Prime editing precisely corrects prevalent pathogenic mutations causing Glycogen Storage Disease Type 1b (GSD1b)

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On behalf of the team at Prime Medicine
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Disclosures

Jeremy Duffield declares he is a current employee of Prime Medicine, Inc. and owns equity in Prime Medicine.
Prime Editors are programmable for both Search and Replace

Gene with mutation

Prime editor complex initiates search for target DNA

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

Nicked DNA strand primes the RT domain for DNA synthesis

Prime edited complex copies in corrective DNA sequence

3' flap preferentially incorporated, excess flap repaired, gene fully corrected

1Completion 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

animated movie available at www.primemedicine.com
Prime Medicine is developing a universal Lipid Nanoparticle (LNP) formulation to deliver Prime Editors for all liver indications.

Universal liver targeted LNP for all liver indications has potential to expedite clinical development of Prime Editing for a broad spectrum of patients.

**Universal LNP for liver indications – Potential advantages**

- Addressing a new liver indication would only require swapping the guide RNAs
- Potential for:
  - Pharmacology/ADME/Safety/Toxicology packages leveraged across multiple programs
  - Faster non-clinical, clinical development and regulatory path for follow on programs
- A modular manufacturing process for universal LNP used across liver indications
- Leverage CMC packages across programs
- Significant cost of goods savings

Prime Medicine universal **liver** LNP contains an active targeting ligand that reduces biodistribution to secondary organs and increases Prime Editing in the liver.
Therapeutic approach: LNP-mediated delivery of Prime Editor components to liver

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

Prime Editor Cargo Components

- mRNA
- pegRNA
- ngRNA

LNP Encapsulated Prime Editor Cargo

Liver Delivery

Precise Correction of Mutation in Hepatocytes

Prime Editing in Hepatocytes

mutated allele

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

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

Quantify precise correction & unintended edit frequency

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

*Based on PK/PD relationships and quantification of cell types in liver:

# Unintended edits = any SNVs or indels within 300bp either side of the edit site

Determine Precise Edit & Unintended Edit Frequency

Dose

Precise Correction

Unintended Edits

Theoretical maximum

Precise Correction %

Unintended Edit (%)

Vehicle
Low
Medium
High

0
20
40
60

0.0
0.5
1.0
1.5
2.0
2.5

0
20
40
60

0
20
40
60

Theoretical maximum

Unintended edits, even at the high dose are below 0.2%
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

Key Takeaways:
• Prime Editing restores expression of G6PT protein
• Extent of G6PT mutation correction correlates with extent of G6PT protein restoration
Safety: Prime’s comprehensive suite of IND-ready assays for off-target discovery

**Local off-targets**

On-target genome

- Targeted Sequencing
  - Targeted Nick Detection Assay*
  - Genome wide DSB detection
  - Genome wide Nick Detection Assay*

- potential off-target editing sites

- Identified Off-targets

**Chromosome scale or structural off-targets**

- cDNA synthesis
  - Translocations
  - Large Deletions

- Reverse Trans. Assay
  - mRNAseq
  - PEG integration

- Chromosomal Integrity Assays
  - Imaging
  - Sequencing based

  - Whole Genome Sequencing
  - Vector Integration Analysis

- Identified Off-targets

*Proprietary assay developed by Prime Medicine
Preliminary analysis showed no detectable off-target editing in patient cells

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

Targeted Sequencing

Genome wide Identification of potential off-targets

potential off-target editing sites

Identified off-targets

GSD1 Program: patient cells

On-target Edit (G6PT)

1Targeted Analysis of potential off-target sites using deep sequencing in Prime Edited human iPSC cells carrying the p.L348fs
Approach to cynomolgus monkey liver Prime Editing to support safety and pharmacology for the GSD1 program

Precisely edit the p.L348 codon in SLC37A4 (G6PT) gene with single base substitution in healthy Cynomolgus adult monkeys

- Prime Editing guide RNAs (pegRNAs) for Cynomolgus monkey are surrogates for the human pegRNAs
- As close to the lead human pegRNA sequence as possible with similar potency and activity
- All other Prime Editing components are held constant
- Wherever possible non-terminal studies are performed

Liver Bx = liver biopsy; GSD1 = glycogen storage disease type I; SLC47A4 = gene name for the glucose 6 phosphate transporter or G6PT
Surrogate Prime Editor efficiently and precisely edits p.L348 in SLC37A4 (G6PT) gene in healthy cynomolgus monkey liver

Up to 50% whole liver precise editing in Cynomolgus monkey

Non-naïve cynomolgus monkeys had previously received Prime’s Universal LNP to target a different gene

Prime Medicine’s liver-targeted LNP-RNA Prime Editor drug was well tolerated by cynomolgus monkeys

- No infusion reactions
- No body weight changes
- Transient, modest liver function test changes and minimal transient cytokine abnormalities
- Redosing of LNP-RNA drug (non-naïve) was tolerated similarly to naïve animals
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 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 protein expression consistent with allele correction

Initial studies from Prime’s off target pipeline have not detected any off-target editing genome-wide

Large animal cynomolgus monkey studies using the universal LNP-RNA platform with a surrogate pegRNA show high levels of up to 50% of precise editing of G6PT at p.L348 in whole liver equivalent to ~83% of hepatocytes at a dose that was well tolerated
THANK YOU!