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 proprietary formulation A (LNP A) is the top performer in G542X/ΔF508 hBE cells**

Fully differentiated cells were evaluated using a commercially available mesh neutrophil assay with ReCode’s LNP formulation containing CFTR mRNA. The top panel, CFTR function was determined as forskolin-induced and bumetanide-suppressed current measured with robotic transmembrane current clamp system (MTEC244). Representative traces are shown on the right. ReCode’s LNP A formulated CFTR mRNA shows the highest rescue of CFTR function.

**Evaluation of modified nucleotides and selection of a lead mRNA**

Native or ReCode-optimized CFTR mRNAs were evaluated 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 (arrow) 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).

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

Three days old confluent FRT cells grown on TransWELL permeable support were transfected with different CFTR mRNA using Lipofectamine 2000. MTET244 assay of the transcellular conductance was performed 24h post-transfection. On the left, transcellular conductance (Gj) responses, bars are 0.1-under the curve area under the forskolin and inhibitor-172 addition time-points. On the right, representative conductance kinetic traces in FRT monolayer after addition of CFTR modulators, as indicated. We identified a CFTR mRNA sequence and composition with improved stability that is stably maintained in functional CFTR protein compared to the wild-type sequence, both in FRT and primary NBE cells (not shown). (Note: the CFTR(wt) mRNA was transcribed in the presence of modified nucleotides to favor the presence of full-length mRNA. Utilization of modified nucleotides also lead to reduced immunoreactivity in vitro (see poster P316: Optimization of DNA1 mRNA Constructs to Treat Primary Ciliary Dyskinesia).

**CFTR mRNA restores function in CF patient-derived hBE cells including genotypes not responsive to modulators**

NBE cells with Trikafta-responsive and non-responsive CF genotypes were grown on permeable support in 24 well TransWELL plate. Fully differentiated cells were evaluated with high dose of HA- CFTR mRNA formulated with ReCode’s proprietary formulation A (LNP A). Analysis was performed 24h post-nebulization. CFTR function was determined as described before. On the left panel, representative traces for the W1282X/W1282X genotype.

**Disclosure**

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