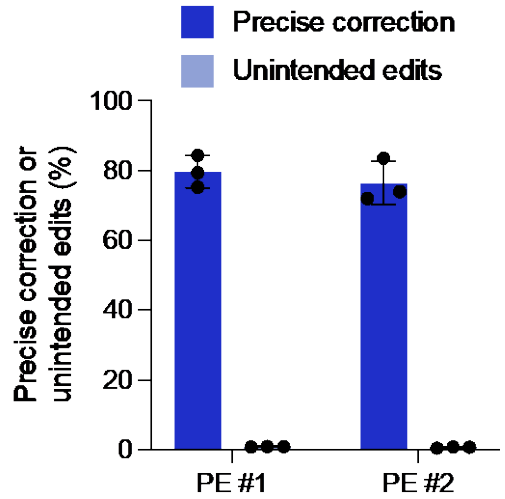


Validation of Prime Editors in primary mouse hepatocytes from humanized mouse model

LNP delivery of RNA Prime Editing components







Prime Medicine's high throughput screening platform identifies multiple Prime Editors capable of efficient correction of H1069Q and R778L in hepatocyte cell lines and primary mouse hepatocytes isolated from WD humanized mice

Prime editing restores ceruloplasmin abundance in patient-derived H1069Q iHeps *in vitro*

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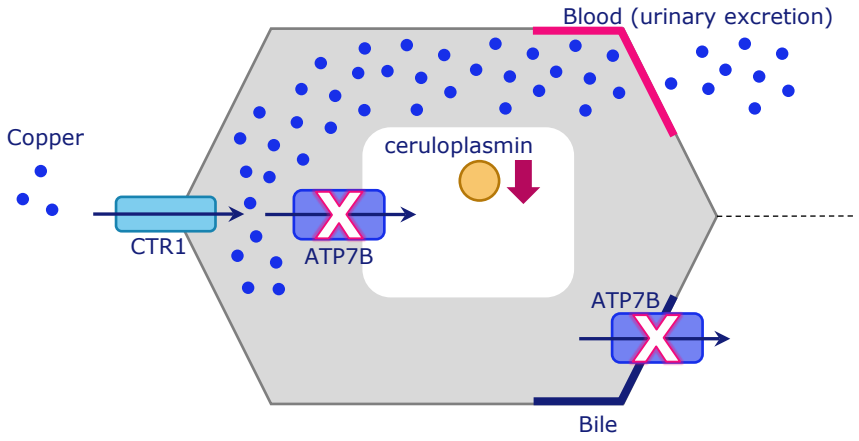
Ceruloplasmin reduction in WD hepatocytes

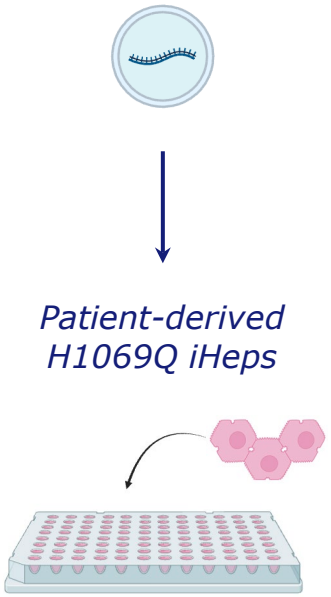


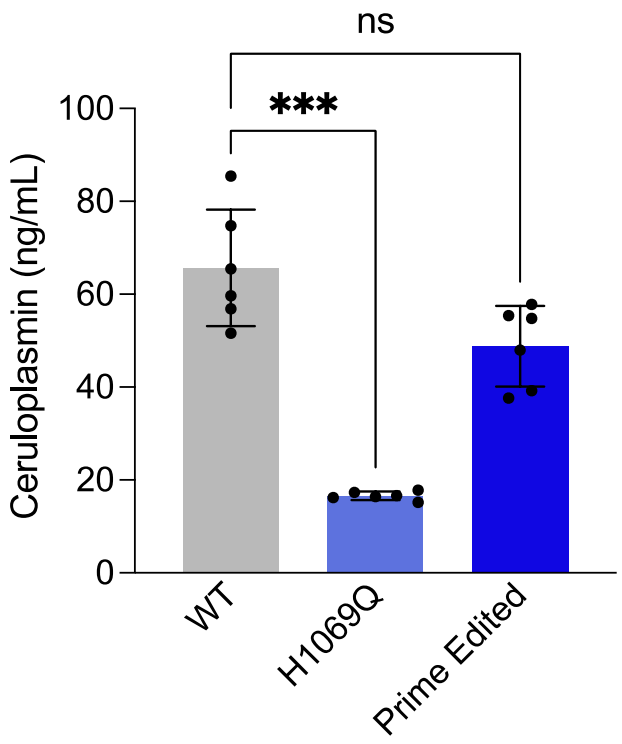
Failure to transport copper in WD reduces ceruloplasmin protein abundance

Restoration of ceruloplasmin levels *in vitro*

RNA Prime Editing components

Patient-derived H1069Q iHeps





Prime Editing in WD patient-derived induced hepatocytes restores ceruloplasmin abundance to levels similar to WT

iHep = human-induced pluripotent stem cell-derived hepatocytes

Prime Editor optimizations enable durable DNA and mRNA correction in a fully humanized *ATP7B* p.H1069Q WD mouse model

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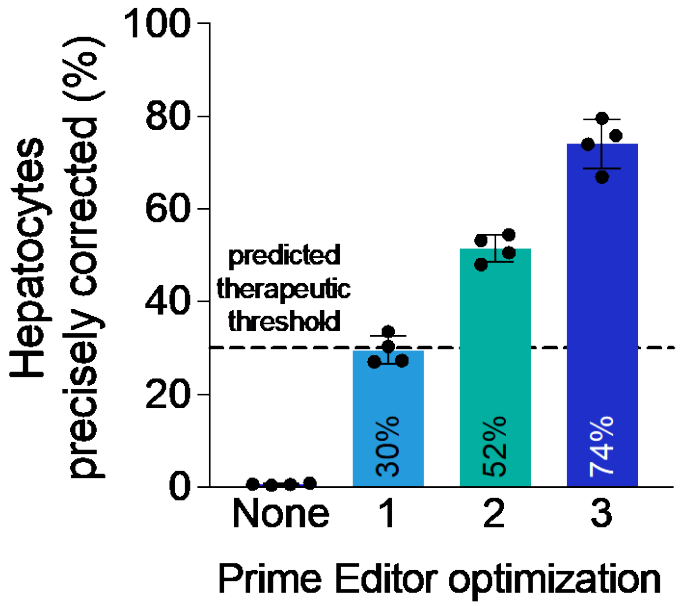
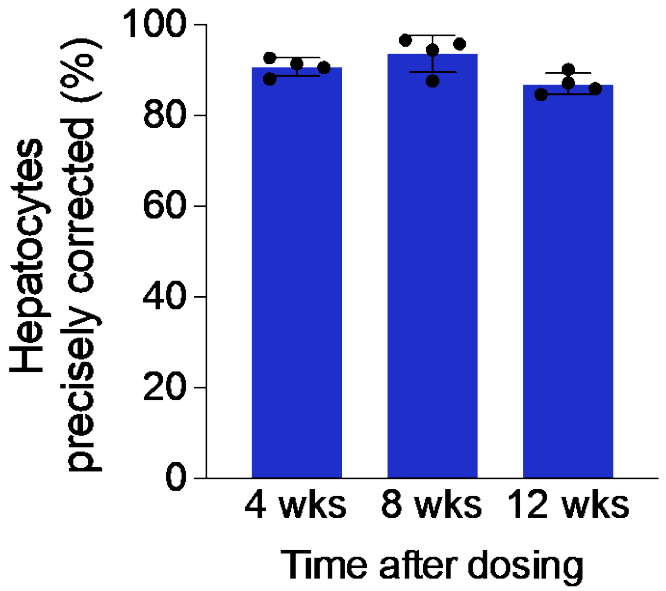
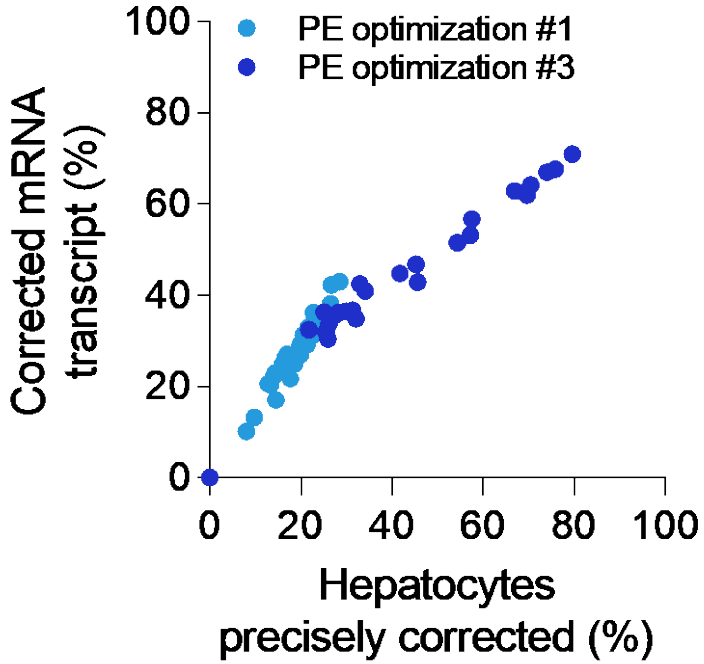
H1069Q correction in WD mouse model



Durability of H1069Q correction over time



Genome and transcript correction of H1069Q



Prime Editors demonstrate efficient and durable *in vivo* correction of *ATP7B* H1069Q in humanized WD mouse model

Hepatocyte correction based on 60% hepatocytes in whole 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, AdvAnat Embryol Cell Biol. (2001) 161:III–XIII. 1–151

Further Prime Editor optimizations substantially improve potency in humanized mice

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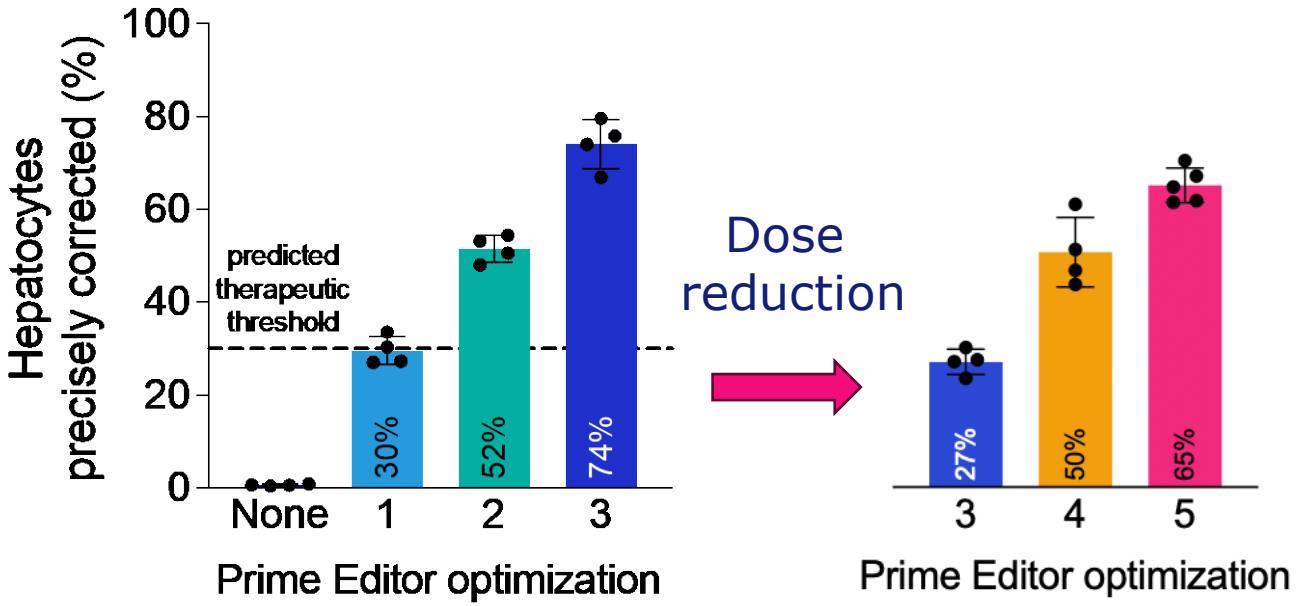
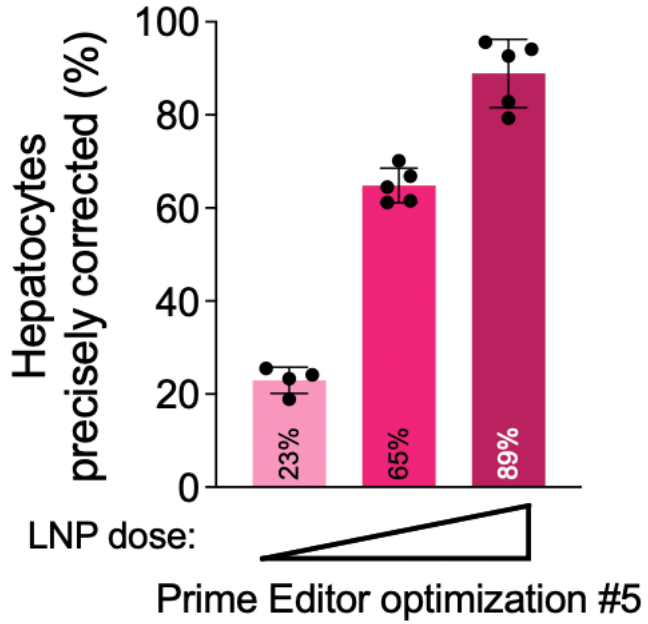
Improved Prime Editors enable therapeutic editing at low dose in WD mouse model



Dose level: **1X**

Dose level: **1/3X**

Optimized Prime Editor dose response



Improved Prime Editors demonstrate efficient *in vivo* correction at low dose of *ATP7B* p.H1069Q in humanized WD mouse model

Hepatocyte correction based on 60% hepatocytes in whole 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, AdvAnat Embryol Cell Biol. (2001) 161:III–XIII. 1–151

Copper homeostasis is restored in humanized WD mouse model following H1069Q correction by Prime Editing

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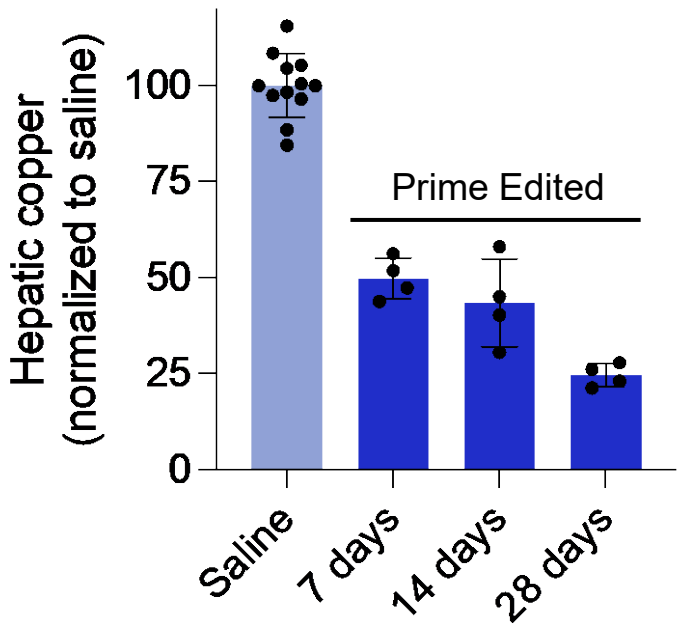
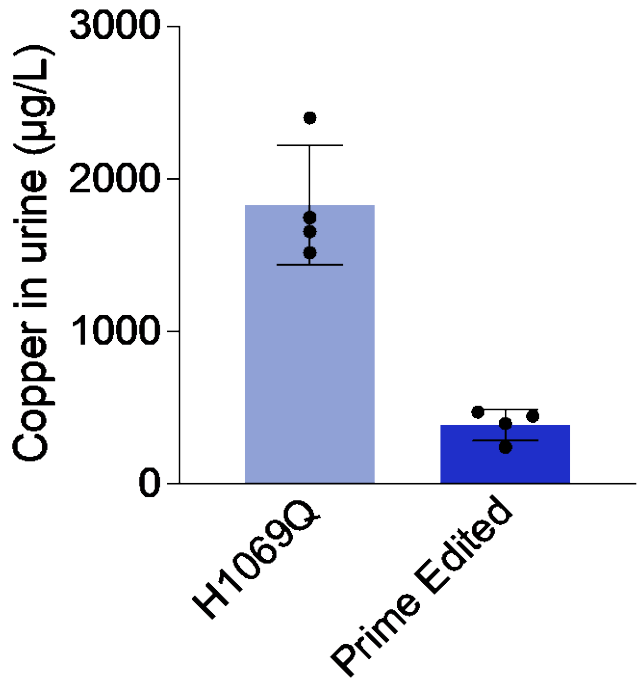
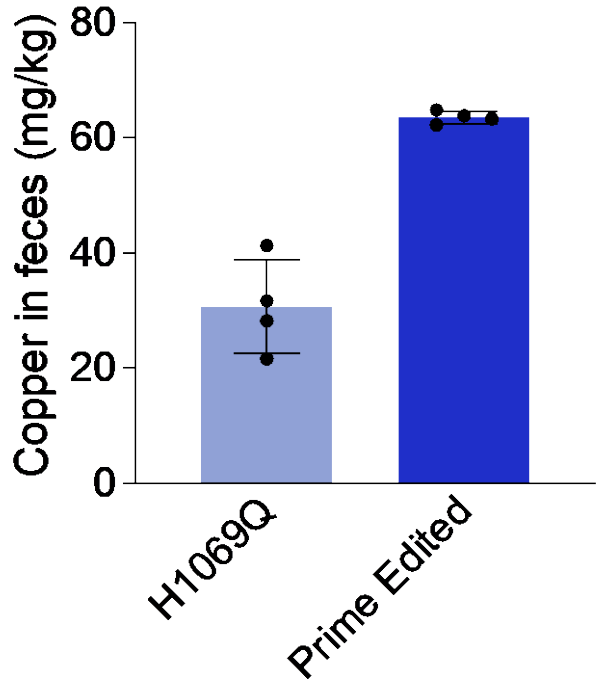
Liver copper



Urinary copper



Fecal copper



Correction of H1069Q in WD mouse model reduces liver copper by >75% and restores copper excretion from urine to feces

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

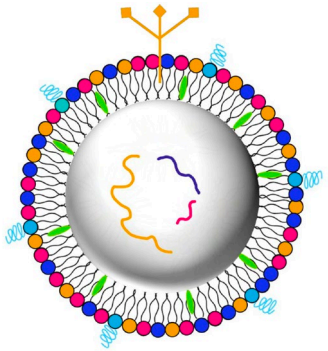
PE mRNA

pegRNA

ngRNA

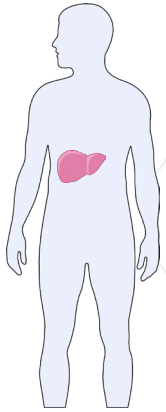
LNP formulation

LNP-encapsulated Prime Editor Cargo

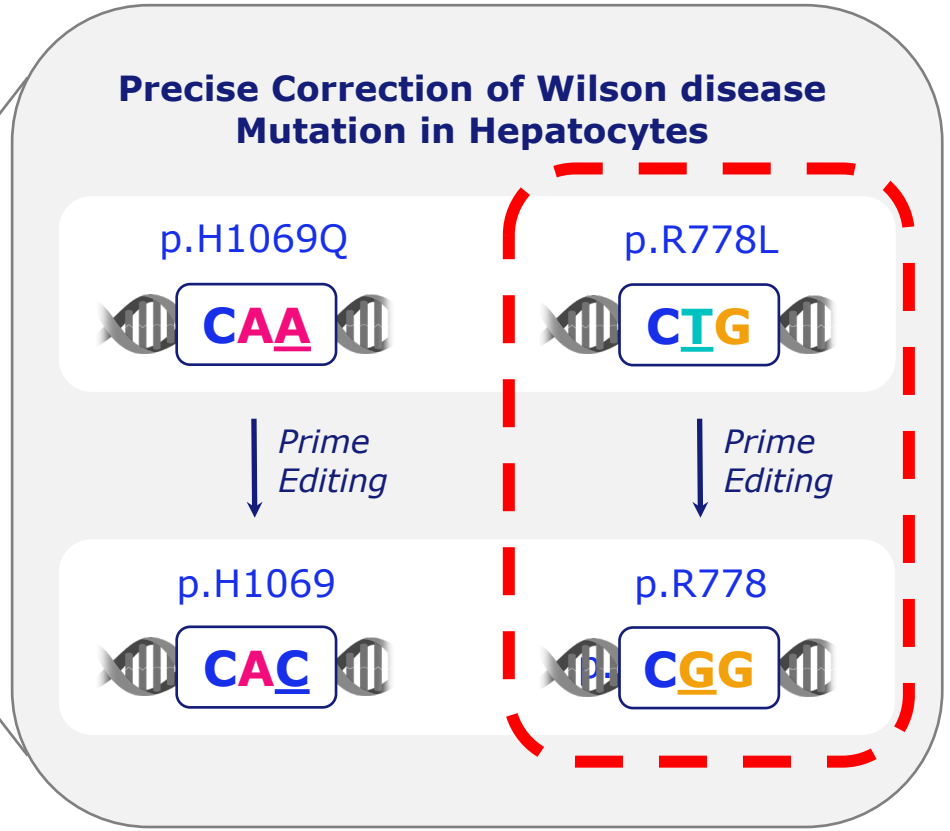


Single IV infusion

Liver Delivery



Precise Correction of Wilson disease Mutation in Hepatocytes



Multiple Prime Editors demonstrate efficient *ATP7B* p.R778L correction *in vitro* and *in vivo*

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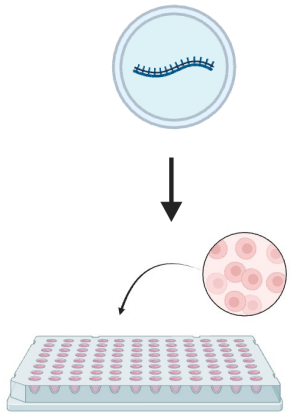
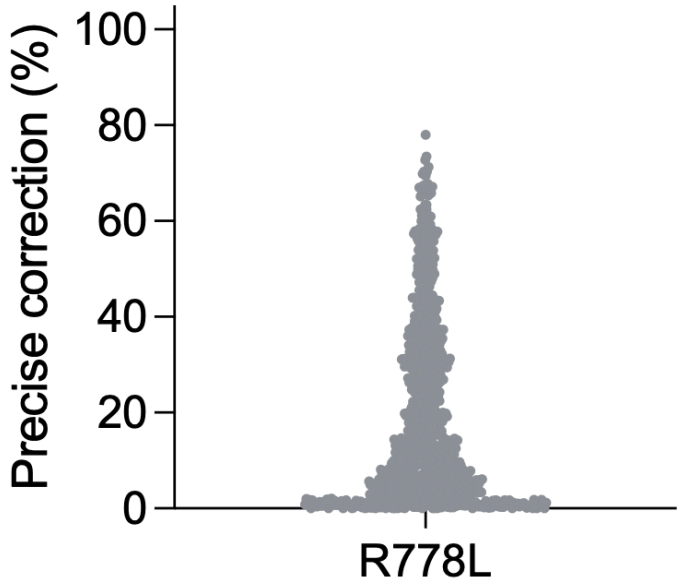
Correction of R778L *in vitro* in immortalized hepatocyte-like cells

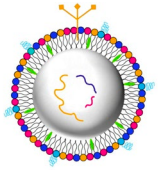
Lipofection of RNA Prime Editing components



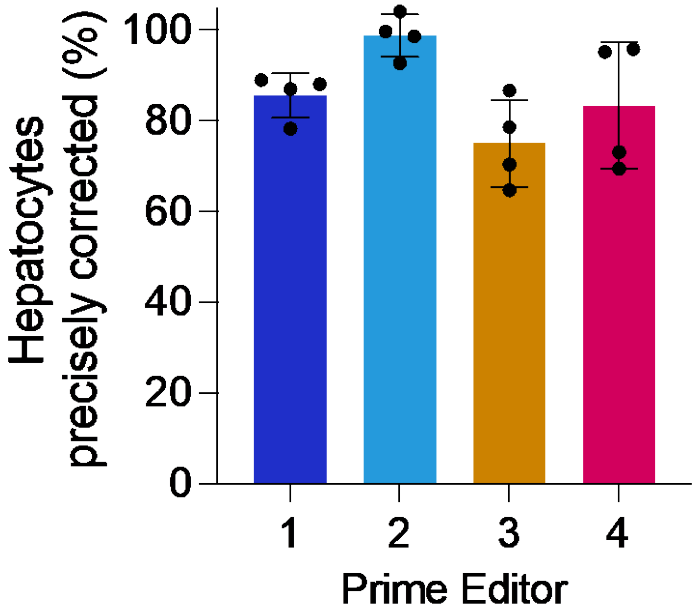


Correction of R778L *in vivo* with LNP delivery to humanized mouse model

LNP delivery of RNA Prime Editing components



Humanized R778L mouse model



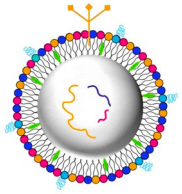
Prime Editors efficiently correct the R778L mutation in humanized mouse model

Hepatocyte correction based on 60% hepatocytes in whole 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, AdvAnat Embryol Cell Biol. (2001) 161:III–XIII. 1–151

Proof of concept for Wilson Disease H1069 using Prime Medicine's Universal LNP-formulated NHP surrogate Prime Editors following single administration

Prime Editing of *ATP7B* H1069 in NHP using unoptimized surrogate

PE RNA encapsulated in universal LNP



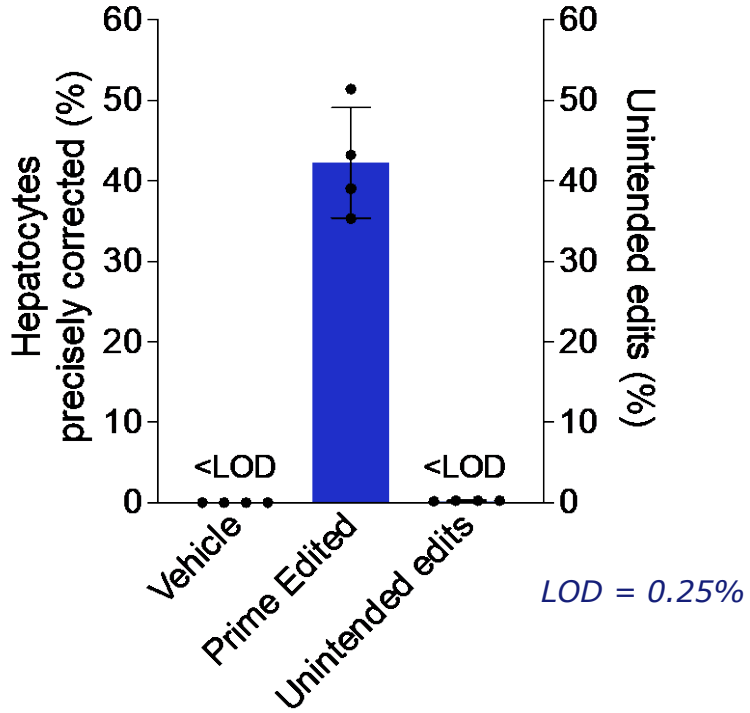
Single IV injection

Cynomolgus macaque

Surrogate Prime Edit at *ATP7B* p.H1069

Prime Editing

Precise *in vivo* NHP editing at *ATP7B** with no detectable unintended edits



LOD = 0.25%

Interim data on WD NHP studies show up to 51% *ATP7B* p.H1069 precise hepatocyte editing

*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; LOD = limit of detection

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

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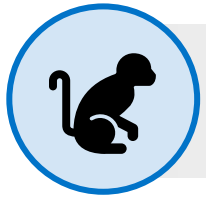
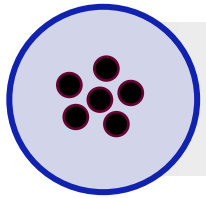
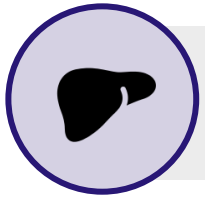


- Well-tolerated with no acute reactions, clinical observations, or body weight changes
- Animals healthy at 54 weeks

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



- Minimal changes in serum IL-6 levels
- No other observed cytokine changes



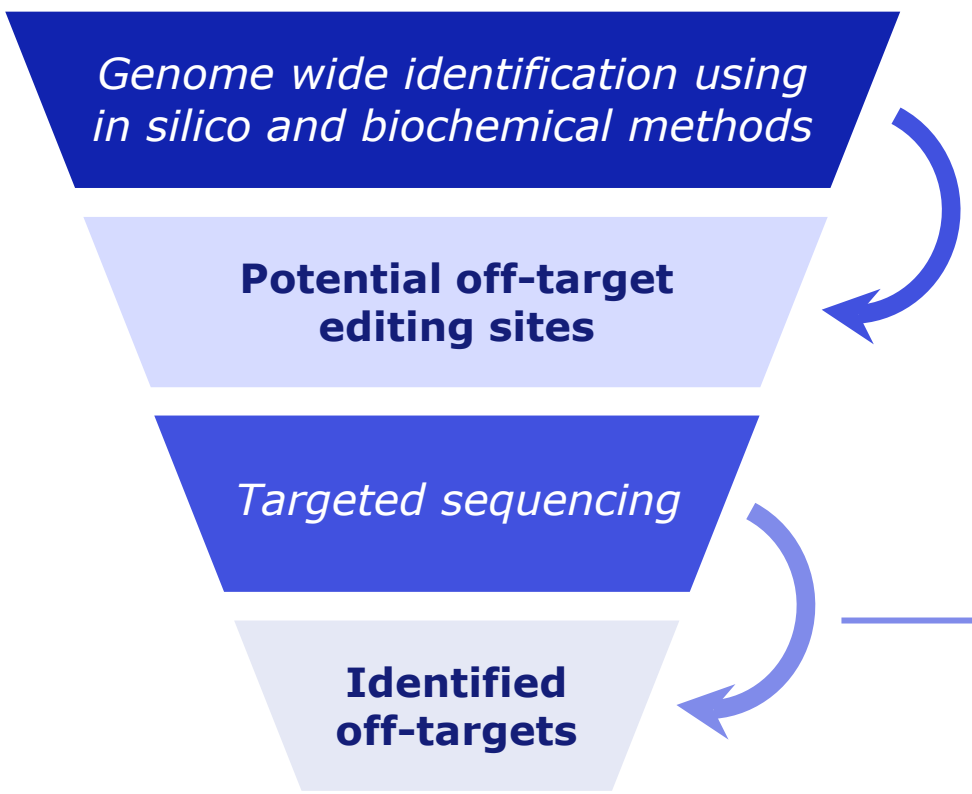
- No changes observed in liver histopathology (H&E)
- Minimal transient LFT elevations

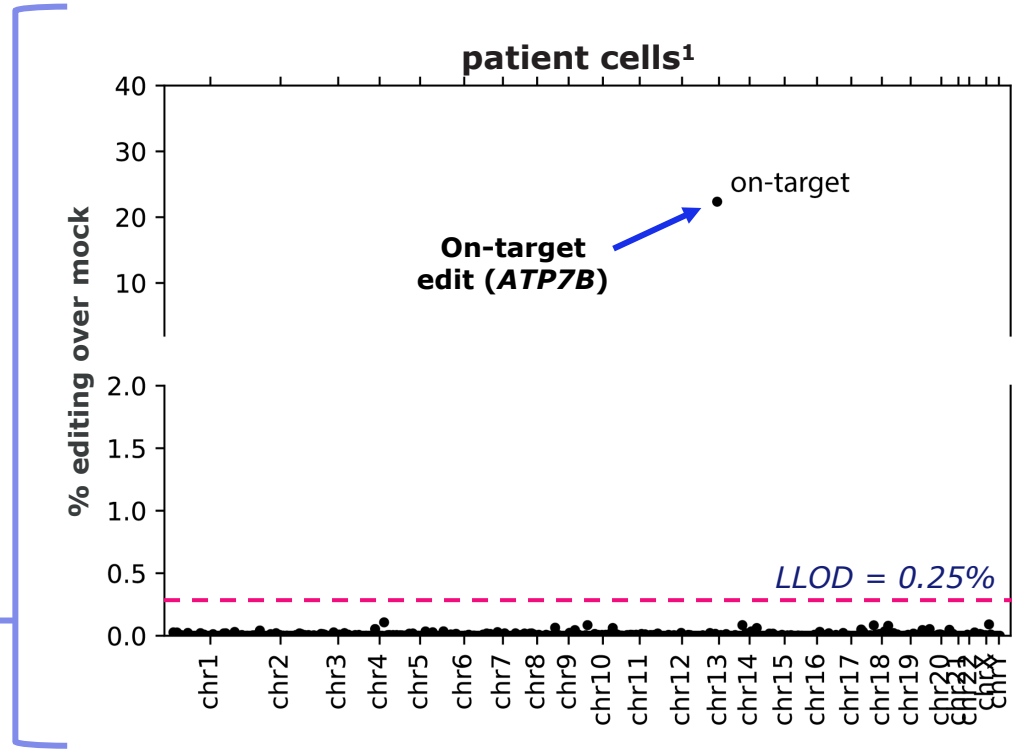
Benchmarked against other LNPs in clinical development

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 Wilson Disease

"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. ATP7B = ATPase copper transporting beta; LLOD = lower limit of detection

Summary

LNP-formulated Prime Editor enables *in vivo* therapeutic precise editing of the *ATP7B* p.H1069Q and p.R778L mutations causing Wilson disease

Modular LNP platform

- Prime Medicine 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 and rodent studies

Wilson Disease

- Prime Medicine's Universal LNP-formulated Prime Editors for Wilson disease precisely correct the p.H1069Q mutation, with up to 80% precise correction *in vivo*, restore wild-type mRNA expression, and reduce hepatic copper levels in p.H1069Q Wilson 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 Wilson disease patients

Wilson disease program on track for IND and/or CTA filing in H1 2026

NHP = non-human primate

prime medicine_

Delivering on the promise
of Prime Editing

Thank you!

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