prime medicine

Delivering on the promise
of Prime Editing

Abstract 287

Developing Hotspot Prime Editors to enable therapeutic correction of multiple *CFTR* mutations in Cystic Fibrosis

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American Society of Gene & Cell Therapy
May 16th, 2025

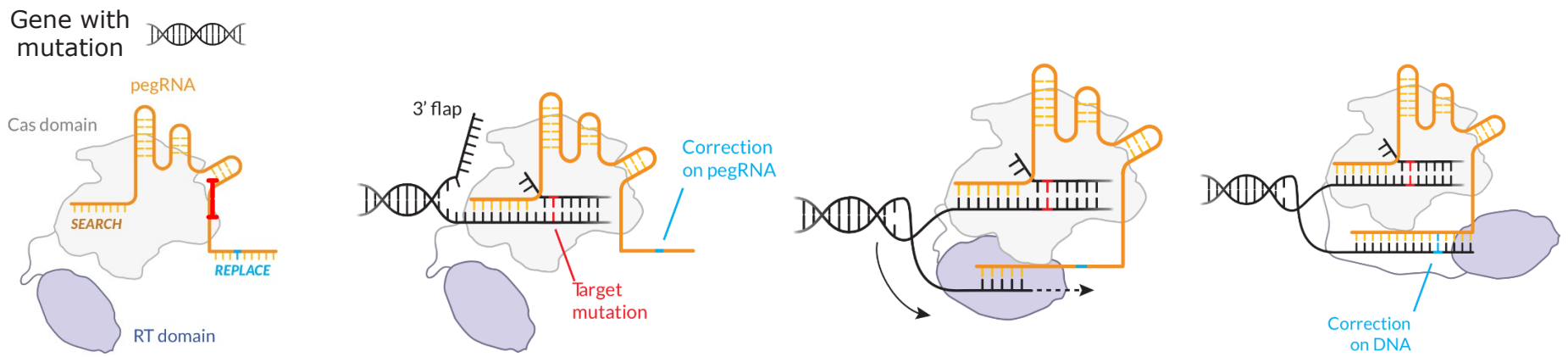
On behalf of CF Project Team

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

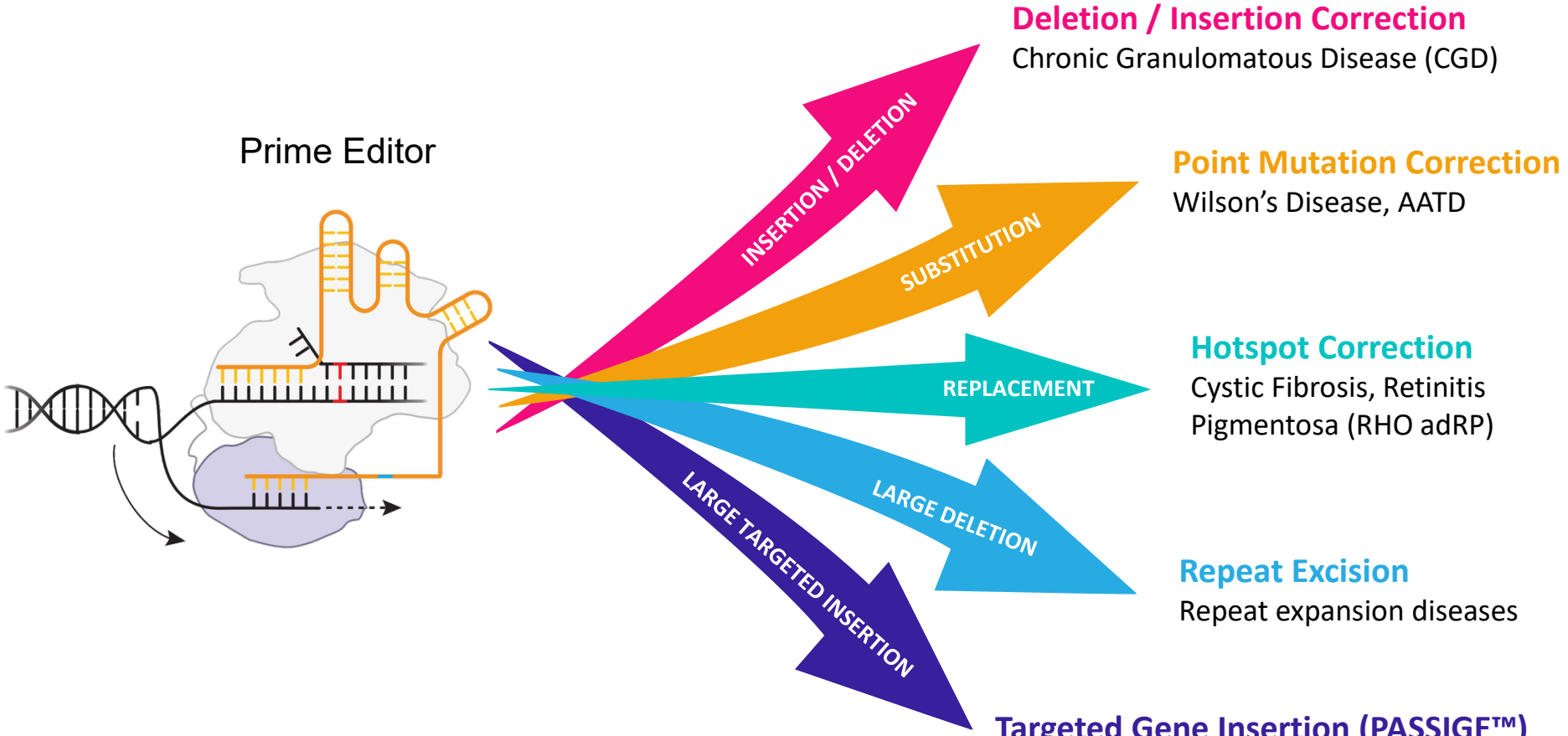
GENE CORRECTED

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's Disease, AATD

Hotspot Correction
Cystic Fibrosis, Retinitis Pigmentosa (RHO adRP)

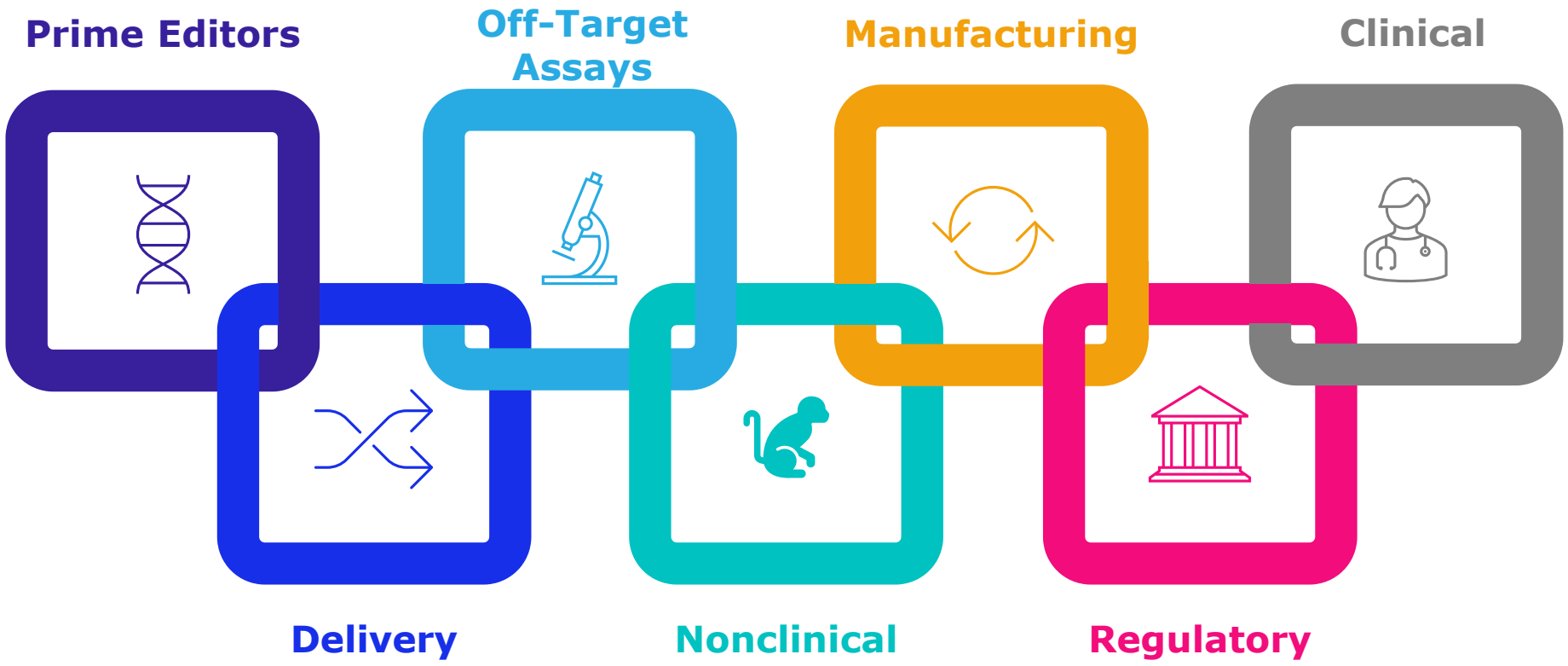
Repeat Excision
Repeat expansion diseases

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



Challenges and opportunities for developing treatment for Cystic Fibrosis

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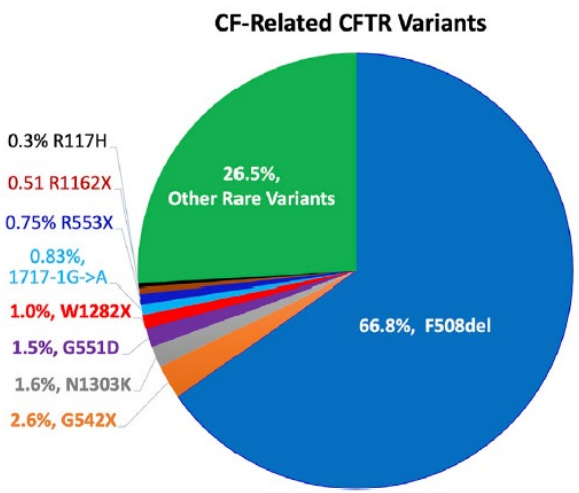
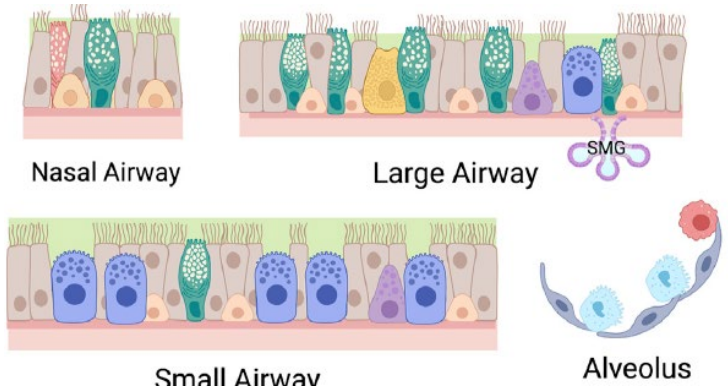
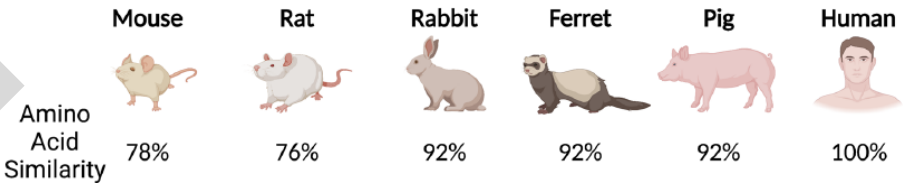


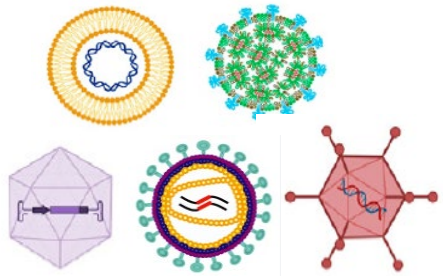
1 **> 360 disease causing mutations**

2 **Barriers to reach the cell types to correct the cause of the disease**



3 **Limited phenotypic translation of rodent disease model to human disease**





4 **Each delivery vehicle encounters different challenges to reach target cell type with safety profile to enable clinical efficacy**

Initial approach: correct prevalent mutational hotspots by Prime Editors

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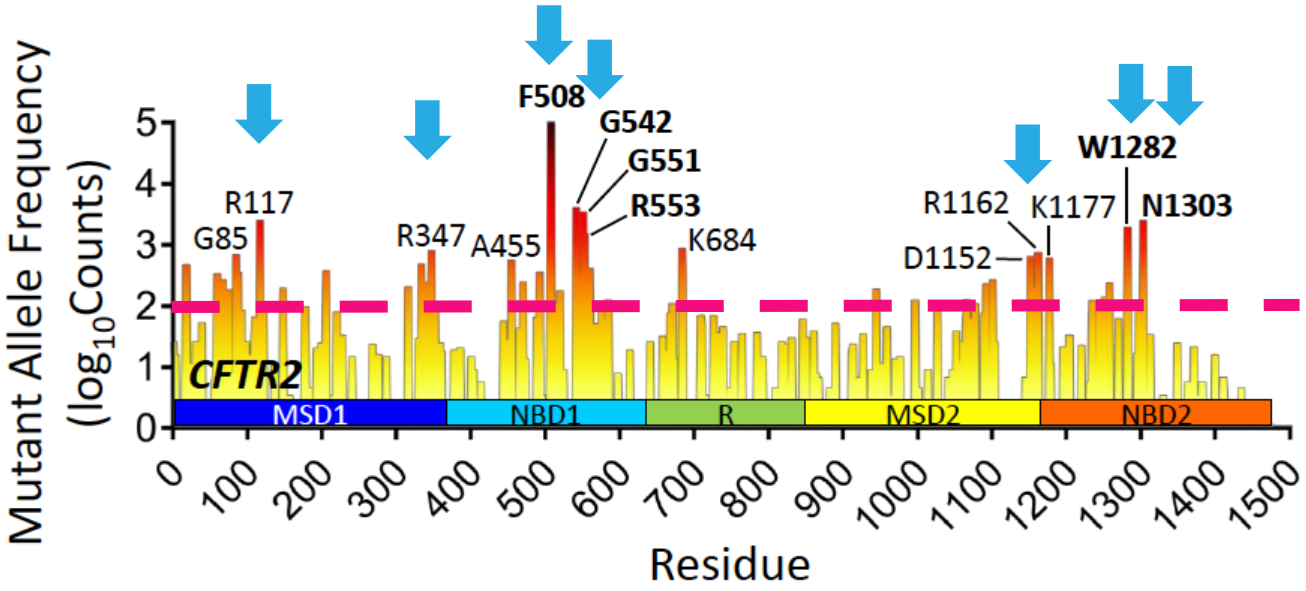
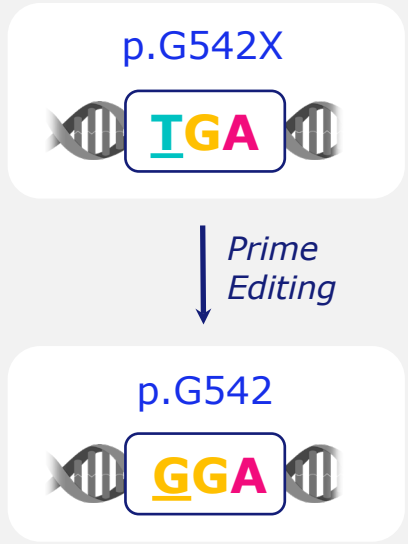
Correct the causative mutation back to normal wild type sequence in lung epithelial progenitors

7 hotspot correcting Prime Editors could address >93% of the patient population*



Precise Correction of *CFTR* p.G542X mutation in lung basal, club and goblet cells by Prime Editing



High unmet need mutations:

- All non-sense and frameshift mutations
- Mis-splicing mutations
- Missense mutations not responsive to current correctors or potentiators

Other high unmet need patients:

- In frame and other mis-sense mutations for patients who are intolerant of current standard of care

*correcting single copy of *CFTR* is sufficient to restore to healthy individual genotype

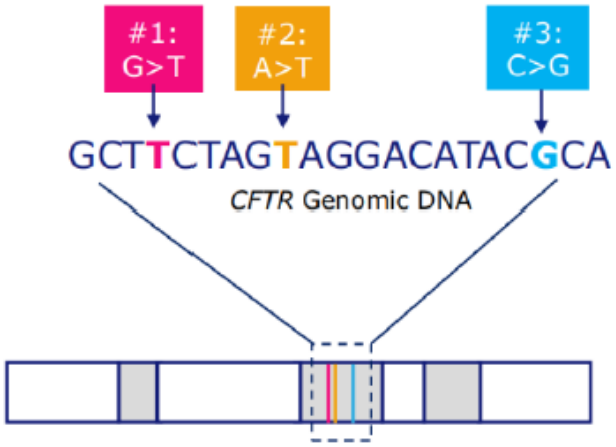
#Figure adapted from Molinski et al 2018

Parallel Prime Editing Approaches to CF: Hotspot & PASSIGE

In 2024, Prime Medicine entered into agreement with CF Foundation to support development of Prime Editors for Cystic Fibrosis

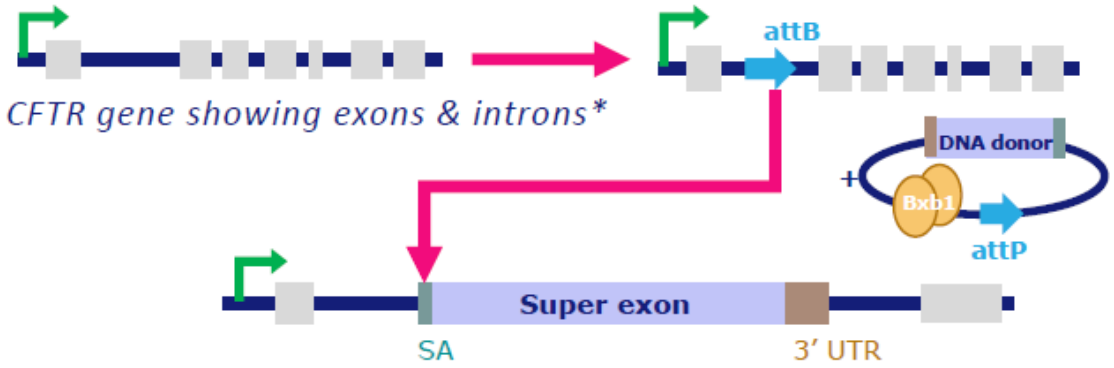
Hotspot

Eight hotspot Prime Editors could address the "high unmet need" mutations; these same Prime Editors could address >93% of all CF patients



PASSIGE

Potential to address nearly all CF patients with a single super exon insertion strategy

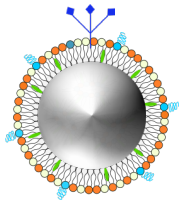


Restoring CFTR function in Prime Edited cells under endogenous control

CF = Cystic fibrosis; attP = complimentary recognition sequence for attb; SA = spliace acceptor; 3'UTR = 3' untranslated region; Exons in gray; introns in black;

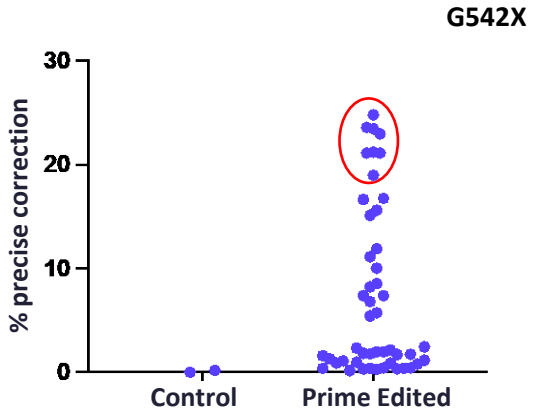
Highly active CFTR Prime Editors for correcting multiple Cystic Fibrosis mutational hotspots*

Exemplary Prime Editors shown here for CFTR G542X correction

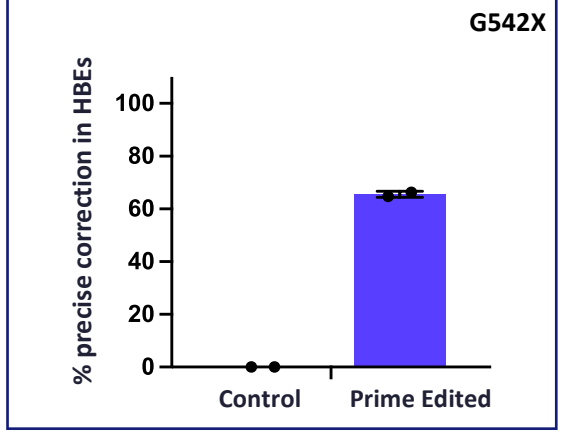


Modular delivery system

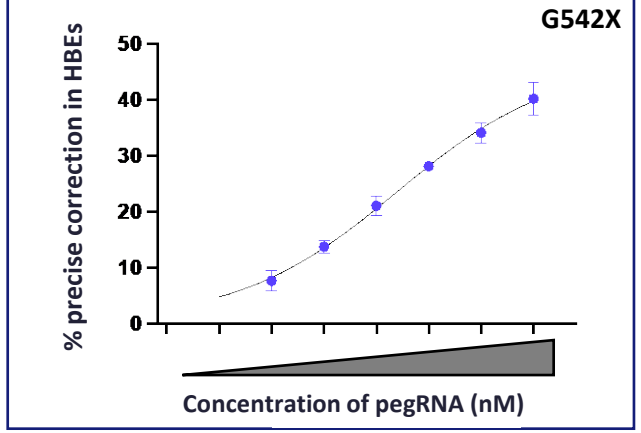
In vitro Prime Editor in initial High Throughput Screen



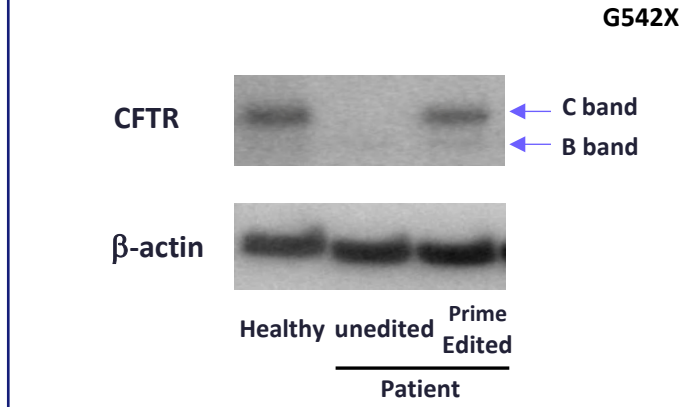
In vitro Prime Editor in Primary Human Lung Progenitors (HBEs)



Highly potent Prime Editor in Primary Human Lung Progenitors (HBEs)



Restoration of CFTR protein level in differentiated ALI culture



In vivo delivery to humanized mice and large animals ongoing

- We believe primary human lung progenitor data most predictive of *in vivo* efficacy
- Comprehensive suite of assays in development to enable selection of development candidate and advance to IND enabling studies
- Humanized mouse colonies, ferret / NHP colonies established for *in vivo* optimization
- Prime's targeted modular lung LNP as well as alternative delivery system are being applied to accelerate CF hotspot editing *in vivo*

CFTR = cystic fibrosis transmembrane conductance regulator; HBEs = human bronchial epithelial cells; ALI = air-liquid interface; Genotype of patient HBEs used in ALI experiment is G542X/621+1G->T
* Approximately 77% of Cystic fibrosis patients have the G542X, R553X, G551D, N1303K, F508del mutations combined in USA and Europe.

Restoration of CFTR function in human bronchial epithelial cells and patient-derived intestinal organoids with unoptimized G542X Prime Editor

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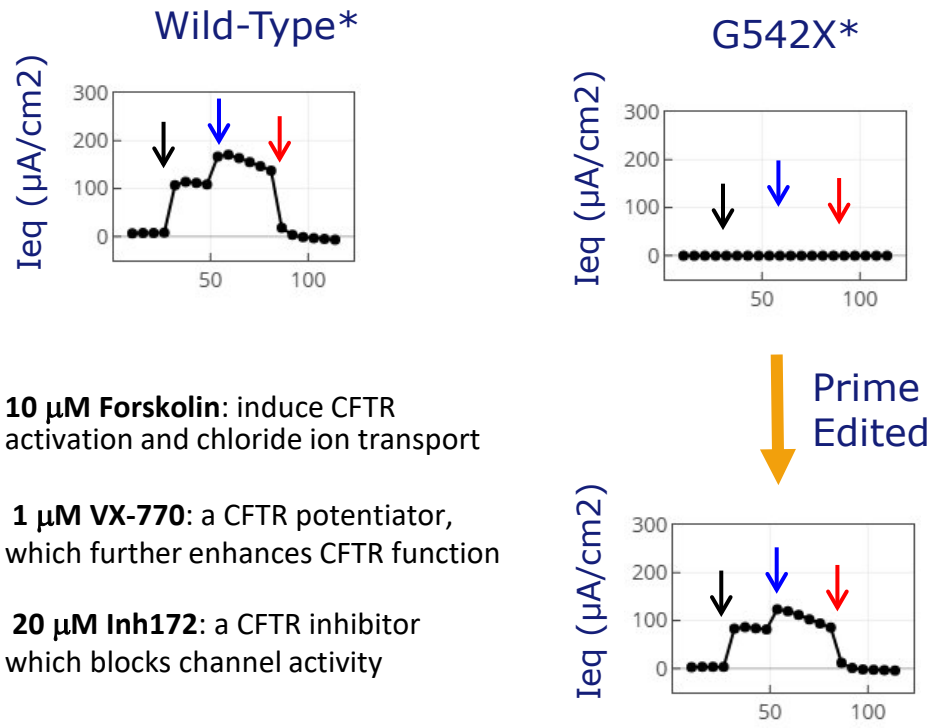
One-time, non-viral delivery to patient *CFTR*$^{G542X/G542X}$ cells restores CFTR function

Delivery of Prime Editors to p.G542X HBEs restores CFTR function and transepithelial chloride conductance in I_{eq} ephys assay



↓ **10 µM Forskolin**: induce CFTR activation and chloride ion transport

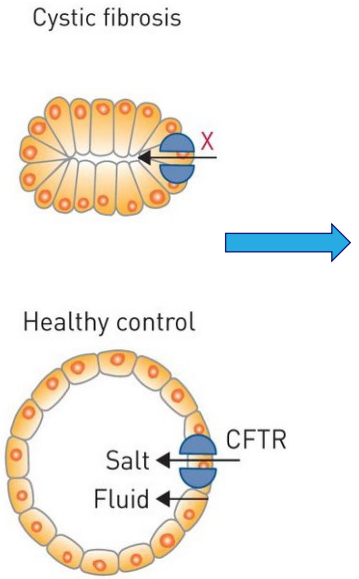
↓ **1 µM VX-770**: a CFTR potentiator, which further enhances CFTR function

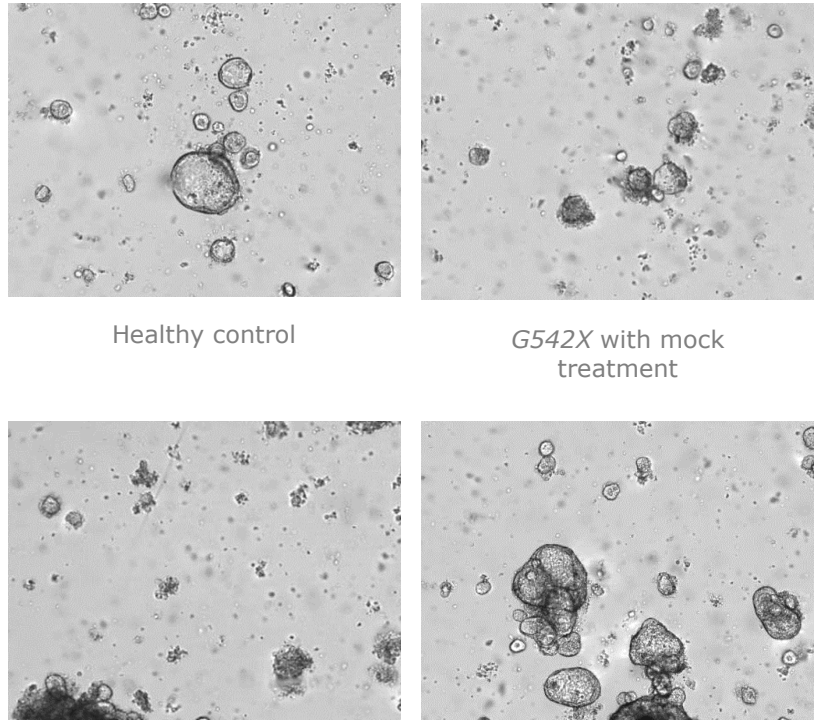
↓ **20 µM Inh172**: a CFTR inhibitor which blocks channel activity

Data generated by CFF lab, Lexington, MA

Delivery of Prime Editors to intestinal organoids derived from primary HBE cells with G542X mutation restores swelling and CFTR function

Intestinal organoids swelling assay for CFTR function





Healthy control

G542X with mock treatment

G542X with TRIKAFTA® treatment

G542X with Prime Editing correction

*16HBE14o- (Wild-Type cells) and 16HBEge G542X/G542X cells were used in I_{eq} assay. Intestinal organoids cartoon were adapted from Van Mourik et al., 2019. Actual time course: 24 hours.
TRIKAFTA® is a registered trademark of Vertex Pharmaceuticals, Incorporated.

Prime's approach is to deliver Prime Editors to critical epithelial cells in patient lung for efficacy and durability

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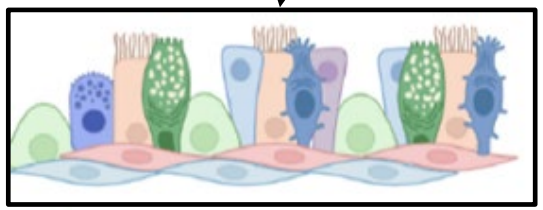
Prime Editors correct the CFTR gene to normal resulting in physiological control of CFTR

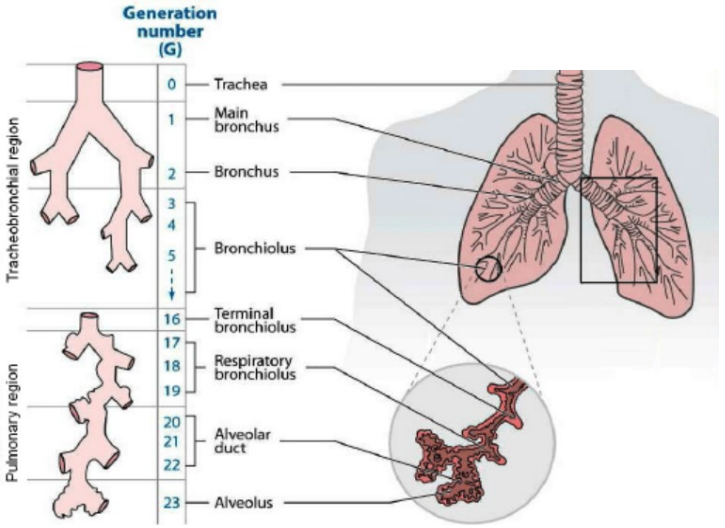
Prime Editors



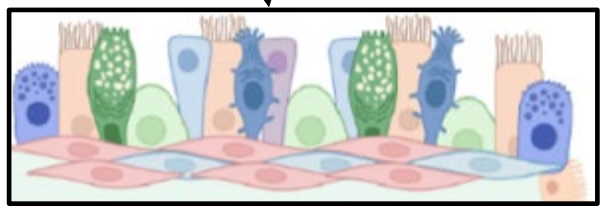
Basal Cells

- containing human lung progenitor cells (HBEs) or lung stem cells

DURABLE (PERMANENT) Correction of CFTR Function



Goal is to target the bronchial airways all the way to the alveolus



Club Cell, Goblet cell & other secretory epithelial cells

SHORT-TERM (ACUTE) Correction of CFTR Function

CFTR = cystic fibrosis transmembrane conductance regulator; HBEs = human bronchial epithelial cells

Viral (AAV) & non-viral (LNP) delivery systems are effective at delivering Prime Editors *in vivo*

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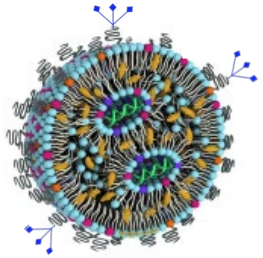
Prime Medicine Lung Delivery Platform



Non-Viral Delivery

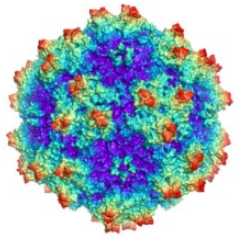
LNP

- LNPs tolerated in clinical trials for mRNA therapies
- Leveraging our internal capabilities

Viral Delivery

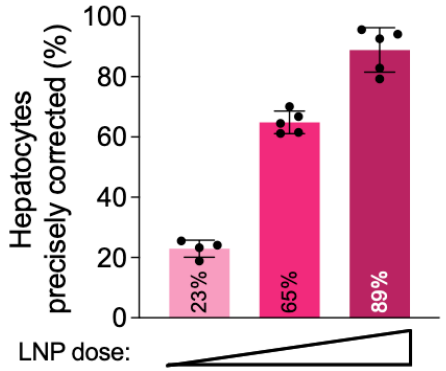
AAV

- Safe and tolerated in clinical trial with lung-trophic AAV capsid



Liver

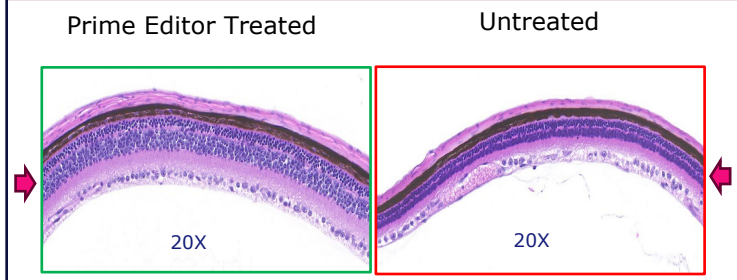
In-vivo editing using an optimized LNP-RNA prime editor for a liver target



➢ Well tolerated, translatable dose for therapeutic use

Eye

Subretinal injection of Dual-AAV Prime Editor preserves the outer nuclear layer photoreceptors in mice harboring pathogenic RHO p.P23H mutation



➢ Well tolerated out to 1 year, translatable dose for therapeutic use

LNP: lipid nanoparticles; AAV adeno associated virus

In vivo LNP screen identified Prime-designed LNPs that effectively deliver Prime Editors to lung epithelium

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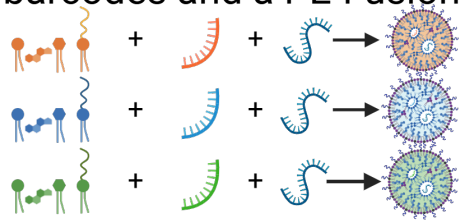
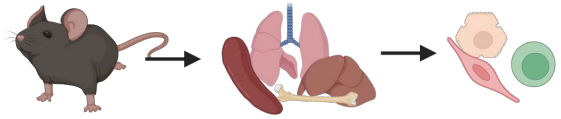
Established an in-vivo LNP HT screening platform tailored for Prime Editing

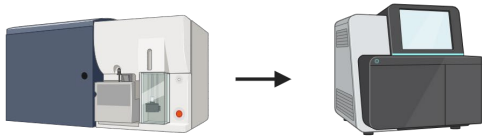
~100 lipid compositions paired with unique barcodes and a PE-Fusion mRNA



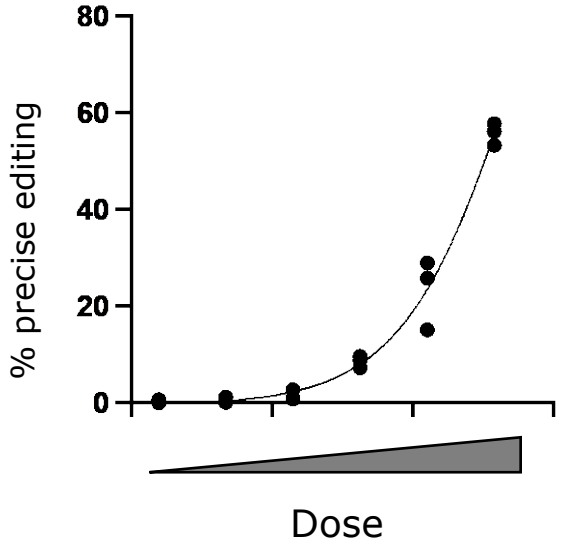
In vivo administration and tissue dissociation



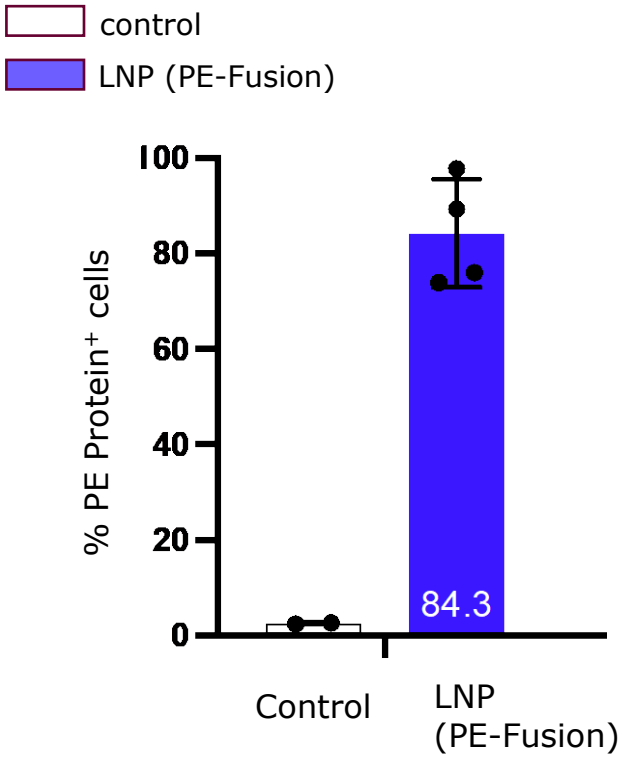
Sorting on functionally delivered cells and sequencing for relative barcode abundance



LNP-RNA prime editors demonstrated efficient G542X editing in patient HBEs



Delivery tropism to ciliated and club cells using of our lead LNP from HT LNP screen



PE = Prime Editor, HT = high-throughput, HBE = human bronchial epithelial cells

Initial PoC in humanized *CFTR* p.G542X mice with an unoptimized CFTR Prime Editor delivered by dual AAV using lung trophic capsid

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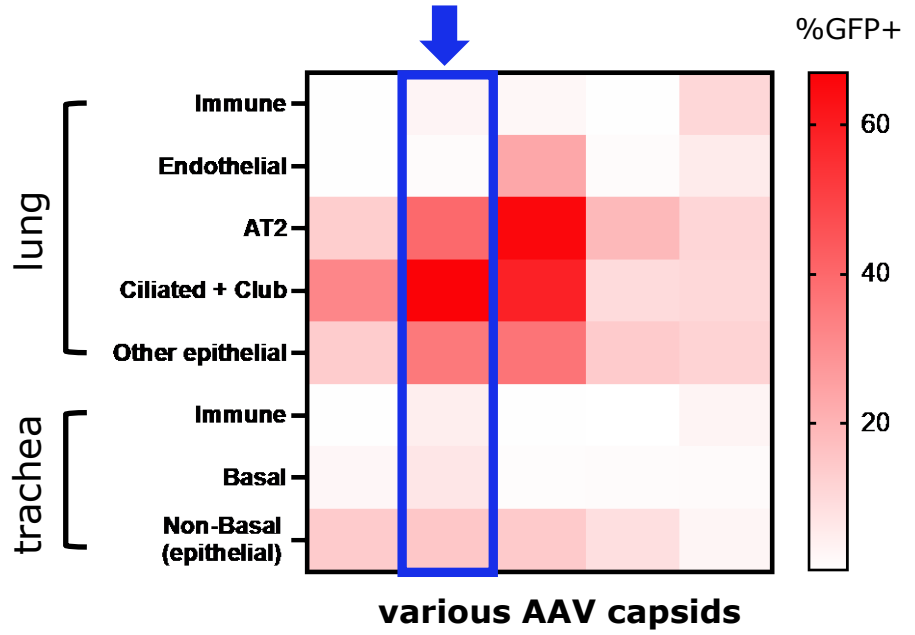
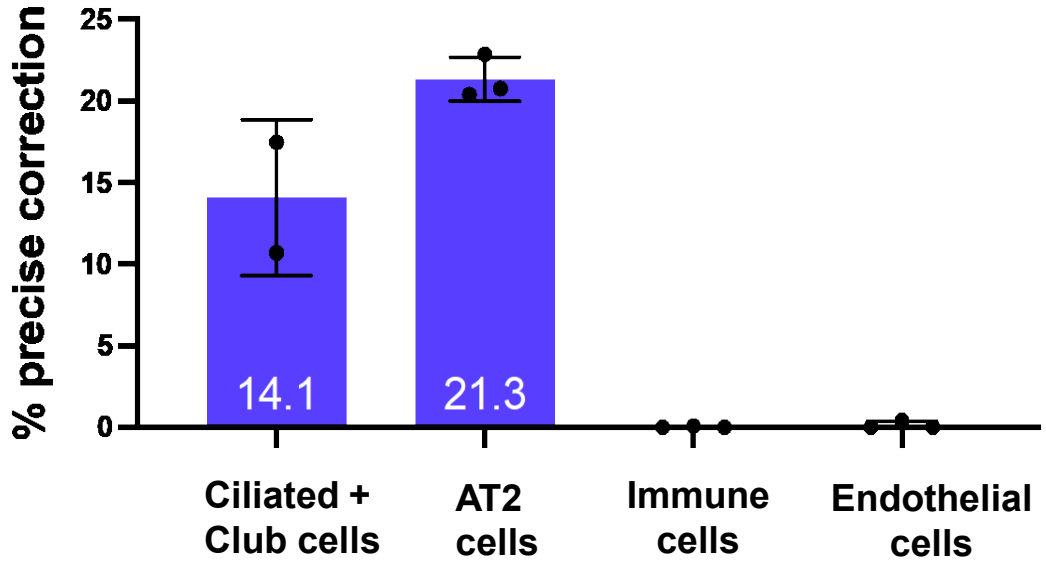
Lead optimization of p.G542X Prime Editors is currently ongoing

Several AAV capsids efficiently transduce mouse lung epithelium and modestly transduce trachea basal cells via airway administration



Single dose, bronchoscopic delivery of Dual-AAV Prime Editor results in precise correction of *CFTR* p.G542X in multiple lung epithelial cell types including club, ciliated and AT2 cells



- Editing detected in lung epithelial cells only, not in immune and endothelial cells

Prepared Dual AAV tool G542X Prime Editor → Delivery to *CFTR* p.G542X mice → 2-3 weeks duration; whole lung digest → Flow sorting of epithelial cell populations → Quantify editing by NGS

AT2 = Alveolar type II cells

Summary

- Prime Editing is a potentially best-in-class gene editing technology
- Hotspot Prime Editing to correct multiple mutations could address many patients with cystic fibrosis: 7 Hotspot Prime Editors could address >93% of patients with cystic fibrosis
- Prime's LNP-RNA capabilities have provided LNPs exhibiting efficient and well tolerated Prime Editing in the liver in animal studies
- Prime has identified LNPs that deliver Prime Editors to secretory and progenitor cells in lung epithelium *in vivo* and human bronchial epithelial progenitors *ex vivo*
- Initial Prime Editors to correct p.G542X delivered by LNP show high editing efficiency and restore CFTR function in patient lung epithelial progenitors and intestinal organoids
- Prime is developing LNP and AAV systems to deliver Prime Editors to secretory and progenitor lung epithelium in vivo
- Established initial proof of concept that Prime Editing can correct CFTR p.G542X in the bronchial epithelium *in vivo* in humanized mice

Thank you!

Marsico Lung Institute

The MLI Tissue Procurement and Cell Culture Core

CYSTIC FIBROSIS FOUNDATION
ADDING TOMORROWS

In collaboration with
Cystic Fibrosis Foundation

CASE WESTERN RESERVE UNIVERSITY

CYSTIC FIBROSIS MOUSE RESOURCE CENTER

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Thank you!

Delivering on the promise
of Prime Editing