Developing Hotspot Prime Editors to Enable Therapeutic Correction of Multiple CFTR Mutations in Cystic Fibrosis

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In collaboration with Cystic Fibrosis Foundation

Abstract

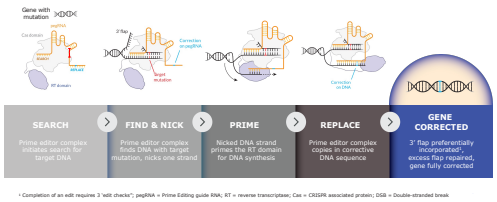
Cystic fibrosis (CF) is a life-threatening, monogenic autosomal recessive disease caused by mutations in the *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) gene. These mutations result in loss of chloride ion transport across epithelial membranes, leading to mucus accumulation in multiple organs, especially lung and pancreas. The mucus in the lung promotes chronic bacterial infections, inflammation and progressive lung damage, which are the primary causes of mortality in CF patients. Despite significant advancements in therapies such as CFTR modulators, patients that are responsive to the modulators require live-long treatment and more than 15% of CF patients have non-responsive mutations and are left without treatment. Genetic therapies for CF have been under development using different delivery vehicles, with limited success. There is a significant unmet medical need for a one-time, disease-modifying therapy for these patients. Prime Medicine is developing Prime Editors to durably and precisely correct multiple mutational hotspots with high unmet need and aiming to achieve permanent restoration of CFTR expression under endogenous control and normal CFTR function. These same Hotspot Prime Editors taken together could address >93% of all CF patients.

Comprehensive high throughput screening for Prime Editors led to identification of potent editors to precisely correct prevalent and non-sense *CFTR* mutations across multiple mutational hotspots including G542X. These Prime Editors demonstrated >60% of precise correction in patient-derived primary Human bronchial epithelial (HBE) cells, and restoration of CFTR expression in HBE cells differentiated air-liquid interface culture. Correction of *CFTR* mutations also leads to normalization of Cl^- current in an electrophysiology assay, indicating a functional rescue of CFTR. We next evaluated our *CFTR* Prime Editors *in vivo* in a humanized mouse model with *CFTR* p.G542X mutation ($Cftr^{CFTR\ p.G542X/Cftr}$). To deliver Prime Editing components *in vivo*, we are developing a lipid nanoparticle (LNP) system formulated with Prime Editor components in the form of RNA (LNP-PE). We also delivered Prime Editors using a dual-AAV system in development. Following single dosing of PE test articles, we observed encouraging editing activity in lung epithelium including epithelial progenitors, prior to *in vivo* optimizations, indicating this approach has potential to be disease-modifying.

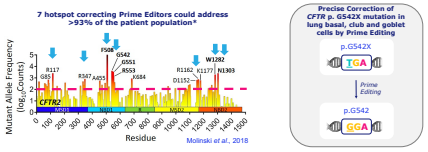
Together, these results highlight that LNP or AAV-delivered Prime Editors have the potential to precisely and efficiently correct the high unmet-need pathogenic mutations causing CF. Prime Editing offers the potential of a safe and effective curative approach for patients impacted by CF.

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

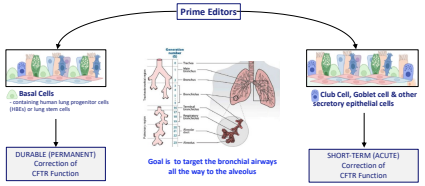


Initial approach: correct prevalent CFTR mutational hotspots by Prime Editors



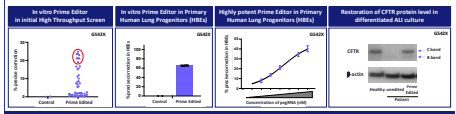
Prime's approach is to deliver Prime Editors to critical epithelial cells in patient lung for efficacy and durability

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



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

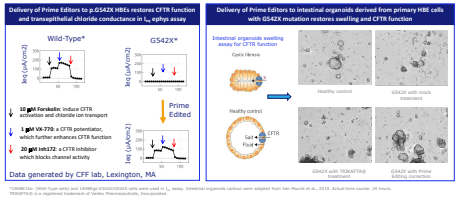
Exemplary Prime Editors shown here for CFTR G542X correction



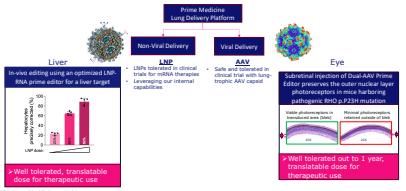
In vivo delivery to humanized mice and large animals ongoing

- 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*

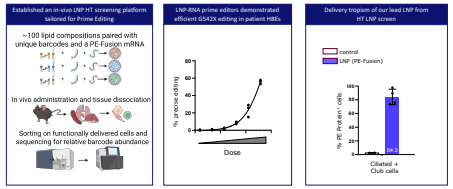
Restoration of CFTR function in human bronchial epithelial cells and patient-derived intestinal organoids



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

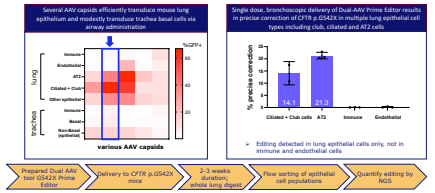


In vivo LNP screen identified Prime-designed LNPs that effectively deliver Prime Editors to lung epithelium



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

Lead optimization of p.G542X Prime Editors is currently ongoing



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

Abbreviations: AAV = Adeno-associated Virus; ALI = Air Liquid Interface; AT2 = Alveolar Type II cells; CFTR = Cystic Fibrosis Transmembrane conductance Regulator; HBE = Human Bronchial Epithelial cells; HT = High Throughput; LNP = Lipid Nanoparticle; NHP – Non-human Primate; PE = Prime Editor