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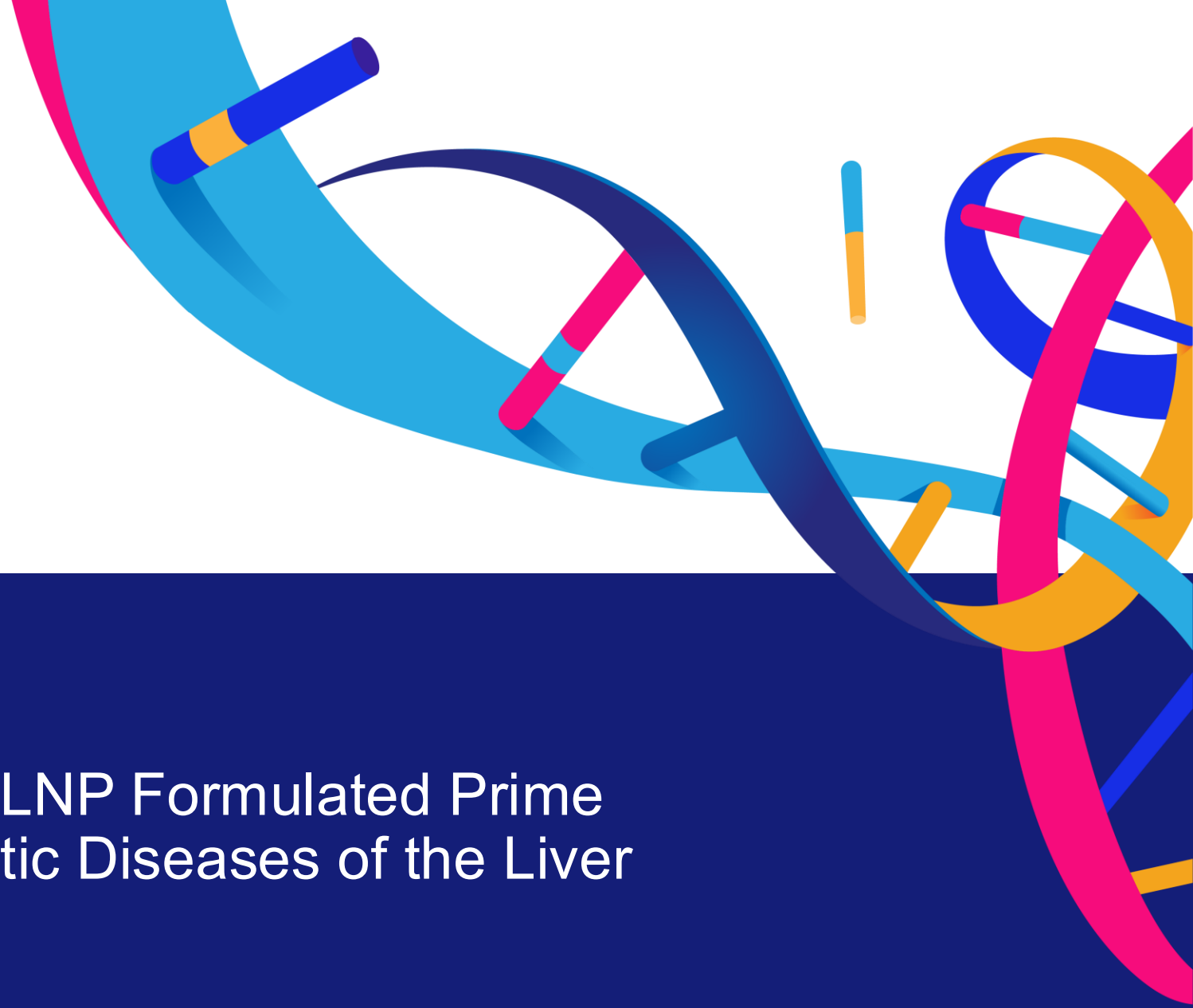
Delivering on the promise
of Prime Editing



Modular Platform for In Vivo LNP Formulated Prime Editors Targeting Rare Genetic Diseases of the Liver

EMA Gene Editing Workshop

September 16, 2025

Prime Editing: gene editing technology that allows personalized therapy that is mutation specific and corrects genetic variants at endogenous genomic locations

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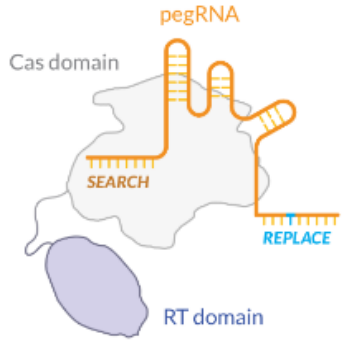
Prime Editor Machinery Overview

Key Components



1. Prime Editor protein (Cas domain + reverse transcriptase domain)
2. Prime Editing guide RNA (pegRNA)
3. +/- nicking guide RNA (ngRNA)

Key Attributes

- Programmable to different DNA targets
- Versatility enables correction of broad mutation classes (point mutations, insertions, deletions)
- Flexibility in PAM targeting through small changes to Cas domain
- Precise edits without “bystanders”
- No requirement for double stranded breaks or donor DNA
- Minimal or no detectable off targets
- Guide discovery needed for each mutation

Steps to Gene Correction

SEARCH	FIND & NICK	PRIME	REPLACE	GENE CORRECTED
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 editor complex copies in corrective DNA sequence	3’ flap preferentially incorporated, excess flap repaired, gene fully corrected

Abbreviations: pegRNA = prime editing guide RNA; RT = reverse transcriptase; Cas = CRISPR associated protein

Source: Anzalone, et al (David R. Liu). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 2019.

How to make it economically viable to ensure that even the subset of patients with the rarest mutation of an ultrarare disease may benefit from a Prime Editing cure

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Where we are now

Only a subset of patients with prevalent mutations in "common" rare diseases can access Prime Editing for an economically viable potential cure

Establish modular genetic medicine approaches

Establish modular genetic medicine approaches through leveraging preclinical, manufacturing, and clinical data across mutations and indications

Call to Action

Streamlining regulatory pathways to enable rapid and viable development of genetic cures for all patients

Challenges for the development of Prime Editing products for rare mutations

- The same Prime Editing mechanism and delivery platform could be applied to many patients that possess different mutations within a disease, where products would differ only in the guide RNAs that are mutation-specific.
- However, the large number of costly and redundant nonclinical, CMC and clinical studies that would be required for each unique product would make it challenging to deliver therapies to patients with rarer mutations.

- **Nonclinical challenges**
  - Tailor-made animal models harboring specific individual mutations are currently used to assess efficacy and potency, but these models are infeasible to scale for many (10s or 100s) different mutations
  - Biodistribution, reproductive toxicology, germline editing, and GLP toxicology studies are resource intensive and time consuming, but largely influenced by common delivery platform, less by differences in guide RNAs
  - Exhaustive off-target risk assessment for every rare mutation would be extremely resource intensive and time consuming

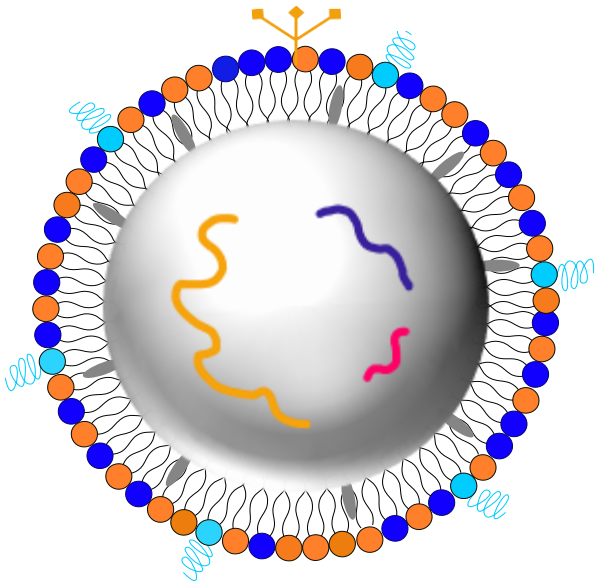
- **CMC challenges**
  - Regulatory expectations for CMC manufacturing processes and analytical assays are disproportionately burdensome, particularly in the context of rare diseases. Under current frameworks, each variant is often treated as a wholly new drug substance and drug product, which triggers a full set of requirements (e.g., process ranges and validation, assay qualification/validation, stability data packages)

- **Clinical challenges**
  - Dose finding and dose escalation for small populations can be difficult or impossible.

Delivery Vehicle: Liver targeted LNPs are unchanged across products

LNP Modularity:

6 out of 8 components within the LNP are the same for liver programs



Ionizable Lipid	• Nucleic acid encapsulation and endosomal escape
Helper Lipid	• Stabilize and improve LNP pharmacokinetics • Facilitate membrane fusion and endosomal escape
PEG Lipids	• Control particle size and stability • Stealth coating reduces serum interactions and increases half-life
Cholesterol	• Improve intracellular delivery • Increase LNP stability
Targeting Ligand	• Improve biodistribution of LNPs to hepatocytes
PE mRNA	• Prime editor enzyme
pegRNA	• pegRNA is disease & mutation specific
ngRNA	• ngRNA is disease & mutation specific; usage is dependent on the Prime Editing strategy applied

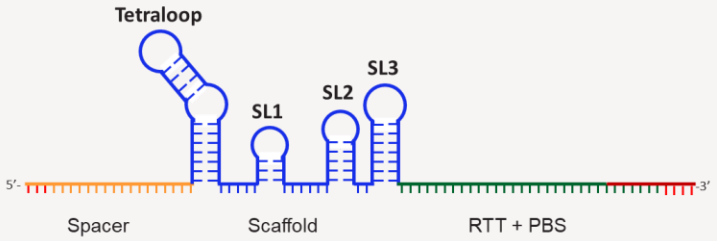
LNP vehicle drives biodistribution and toxicity, and is the major contributor to any immunogenicity

Prime Editing component modularity

Each RNA component making up the LNP cargo is designed with modularity

Exemplary Prime Editing Components

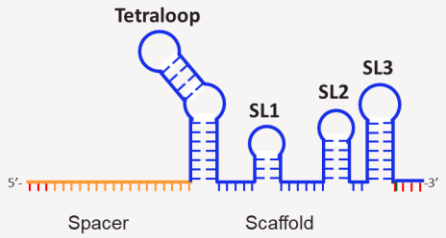
Prime editing guide (peg) RNA



pegRNA complexes with translated **Prime Editor (PE) Protein** *in vivo* to form active **Prime Editor Complex**

- **Scaffold** is considered *fixed*, enabling Prime Editor loading
- **Spacer**, **RTT**, **PBS** uniquely target the desired gene loci
- **RTT** serves as a template to reverse transcribe the correct genetic sequence

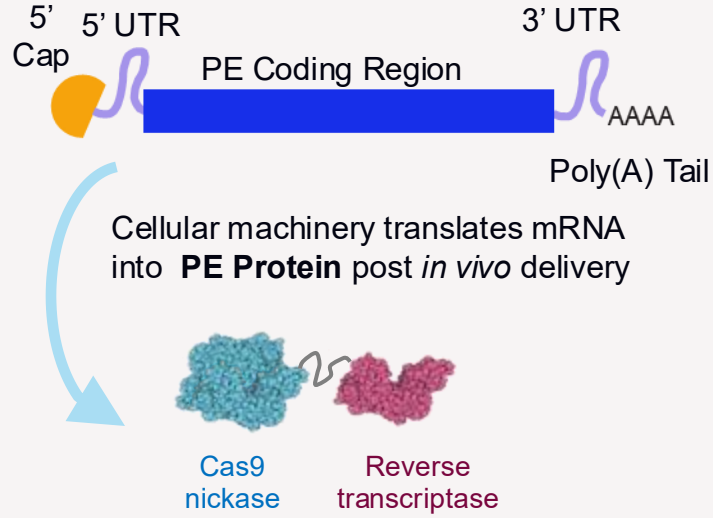
nick guide (ng) RNA



ngRNA complexes with translated **PE Protein** *in vivo* to form Prime Editor Complex which enhances efficiency of edit

- **Scaffold** is considered *fixed* and common to the pegRNA scaffold sequence, enabling Prime Editor loading
- **Spacer** uniquely target the desired gene loci

Prime editor mRNA



- **Cap**, **Tail** and **UTRs** are *fixed* across all Prime Editor mRNAs for a target tissue
- **Coding region** is *fixed* except for minimal changes required for a different PAM

Clinical overviews of Wilson's disease and glycogen storage disease type 1b

Wilson's Disease

Disease severity and opportunity

- Causes liver failure, neurocognitive decline, premature death
- Affects ~20,000 patients in the US and Europe, with 30-50% carrying the H1069Q mutation
- R778L is most common mutation in the Asian population

Unmet need

- Liver transplant is only curative option, but with high morbidity/ mortality
- No approved disease-modifying therapies
- Current care focuses on preventing copper buildup with chelating agents and a low-copper diet

Human biology

- Autosomal recessive disorder caused by loss-of-function mutations in *ATP7B*
- Disrupts copper homeostasis, causing toxic buildup in the liver and brain
- Correcting 20-30% of hepatocytes could be curative

Glycogen Storage Disease Type 1b

Disease severity and opportunity

- Causes life-threatening hypoglycemia, hepatomegaly and growth delay
- Affects fewer than 1,000 patients in the US and Europe
- Patients often experience neutropenia, leading to increased infection risk, as well as inflammatory bowel-like syndrome

Unmet need

- Patients rely on lifelong dietary management and frequent cornstarch supplementation to prevent hypoglycemia
- No curative treatments, only dietary and infection management
- Care focuses on blood sugar control and infection prevention with G-CSF

Human biology

- Autosomal recessive disorder caused by *SLC37A4* mutations
- Disrupts glucose-6-phosphate transport, leading to glycogen buildup
- Restoring this pathway could normalize glycogen metabolism and improve outcomes

Potential to reach all patients through gene correction regardless of mutation and population size

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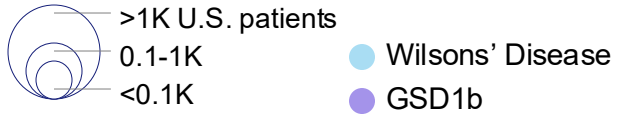
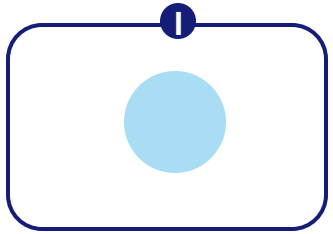
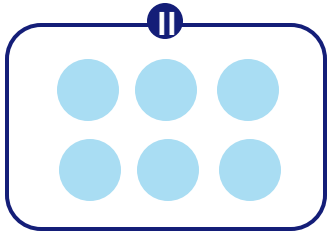
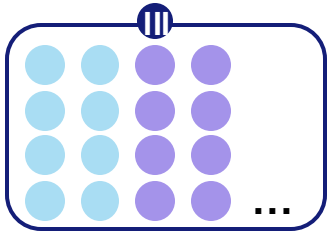
Exemplary Gene Correction Patient Accessibility

Illustrative, not to scale



	I Prevalent mutation(s) in Wilsons' Disease	II Less prevalent mutations in Wilsons' Disease	III Other rare mutations in Wilson's Disease or liver-targeting diseases
Description	**Establishing clinical safety and optimal dose for LNP delivery of Prime Editor**	**Demonstrating modularity to follow on mutations within the same disease**	**Providing benefit to all patients across different mutations and liver diseases**
Disease mutation population			
Expanding *in vivo* LNP Prime Editor	Prime editor targeting the prevalent or hotspot mutation with the largest patient population for one disease	Prime editor targeting multiple mutations that meet a patient population threshold for one disease	Potential to efficiently develop Prime Editors targeting all mutations, addressing the entire patient population in more than one disease

In vivo correction of disease-causing mutations in Wilson's Disease and Glycogen Storage Disease Type 1b (GSD1b) mouse models with Prime Editing

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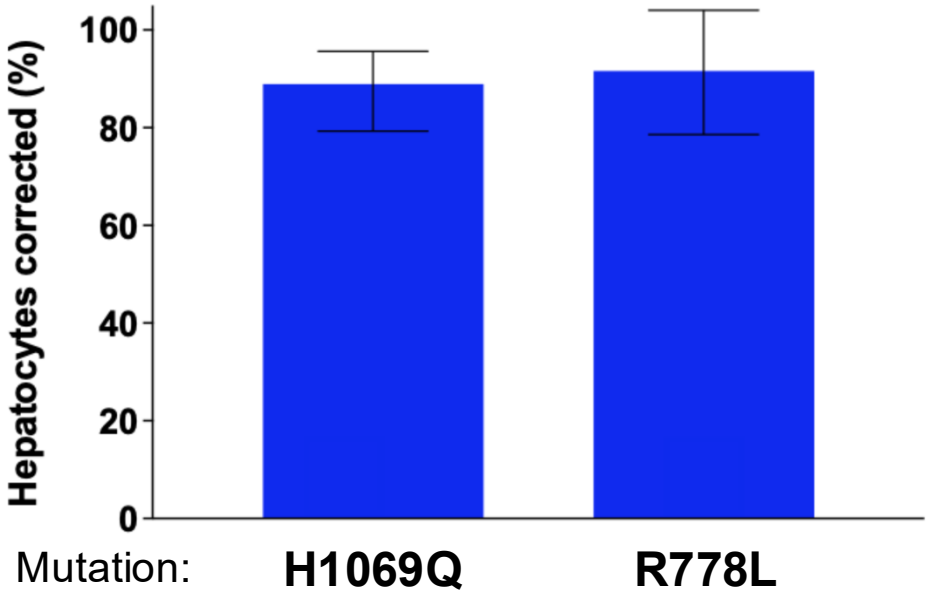
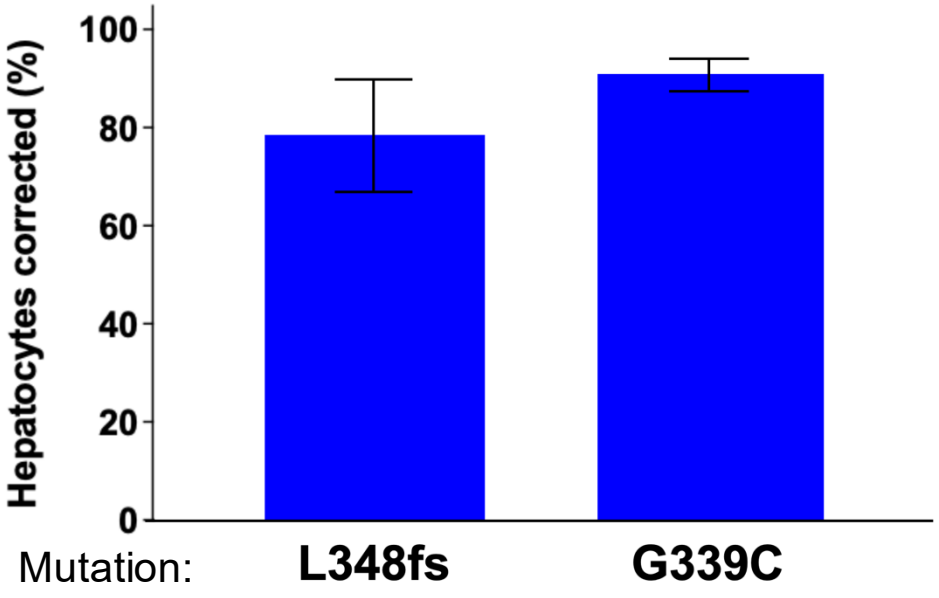
Mouse models harbor humanized genes (*ATP7B* for Wilson's Disease models, *SLC37A4* for GSD1b models) and human disease-causing mutations

Efficient *in vivo* correction of the H1069Q and R778L mutations in Wilson's Disease mice



Efficient *in vivo* correction of the L348fs and G339C mutations in Glycogen Storage Disease 1b mice



Benefits of Prime Editing platform and shared delivery system for preclinical development

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Leveraging shared delivery system streamlines NCD across programs and diseases

Pharmacology

Same prime editing mechanism across all mutations, tissues/organs, indications:

- Standardized assay measures biological activity
- Target engagement, pharmacodynamic, and efficacy
- PK/PD modeling for dose projection

DMPK

Toxicokinetic and pharmacokinetics are driven by shared delivery system:

- Limited biodistribution to non-target tissues
- Bioanalytical assays
- ADME properties

Toxicology

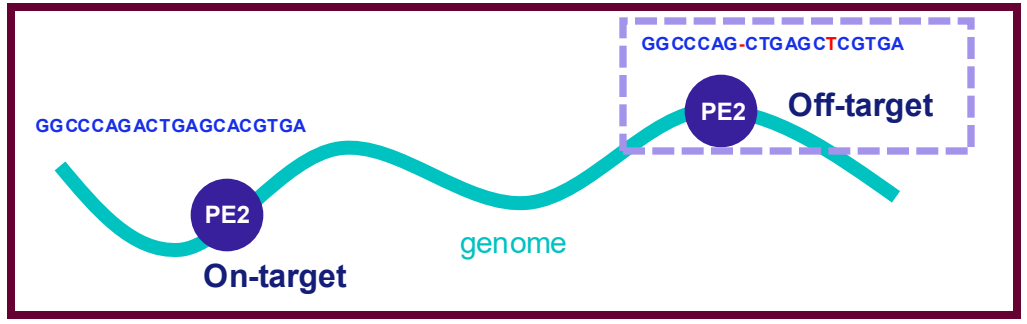
Safety expected to be driven by shared delivery system:

- Target tissue safety
- Germline transmission
- Safety assessments and biomarkers
- TK/TD modeling
- Immunogenicity

DP: Drug Product; PK/PD: Pharmacokinetic/Pharmacodynamic; TK/TD: Toxicokinetic/Toxicodynamic; ADME: Absorption, Distribution, Metabolism, and Elimination; Drug metabolism and pharmacokinetics; NCD: Nonclinical Development; NGS, Next Generation Sequencing

Identifying the meaningful risks in gene editing

Off Targets (bp scale)



Chromosome Scale or Structural Off Targets

Assays for off-target detection

Targeted Nick Detection Assay	Genome wide DSB detection	Genome wide Nick Detection Assay

Potential off-target editing sites

Targeted Sequencing

Identified Off-targets

Reverse Trans. Assay

mRNAseq PEG integration

Chromosomal integrity assays

Imaging based	Sequencing based

Whole Genome Sequencing | Long read sequencing

Identified Off-targets

Comprehensive suite of off-target assays applied to initial product(s) powers data-driven risk assessment for later products. Proposing simplified off-target assays for later products.

Stewart-Ornstein et al., 3'-end ligation sequencing is a sensitive method to detect DNA nicks at potential sites of off-target activity induced by prime editors. Genome Research (2025)

Prime Editing Has Highly Differentiated Safety Profile: No off-target activity detected in any lead program

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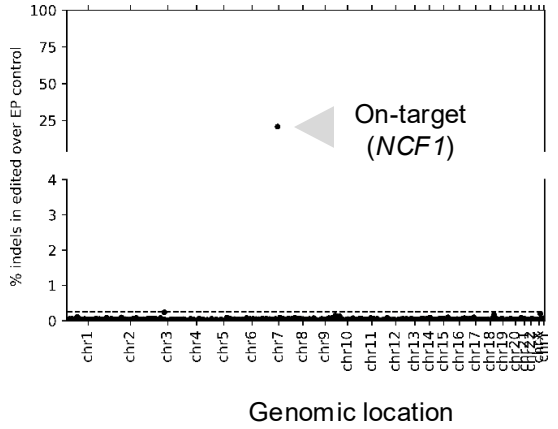
- **No** detectable double strand breakage
- **No** detectable off-target edits
- **No** detectable bystander edits
- **No** detectable off-target deletions, chromosomal translocations or rearrangements

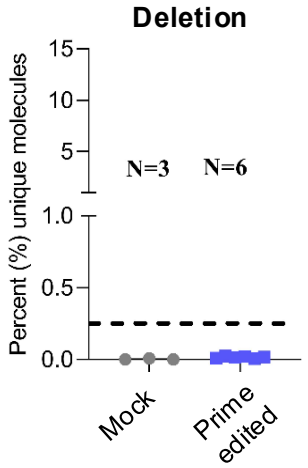
Examples from CGD program used to support IND/CTA filings

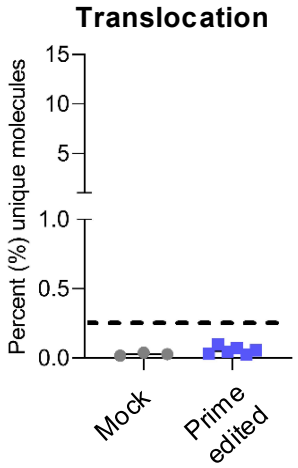
No off-target editing detected in healthy human donor CD34+ cells[1]

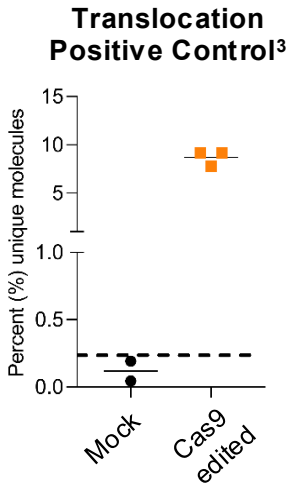
No large deletions or translocations observed in bone marrow engrafted **Prime-Edited** LT-HSCs[2] vs. Cas-9 nuclease edited cells

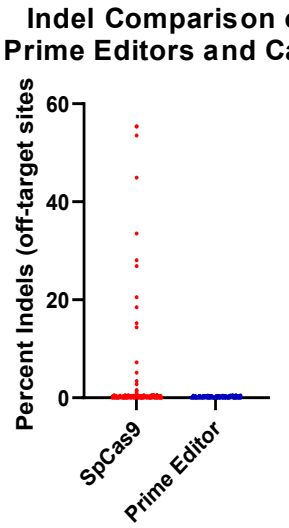
No Off-Target Edits detected with Prime Editing vs. Cas9











Phase I/II program

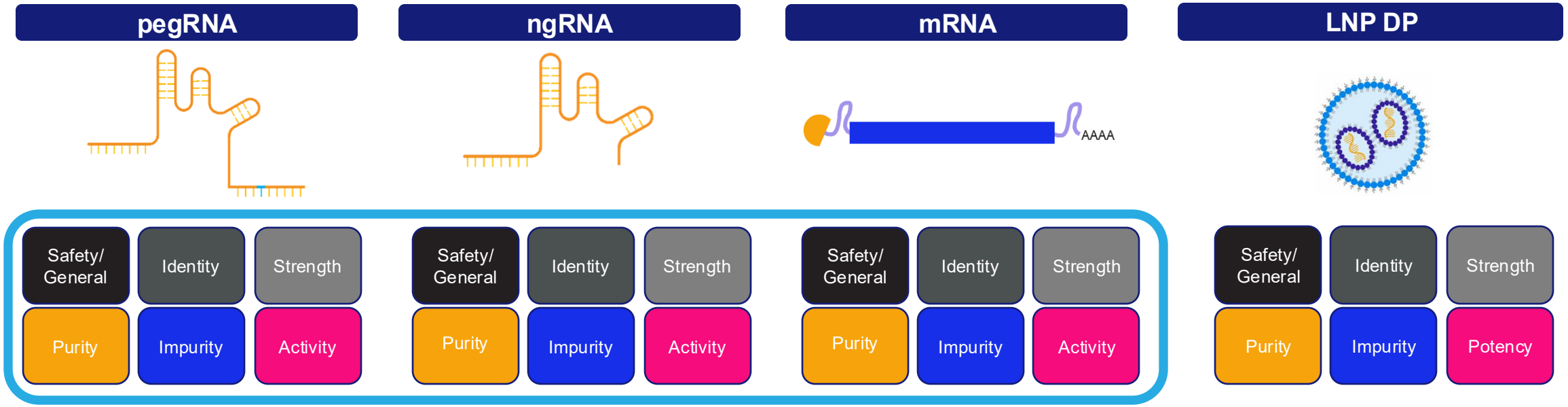
[1]Analysis of edited CD34+ cells from CGD program: Targeted *in vitro* Analysis of 550 potential off-target sites of off-target editing. [2]Data from *in vivo* analysis from mouse bone marrow harvested 16 weeks after engraftment was complete. [3]Cas9 nuclease-edited cells, generated by transfecting HEK293T with sgRNA targeting *NCF1* and SpCas9 mRNA.
CGD = chronic granulomatous disease; HSC = hematopoietic stem cell; IND = investigational new drug; CTA = clinical trial application

LNP-PEs are complex drug products requiring multiple components that need to be controlled and tested

Need for pragmatic risk-based reduction in total number of assays when addressing all mutations in an indication

Common assays for mRNA, pegRNA, DPs

Potency, identity testing methods – needed for individual programs



Same types of assays used across all LNP based programs

Clinical modularity for platform trial

Steps to enable clinical modularity

- Multiple mutations and diseases studied in parallel or sequentially
- Safety aggregated across liver-directed LNP products
- Clinical experience in initial product leveraged to expedite dose finding in subsequent DP
- Biomarker/ efficacy outcomes pooled for same disease product
- Approval of products for rare variants based on non-clinical and CMC comparability

Streamline clinical trials from rare disease mutations to ultra rare disease mutations

	I Prevalent mutation(s) in WD	II Less prevalent mutations in WD	III Other rare mutations in WD or liver diseases
Indication / Mutation	Wilson's Disease H1069Q[2]	WD R778L, M769fs	WD and GSD1b rare variants
MRSD[1] / Dose Finding	MRSD based on toxicology; conventional dose finding	MRSD **informed by initial program**; abbreviated dose finding	**No clinical investigation required for WD**; leverage nonclinical mutation-specific assays, CMC comparability on key quality attributes (including potency), and use clinical safety and efficacy data from studies on common variants; **Fasting duration** to hypoglycemia for GSD1b surrogate biomarker
Surrogate Biomarker	Urinary and/or serum copper	Wilson's Disease: **Urinary** and/or **serum copper**	
Safety	Aggregate across all liver LNPs	Aggregate across all liver LNPs	
Additional Efficacy	Aggregate across WD variants	Wilson's Disease: Aggregate across WD variants	

1. Maximum recommended starting dose
2. Wilson's Disease and GSD1b are used as examples in this platform schematic

Regulatory challenges and proposed solutions

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Challenge	Proposed solution(s)
Diverse gene editing mechanisms exist, including nuclease, base and Prime Editing	Mechanism-specific risk assessment, e.g., less focus on double-strand break toxicity for modalities like base and Prime Editing, which do not generate double-strand breaks
Creating a preclinical animal model for every mutation is infeasible	Establish potency by correlation to well-established benchmarks in cell lines; use **surrogate editors** instead of tailor-made animal models
High burden of nonclinical studies for guides targeting different mutations	Within same delivery platform (e.g., liver-targeted LNP), **leverage data** for biodistribution, reproductive toxicology, germline editing, and GLP toxicology from earlier studies
Exhaustive genotoxicity risk assessment (off-targets, chromosomal changes)	Mechanism-based risk assessment, simplify assays for later Prime Editing products (e.g., more in silico approaches)
High burden of repetitive CMC data generation for platform manufacturing process and assays	Recognize platform-based CMC**:** once editor and delivery system are established, adding new guides is minor change - leverage process data, assay qualification and stability data; allow risk-based reduction of assays for additional variants (e.g., eliminate need for orthogonal assays)
Dose escalation and safety studies for small patient populations with rare mutations	Expedite or **eliminate the need for dose escalation**, especially for ultra-rare variants and leverage correlation in preclinical studies for dosing, and leverage aggregate data from common delivery platform
Multiple regulatory authorities with distinct expectations increasing cost and complexity for multiple individual filings	Regulatory agencies **harmonize** and align on common expectations, terminology, and IND/CTA format to reduce re-formatting and paperwork exercises for submissions