Prime Editing precisely corrects prevalent mutations observed in Glycogen Storage Disease Type 1b (GSD1b) patients

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prime_ medicine

Abstract

Glycogen Storage Disease Type 1b (GSD1b) is an autosomal recessive disorder caused by mutations in the *SLC37A4* gene encoding the glucose 6-phosphate translocase (G6PT) which is required for normal glucose-6-phosphate metabolism, including hepatic glycogenolysis. Patients exhibit multiple clinical manifestations including severe hypoglycemia resulting in seizures and cognitive impairment. Without an approved treatment for GSD1b, patients maintain metabolic control with a special diet and with medications that alleviate secondary complications such as neutropenia. The most prevalent mutations include p.G339C and/or p.L348fs, observed in ~50% of patients. A gene editing approach to correct the mutations in the affected cells to restore G6PT function would directly address the underlying genetic cause of the disease.

Prime Editing (PE) is a next generation gene editing technology that can precisely correct more than 90% of all pathogenic human mutations without the need for double strand breaks (DSBs), with the potential for minimal byproducts at the edit site, and low off-target activity and chromosomal alterations or genotoxicity sometimes observed with CRISPR-based editing. We have developed a universal liver-targeted lipid nanoparticle (LNP) for delivery of Prime Editors to the liver for multiple indications. LNP-RNA PE candidates, that are LNPs formulated with an engineered mRNA encoding the Prime Editor protein and the Prime Editor guide RNA (pegRNA), were developed to correct the mutated *SLC37A4* gene in hepatocytes.

Comprehensive high-throughput screening for pegRNAs identified initial hits that correct either the p.G339C or p.L348fs mutation. Initial PE lead assessment in primary hepatocytes isolated from humanized mice in which the mouse *Slc37a4* gene was replaced with the human *SLC37A4* gene harboring either the G339C or L348fs mutations, or in iPSC-derived hepatocytes, resulted in editing efficiencies up to 80% *in vitro*. A similar assessment was performed *in vivo* following intravenous delivery of the LNP-RNA PE candidates to humanized mice. Genomic correction of the L348fs mutation was observed in whole liver at an average correction rate of 47% (total liver alleles) and an associated correction of *SLC37A4* transcripts, transcript levels and protein expression. Intravenous administration of LNP-RNA to cynomolgus monkey was well tolerated and resulted in precise editing rates in whole liver of up to 50% at day 14 in animals without significant on-target unintended edits detected. These results demonstrate efficient and safe LNP-mediated delivery of Prime Editor candidates to the liver and that Prime Editing can efficiently and precisely correct pathogenic mutations causing GSD1b at rates exceeding those believed to reverse manifestations of disease.

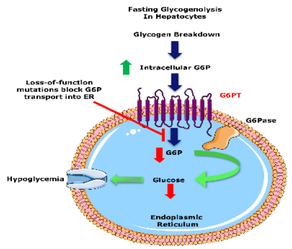
Glycogen Storage Disease Type 1b (GSD1b)

Description:
- Rare disease preventing production of glucose during fasting state, causing hypoglycemic episodes and associated seizures

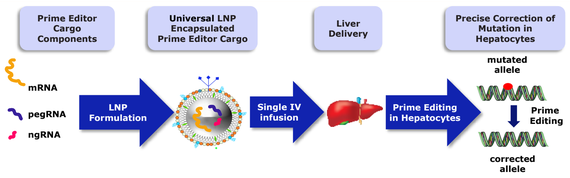
Human Genetics and Biology:
- Autosomal Recessive, caused by mutations in the *SLC37A4* gene encoding G6PT, a glucose-6-phosphate transporter
- p.L348fs and p.G339C mutations found in ~50% of GSD1b patient population

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

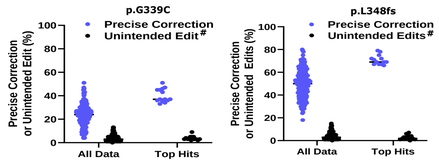


Therapeutic approach: LNP-mediated delivery of Prime Editor components to liver



Results

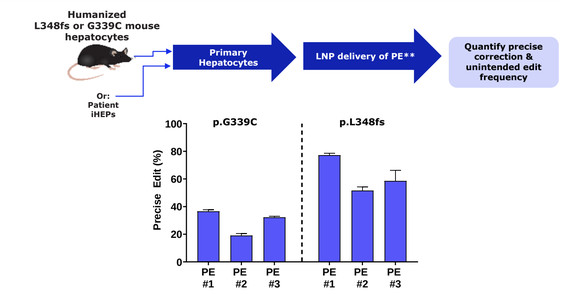
Figure 1: Guide Screening Pipeline Identified Prime Editors That Precisely Correct the G339C and L348fs Mutations



Key Takeaways:
- Guide screening pipeline identified highly active candidates that can precisely correct the p.G339C and p.L348fs mutations.

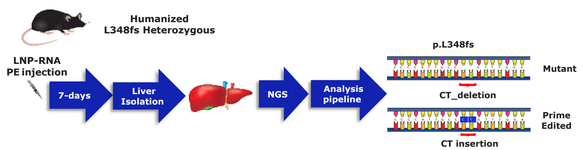
#Unintended edits = any SNVs or indels within 300bp either side of the edit site; *Data shown using humanized primary mouse hepatocytes; **PE = Prime Editor

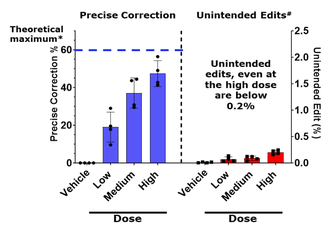
Figure 2: Evaluation of pegRNA Potency in Primary Hepatocytes from Humanized Mice



Key Takeaways:
- 77% and 37% precise correction observed in primary hepatocytes from humanized mice for the p.L348fs and p.G339C mutations, respectively.
- 52% precise correction of the p.G339C mutation following HTS, and 37% in primary hepatocytes – further optimizations underway

Figure 3: LNP delivery of Prime Editor Components to the Liver Precisely Corrects the p.L348fs Mutation in Humanized Mice

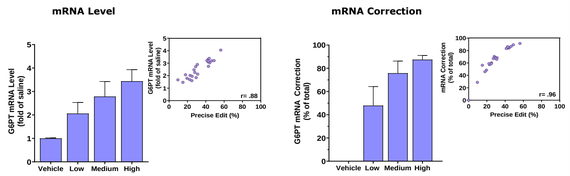




Key Takeaways:
- A dose-dependent increase in precise correction of the p.L348fs mutation is observed following LNP-mediated delivery of Prime Editor components to the liver
- Unintended edit rates are below 0.2% at every dose tested

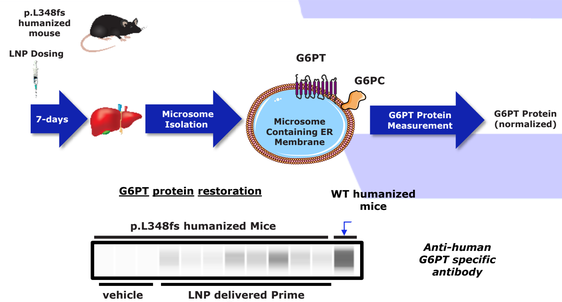
*Based on PK/PD relationships and quantification of cell types in liver: Wang et al Sci. Rep. (2021) 11:19396; MacParland et al Nat Commun (2018) 9:4383; Hansel et al, Curr Protoc Toxicol (2014) 62:14.12.1; Kmiec, Adv Anat Embryol Cell Biol. (2001) 161:III–XIII. 1–151 # Unintended edits = any SNVs or indels within 300bp either side of the edit site

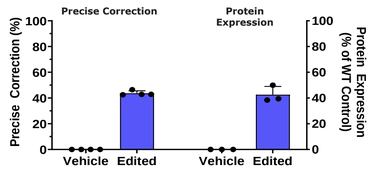
Figure 4: G6PT mRNA Correction Increases G6PT Transcript Levels Following Precise Correction of the L348fs Mutation in the Liver of Humanized Mice



Key Takeaways:
- Precise correction of the p.L348fs mutation is associated with increased G6PT mRNA levels due to correction and stabilization of the G6PT transcript
- G6PT mRNA and transcript correction levels correlate with precise correction of the p.L348fs mutation

Figure 5: Precise Correction of the p.L348fs Mutation in the Liver of Humanized Mice Restores G6PT Protein Expression

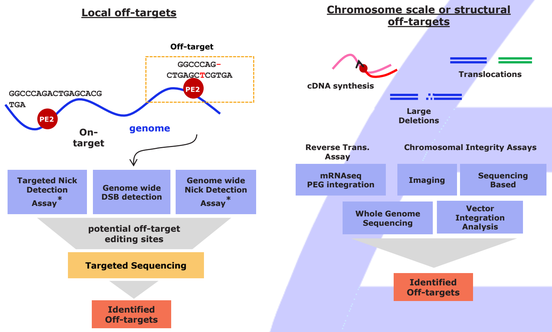




Key Takeaways:
- Prime Editing restores expression of G6PT protein
- Extent of G6PT mutation correction correlates with extent of G6PT protein restoration

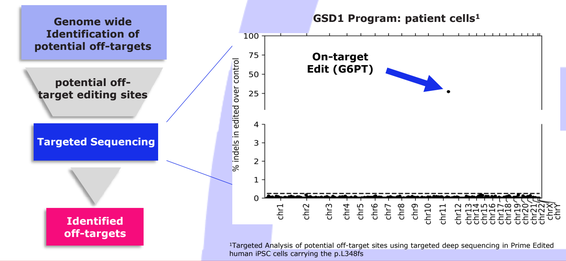
G6PT: glucose-6-phosphate translocase, het: heterozygous, WT: wild-type, ER: endoplasmic reticulum

Figure 6: Safety: Prime's Comprehensive Suite of IND-ready Assays for Off-Target Discovery



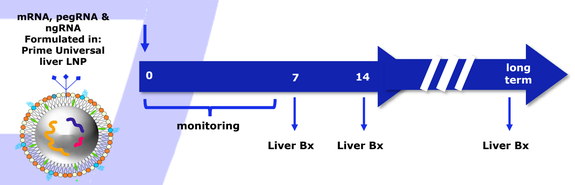
*Proprietary assay developed by Prime Medicine

Figure 7: Preliminary Analysis Showed No Detectable Off-target Editing in Patient Cells

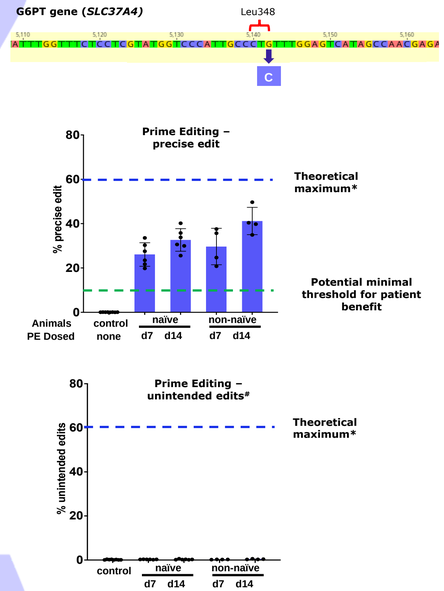


1Targeted Analysis of potential off-target sites using targeted deep sequencing in Prime Edited human iPSC cells carrying the p.L348fs

Figure 8: Surrogate Prime Editor Efficiently and Precisely Edits p.L348 in *SLC37A4* (G6PT) Gene in Healthy Cynomolgus Monkey Liver



- 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
*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

Conclusions

- 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 showed 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

All authors are current employees of Prime Medicine Inc, and own equity in Prime Medicine