Functional Rescue of CFTR by Aerosolized Delivery of Optimized CFTR mRNA Using ReCode LNPs in Primary Human Bronchial Epithelial Cells Derived From Patients With Cystic Fibrosis

Daniella Ishimaru1, Dmitri Boudko1, Ella A. Meleshkevitch1, Maninder S. Sidhu1, Julia R. Poniatowski1, Peiyang Gao1, Touhidul I. Molla1, Sierra R. Comini1, Harriet E. Lister1, Melissa L. Coquelin1, Crystal Johnson1, Ali Alfai1, Omid M. Mousa1, Xueliang Yu1, Rumpa B. Bhattacharjee1, David Liston1, Jackson K. Eby1, Mirko Hennig1, Robert J. Bridges2, Philip J. Thomas3, Vladimir G. Kharitonov1, Brandon A. Wustman1, David J. Lockhart1, Michael J. Torres1
1ReCode Therapeutics, Inc, Dallas TX and Menlo Park, CA, United States; 2Rosalind Franklin University of Medicine and Science, North Chicago, IL, United States; 3University of Texas Southwestern Medical Center, Dallas, TX, United States

Cystic fibrosis (CF) is a progressive, genetic disease affecting a chloride channel, CFTR, located on the apical plasma membrane of specialized epithelial cells. Defective pulmonary mucociliary clearance (MCC) is one of the main phenotypes in CF patients. Because a significant fraction of the CF patient population is not amenable to currently approved CFTR modulators such as Trikafta, the search for universally applicable therapies that promote CFTR function and mucus clearance remains a goal. To address this challenge, ReCode Therapeutics is advancing an mRNA-based treatment to rescue CFTR function using its proprietary lipid nanoparticle (LNP) platform and optimized CFTR mRNA sequences delivered as an inhaled aerosol.

ReCode’s sequence optimization of CFTR mRNA eliminates hydrolysis hot-spot

Native or ReCode-optimized CFTR mRNAs were in vitro transcribed in the presence of unmodified or modified nucleotides, where indicated. Purified samples were analyzed on a Fragment Analyzer (Agilent). Larger peak corresponds to full-length CFTR mRNA. A specific hydrolysis hot-spot (arrows) was observed with the wild-type, native CFTR mRNA sequence. In vitro transcription of CFTR(wt) mRNA in the presence of modified nucleotides reduced the observed hydrolysis (middle panel). Combination of ReCode’s optimized sequence and modified nucleotides allowed for synthesis of full-length CFTR mRNA (bottom panel).

Higher doses of LNP A formulated CFTR mRNA are well-tolerated in G542X/ΔF508 cells

Fully differentiated cells were nebulized with low, medium, or high doses of HA-CFTR mRNA formulated with ReCode’s proprietary formulation A (LNP A). Analysis was performed 24h post-nebulization. CFTR function was described as above. On the top right panel, representative traces. Bottom left, Percentage of LDH release relative to whole cell lysate (=100%). For reference, historical data with cystic fibrosis LNPs showed values for released LDH at ~60%. Here we show that even at the highest dose delivered, no cytotoxicity was detected with ReCode’s LNP. TEER measurements show similar values among all conditions (<500 Ohm/cm).

Evaluation of modified nucleotides and selection of a lead mRNA

Three days old confluent FRT cells grown on TransWell permeable support were transfected with different CFTR mRNAs using Lipofectamine 2000. MTECC24 assay of the transepithelial resistance (R) was performed in the presence of forskolin 5 µM. On the left, transfection efficiency of different amounts of optimized CFTR mRNA was performed 24 h post-transfection. On the right, functional rescue of CFTR was done using the wild-type, native CFTR mRNA sequence (A) in FRT and primary hBEs. Both FRT and primary hBEs show rescued CFTR function with modified nucleotides. CFTR mRNA restores function in CF patient-derived hBE cells including genotypes not responsive to modulators

Conclusions

Our results demonstrate the capability of the ReCode’s LNP platform to deliver optimized functional CFTR mRNA in well-differentiated CF hBE cultures as an aerosol. These preclinical data support further investigation and provide a practical approach to target a significant patient population that does not benefit from current CFTR modulator therapy.

Disclosures