prime medicine

Delivering on the promise
of Prime Editing

Advances in Prime Editing enable *in vivo* therapeutic correction of the *ATP7B* p.H1069Q and p.R778L mutations causing Wilson's Disease

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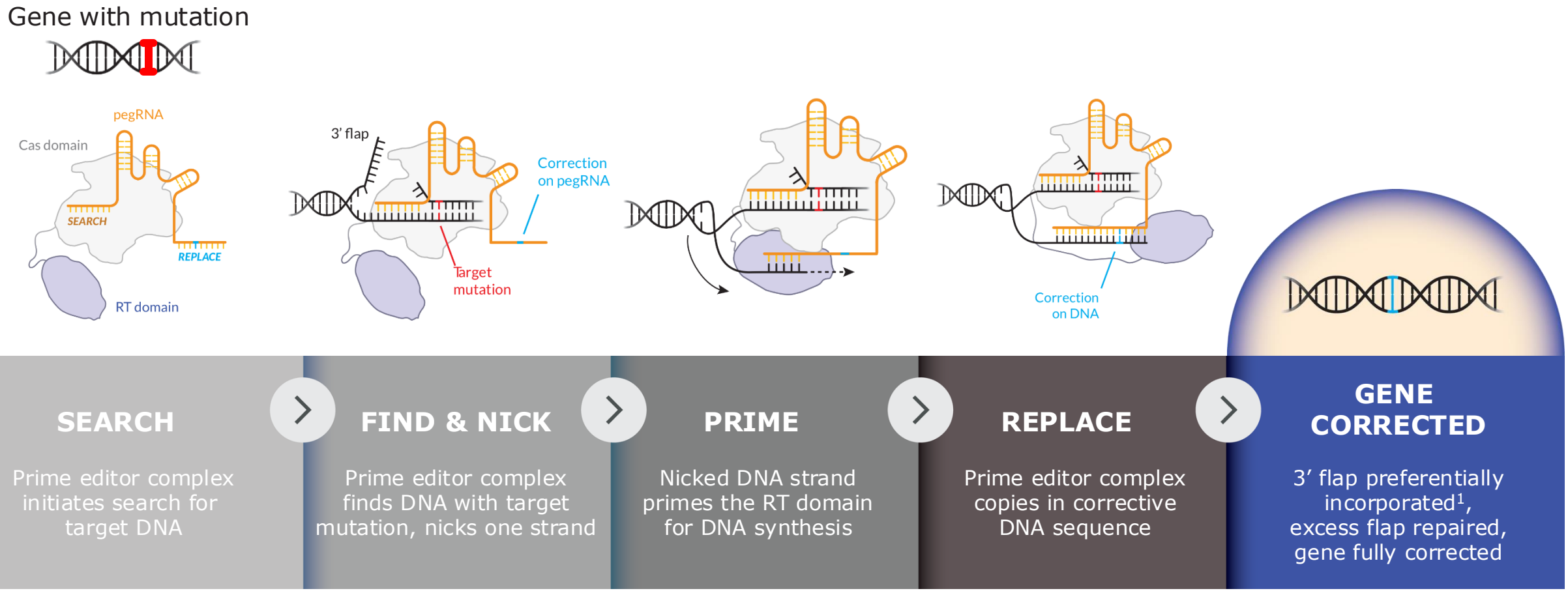
On behalf of the team at Prime Medicine

Disclosures

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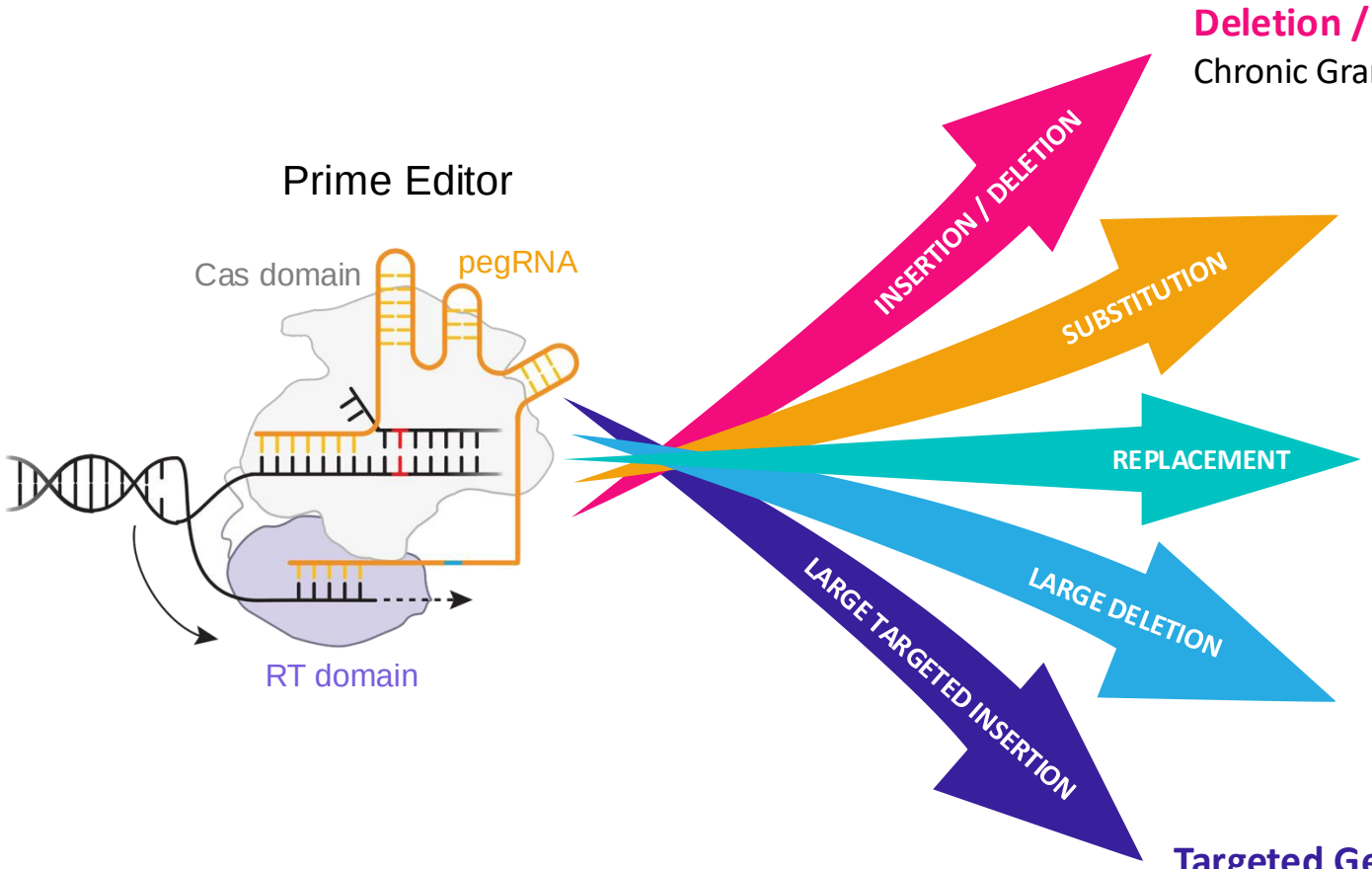
Corrects mutations across many tissues, organs and cell types, in dividing and non-dividing human cells



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

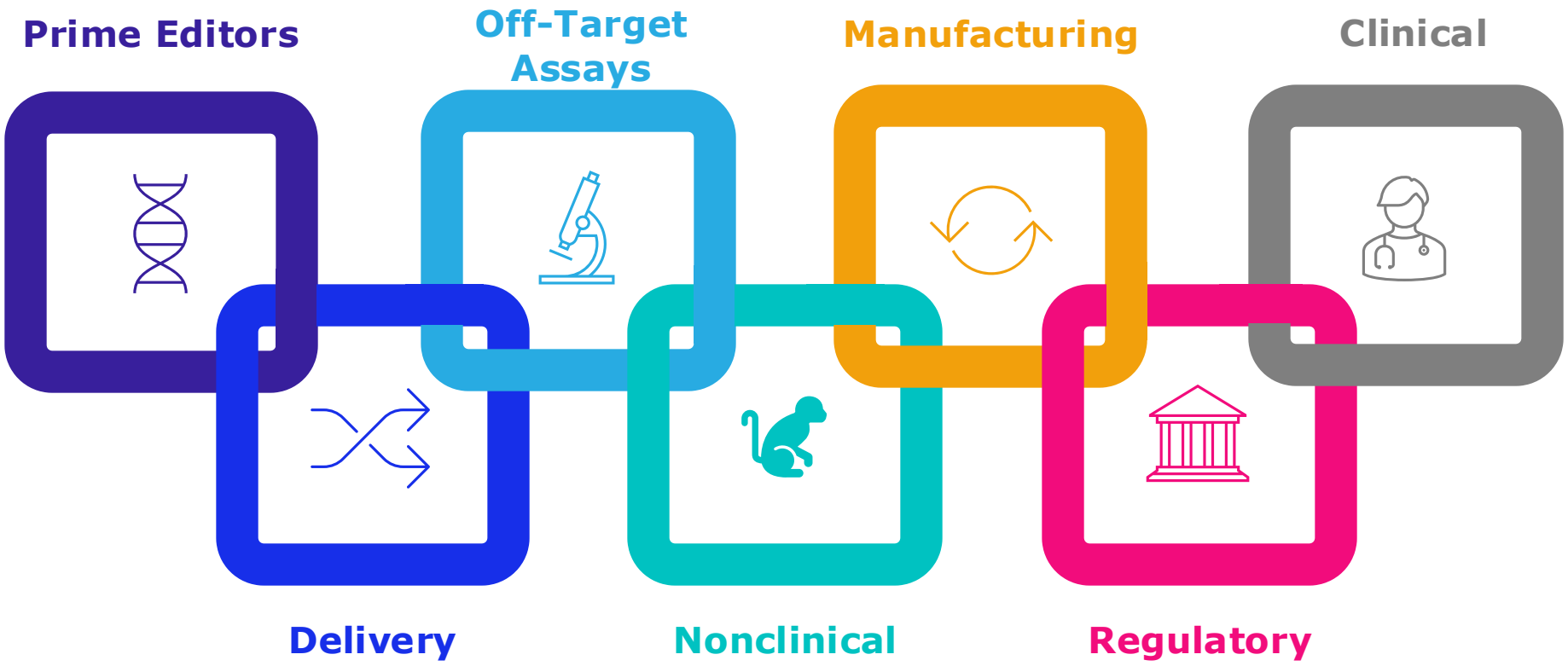
Broad and versatile editing capabilities unlock opportunities across **thousands of indications,** including genetic diseases, infectious diseases, cancers and immunological diseases

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

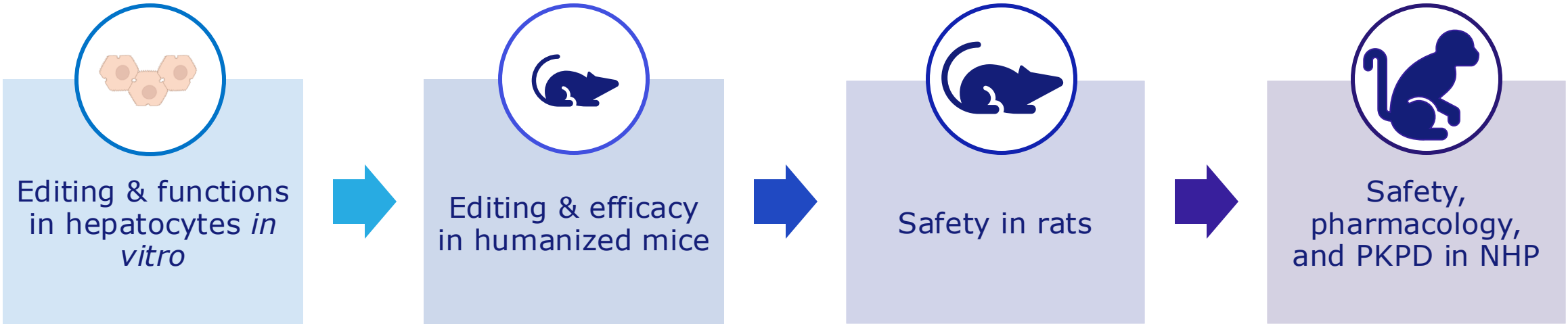
Prime Editing platform modularity accelerates and de-risks ongoing efforts, enabling rapid generation of new product candidates

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

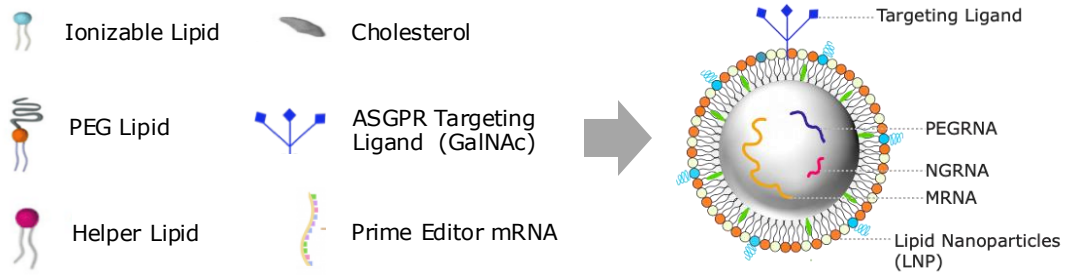


- Establish potency of lead Prime Editor drug candidates
- Establish genotype to phenotype correlation and off-target profile
- Establish pharmacology, safety, tolerability
- Determine biodistribution, drug pharmacokinetics
- Determine PK/PD relationships, human dose projections

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

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

Shared LNP/PE components

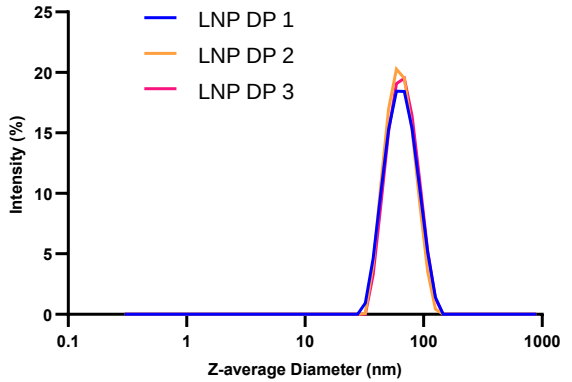


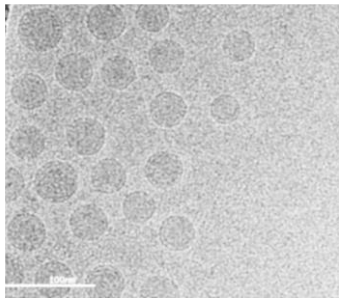
Compared to LNPs without a targeting ligand, Prime's Universal LNP*:

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

Multiple different drug product candidates (DP) from one LNP composition

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





By swapping only the guide RNAs while keeping the other components constant, we have a new drug 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

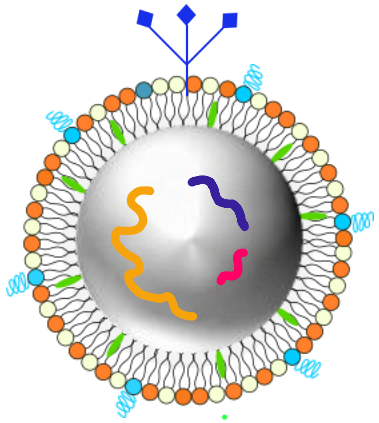
mRNA

pegRNA

ngRNA

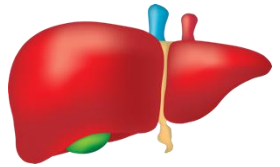
LNP formulation

LNP-encapsulated Prime Editor Cargo



Single IV infusion

Liver Delivery



Prime Editing in hepatocytes

Precise Correction of Mutation in Hepatocytes

p.H1069Q

AGCAGTGAACAACCCTTGGGC
S S E Q P L G

C/A substitution

Prime Editing

AGCAGTGAACACCCCTTGGGC
S S E H P L G

A/C correction

Precise Correction of Mutation in Hepatocytes

p.R778L

GCCCTGGGCCTGTGGCTGGAA
A L G L W L E

G/T substitution

Prime Editing

GCCCTGGGCCGGTGGCTGGAA
A L G R W L E

T/G correction

LNP-Formulated 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

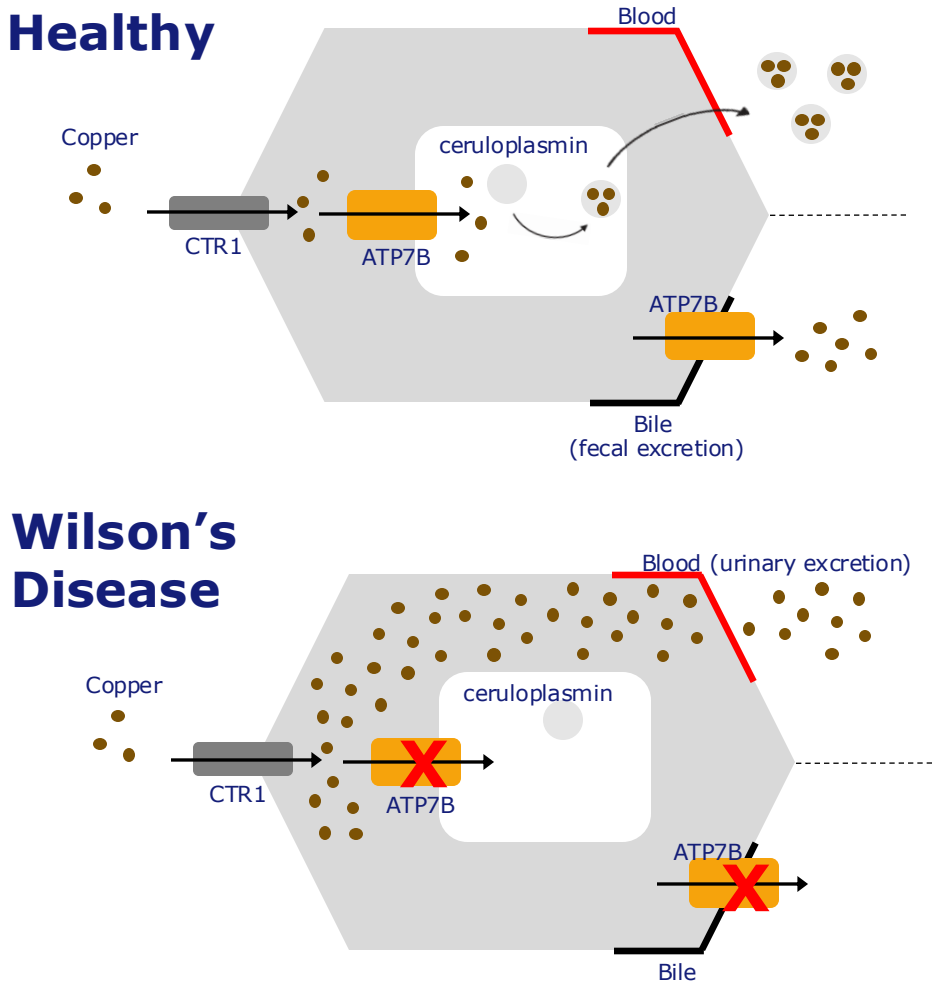
- Many patients die without liver transplant. No approved disease-modifying therapies

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



Identification of lead Prime Editors for correction of *ATP7B* H1069Q and R778L

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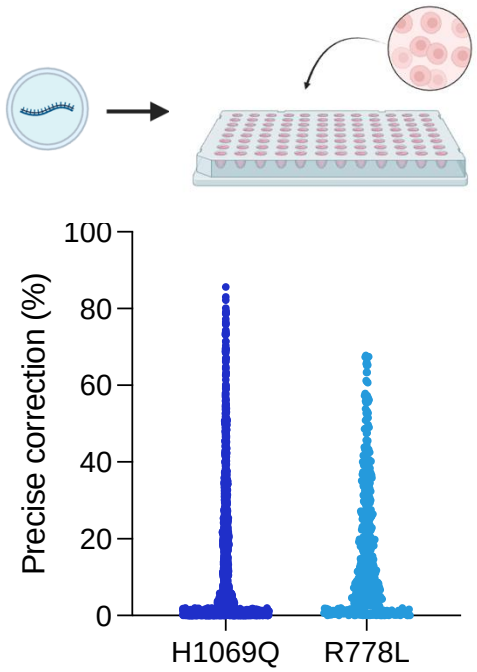
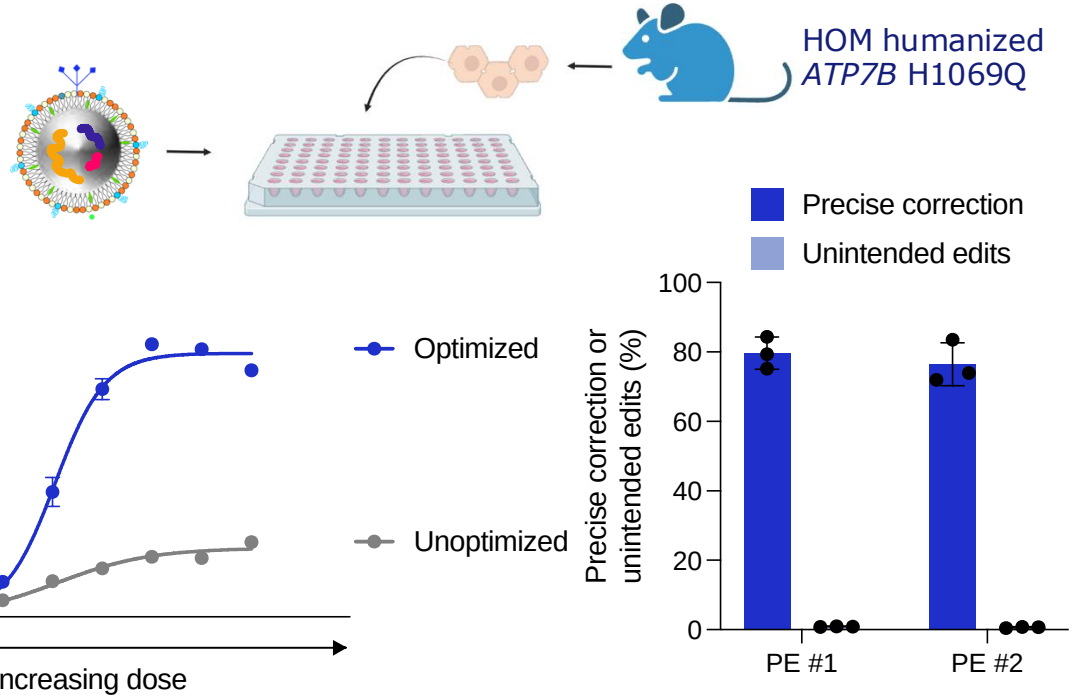
High throughput screening in hepatocyte cell line

Delivery of RNA Prime Editing components



Validation in primary humanized mouse hepatocytes

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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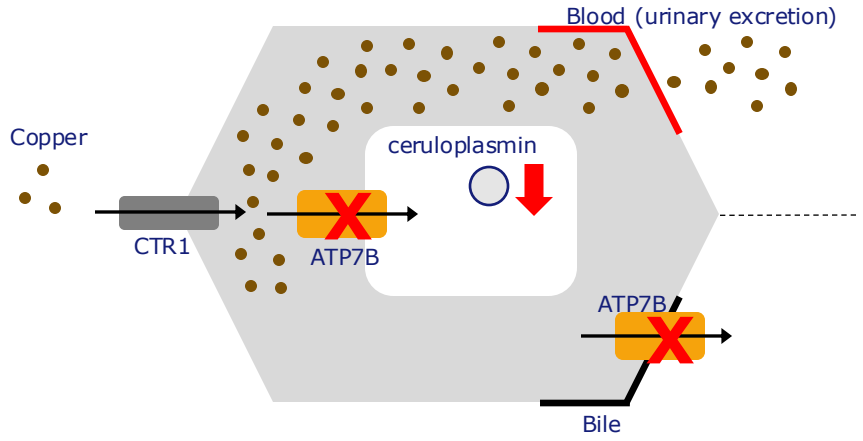
Reduction in ceruloplasmin in WD hepatocytes

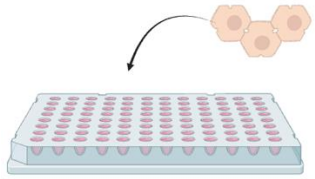


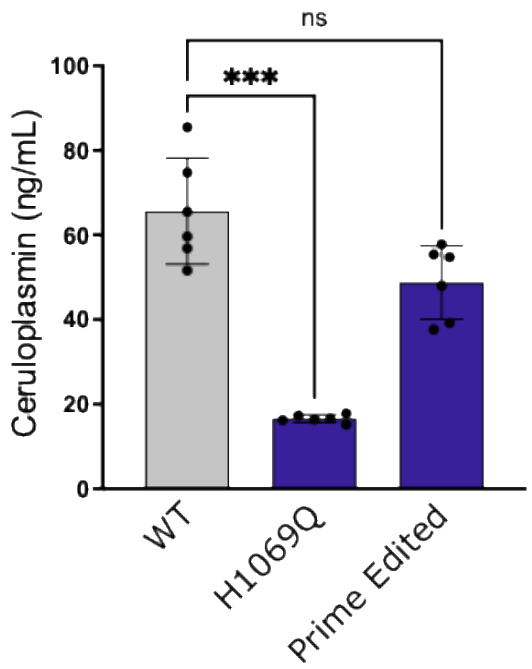
Failure to transport copper in WD reduces ceruloplasmin protein abundance

Restoration of ceruloplasmin levels *in vitro*

RNA Prime Editing components

Patient-derived H1069Q iHeps





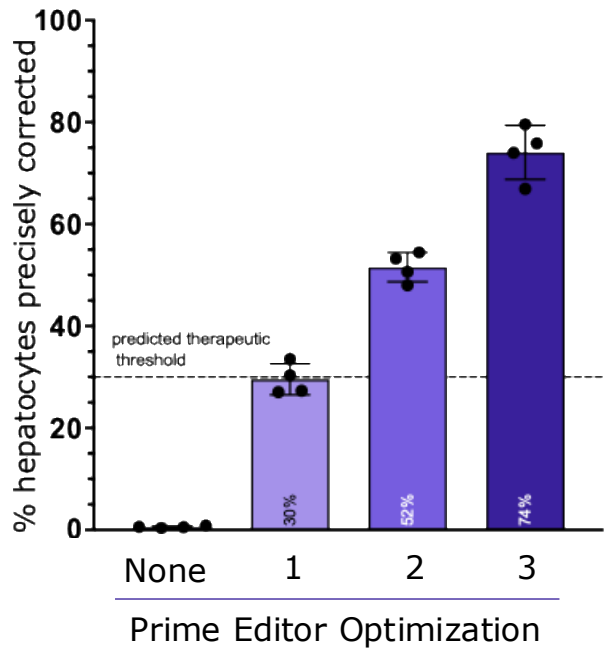
Prime Editing in WD patient-derived induced hepatocytes restores ceruloplasmin abundance to levels similar to WT

iHep = human-induced pluripotent stem cell-derived hepatocytes

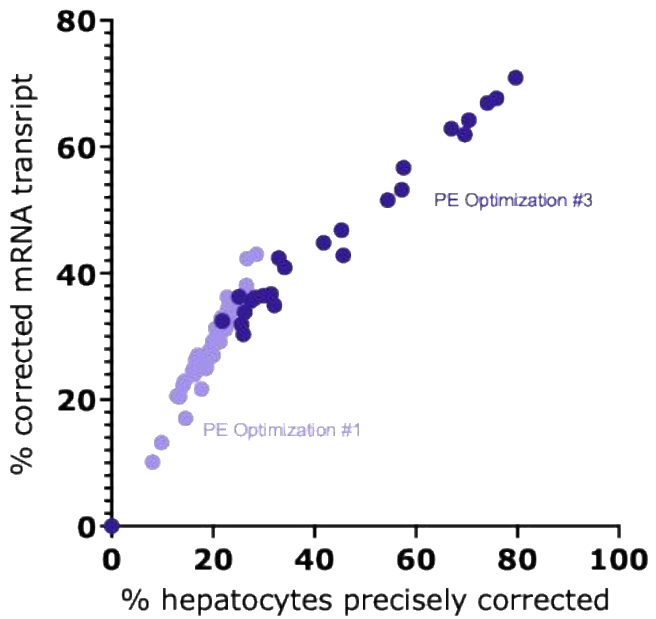
Prime Editors demonstrate efficient DNA and mRNA correction and reduce liver copper in humanized Wilson's Disease mouse model

Fully humanized homozygous p.H1069Q *ATP7B* mouse model

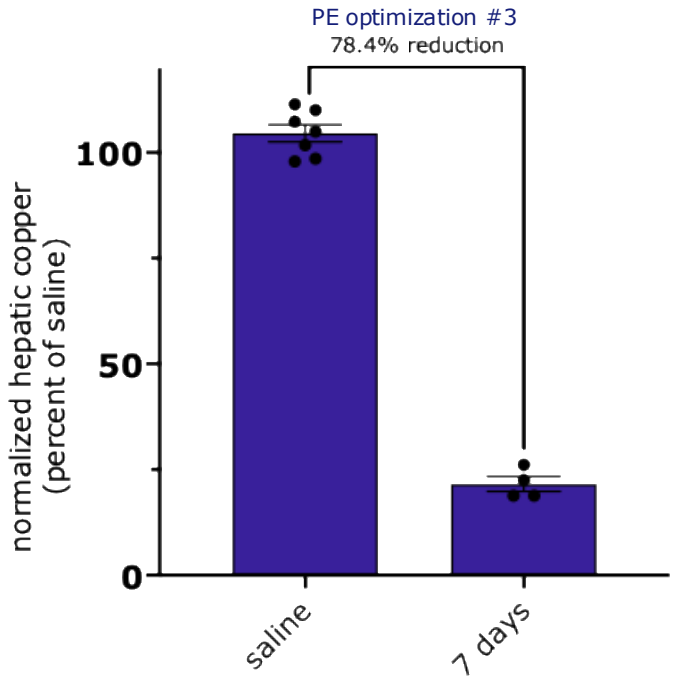
Correction of H1069Q transversion mutation



High efficiency of genome and transcript correction



>75% reduction in liver Cu^{2+} after 7 days



Long term studies ongoing with Prime Editor Optimization #3

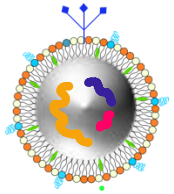
PE optimizations enable efficient genome and transcript correction as well as liver copper reduction in humanized mice

*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; PE = Prime Editor; RNA = ribonucleic acid; mRNA = messenger RNA

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

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

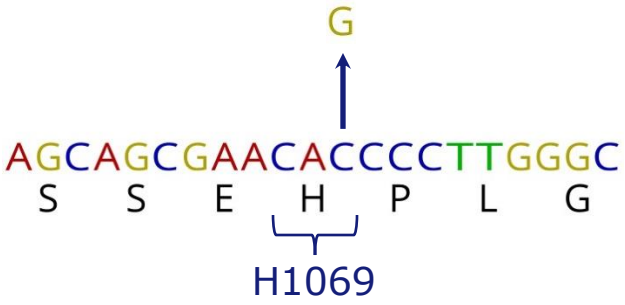
IV injection of PE RNA encapsulated in Universal LNP



mRNA, pegRNA & ngRNA Formulated in Prime Universal liver LNP

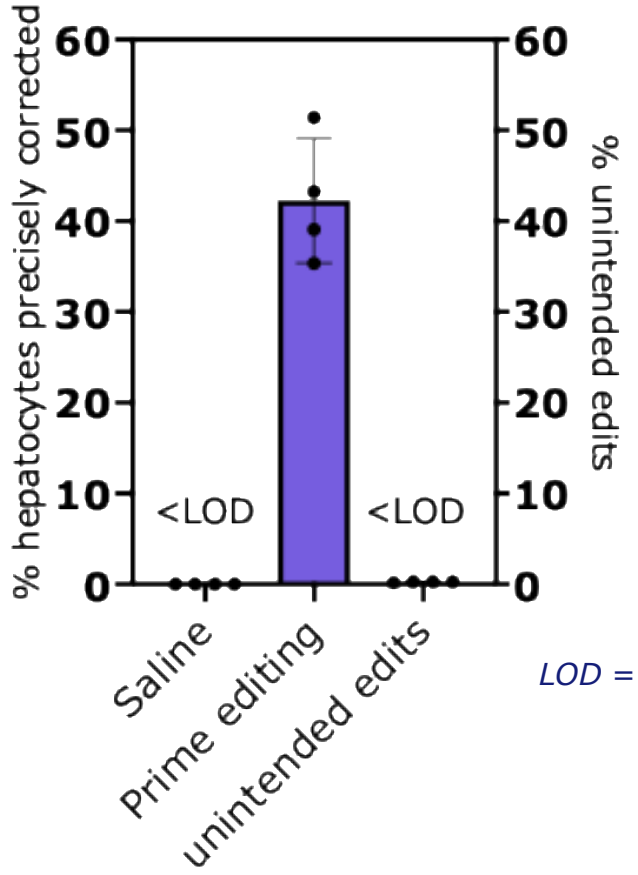
Cynomolgus macaque

Prime Editing of *ATP7B* H1069 in NHP liver hepatocytes



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

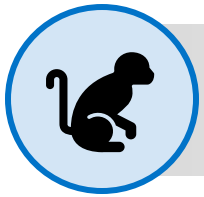
***ATP7B* *in vivo* Prime Editing in NHP Precise Editing at H1069 with no detectable unintended edits**



LOD = 0.25%

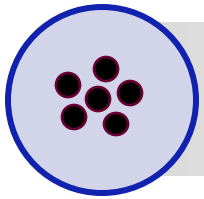
*Based on PK/PD relationships and quantification of cell types in liver: Wang et al Sci. Rep. (2021) 11:19396; MacParland et al Nat Commun. (2018) 9:4383; Hansel et al, Curr Protoc Toxicol (2014) 62:14.12.1; Kmiec, Adv Anat Embryol Cell Biol. (2001) 161:III–XIII. 1–151. ** Calculation based on 60% of 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)

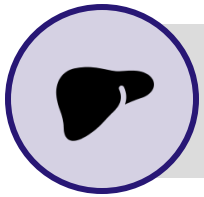


- Well-tolerated with no acute reactions, clinical observations, or body weight changes
- Animals healthy at 54 weeks

- 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's Disease

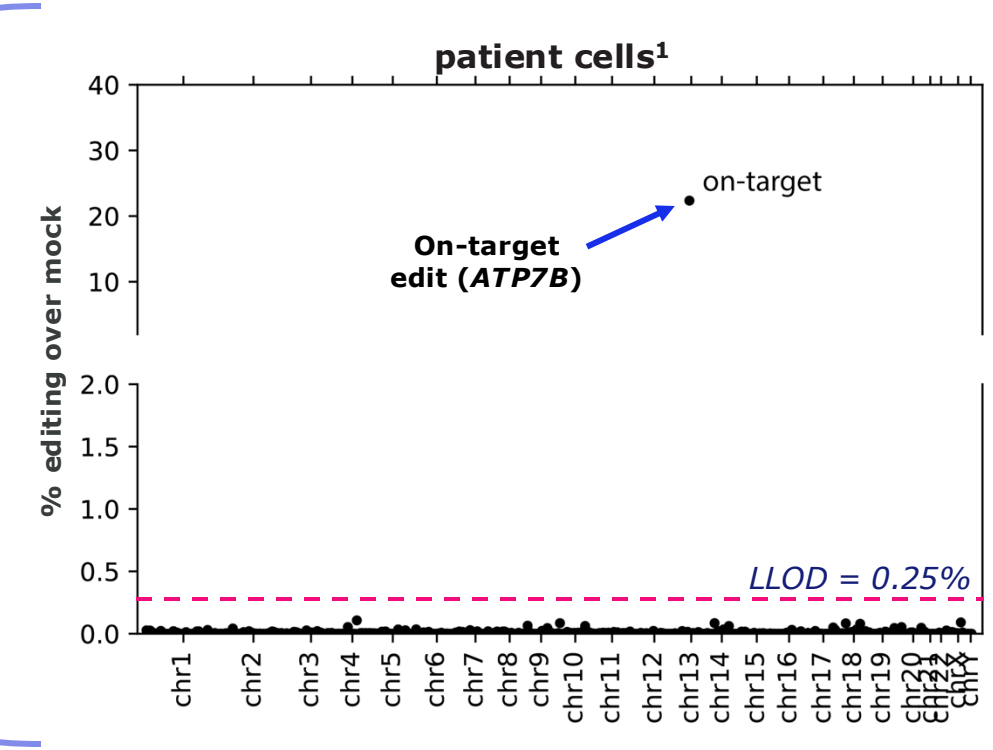
"IND ready" set of comprehensive off-target assays

Genome wide identification using in silico and biochemical methods

Potential off-target editing sites

Targeted sequencing

Identified off-targets



[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

Advances in Prime Editing enable *in vivo* therapeutic correction of the *ATP7B* p.H1069Q and p.R778L mutations causing Wilson's 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's Disease

- Prime Medicine's Universal LNP-formulated Prime Editors for Wilson's 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'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 Wilson's Disease patients

NHP = non-human primate

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Delivering on the promise
of Prime Editing

Thank you!

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