Aerosolized lipid nanoparticle/mRNA therapy to treat patients with Cystic Fibrosis

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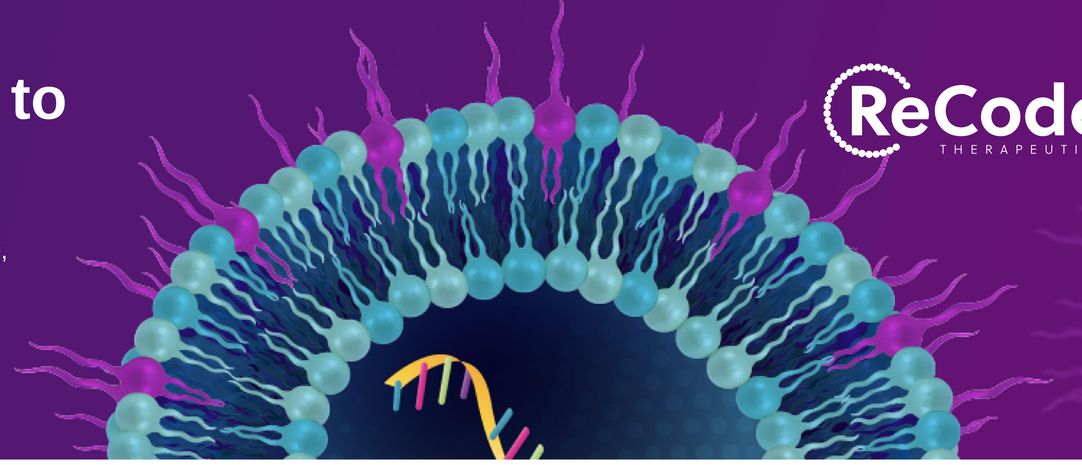
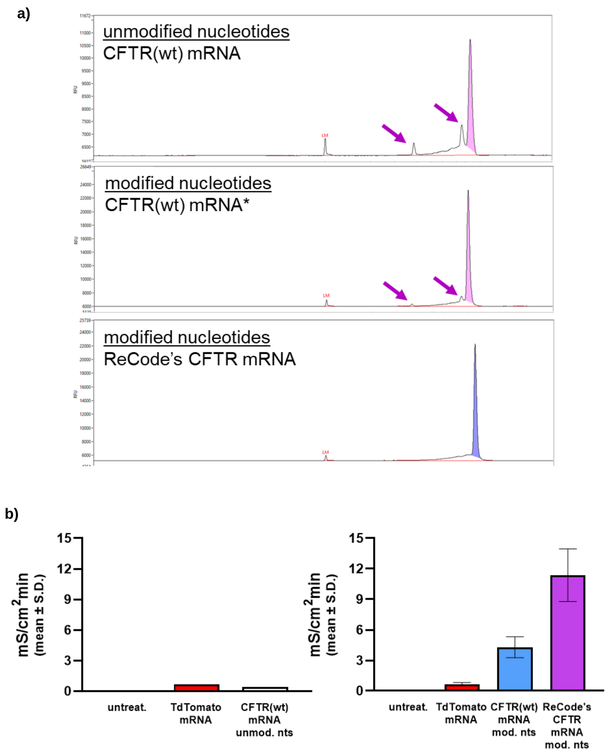


INTRODUCTION

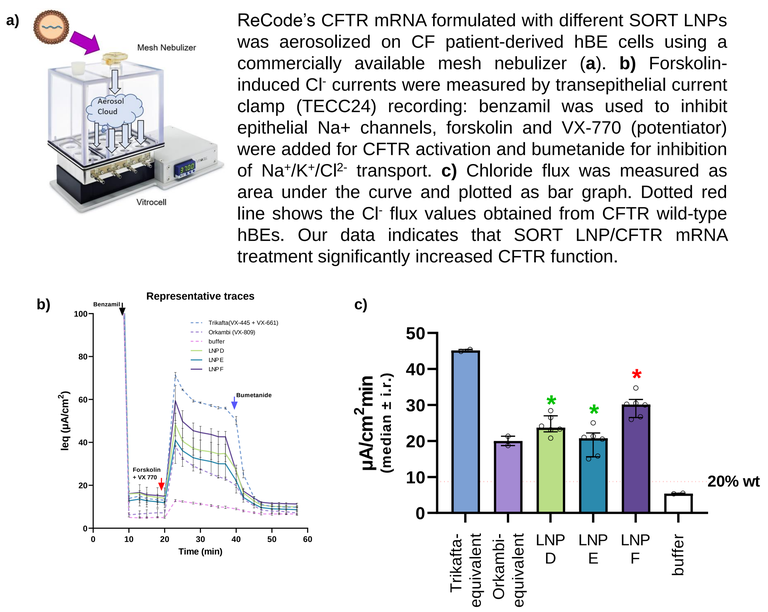
Cystic fibrosis (CF) is an inherited disease caused by mutations in the CFTR gene that encodes a chloride channel, CFTR, located on the apical plasma membrane of specialized epithelial cells. CF causes grave damage to the lungs, digestive system, and other organs, with defective pulmonary mucociliary clearance (MCC) being one of the main causes of morbidity and mortality in CF patients. The approval of small-molecule CFTR modulators such as Trikafta significantly improved the quality of life for most CF patients with access to these drugs. However, a significant fraction of the CF patient population is not amenable to the currently approved CFTR modulators. Therefore, the search for universally applicable therapies that promote CFTR function and MCC remains a goal. To address this challenge, ReCode Therapeutics is advancing an mRNA-based treatment to restore CFTR function using optimized CFTR mRNA encapsulated in proprietary lipid nanoparticles (SORT LNPs) delivered as an inhaled aerosol directly to the target epithelial cells of the conducting airways.

ReCode's CFTR mRNA optimization eliminates hydrolysis hot-spot



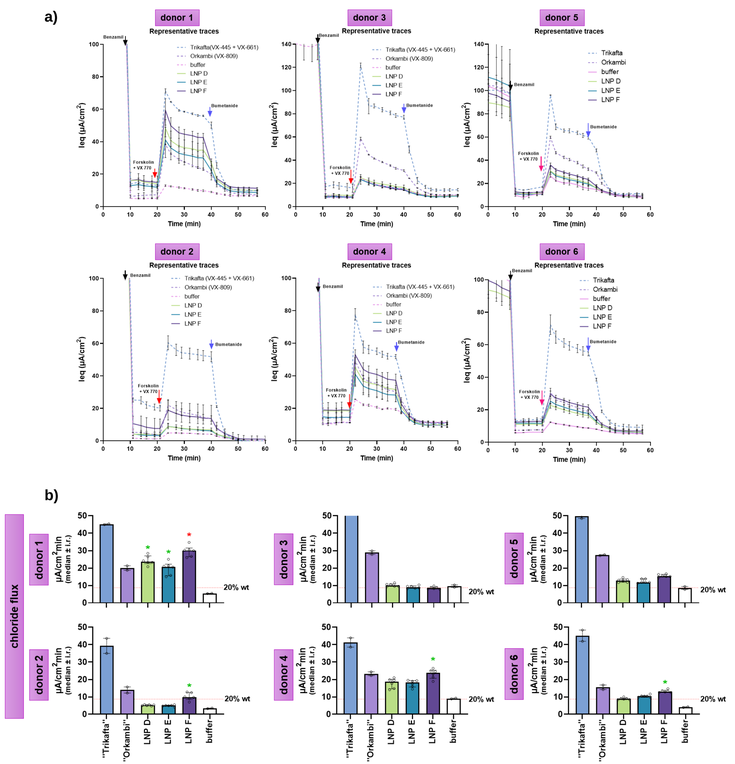
Native (wild-type, wt) or ReCode-optimized CFTR mRNAs were *in vitro* transcribed in the presence of unmodified or modified nucleotides, where indicated. **a)** Purified mRNA samples were analyzed on a Fragment Analyzer (Agilent). Larger peak corresponds to full-length CFTR mRNA (LM = lower marker). A specific hydrolysis hot-spot (arrows) was observed with the wild-type, native CFTR mRNA sequence (top and middle panels). *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 eliminated the presence of the hydrolysis hot-spot, increasing the amount of full-length mRNA (bottom panel). **b)** Fischer Rat Thyroid (FRT) cells were transfected with the mRNAs described on **a)** and CFTR-mediated conductance was measured. Graphs show a direct correlation between amount of full-length CFTR mRNA and conductance, indicating that the highest electrical current was observed when cells were transfected with ReCode's sequence-optimized mRNA synthesized with modified nucleotides. TdTomato mRNA was used as negative control. Data is plotted as mean ± standard deviation.

SORT LNPs are successfully delivered as aerosol to CF patient-derived cells



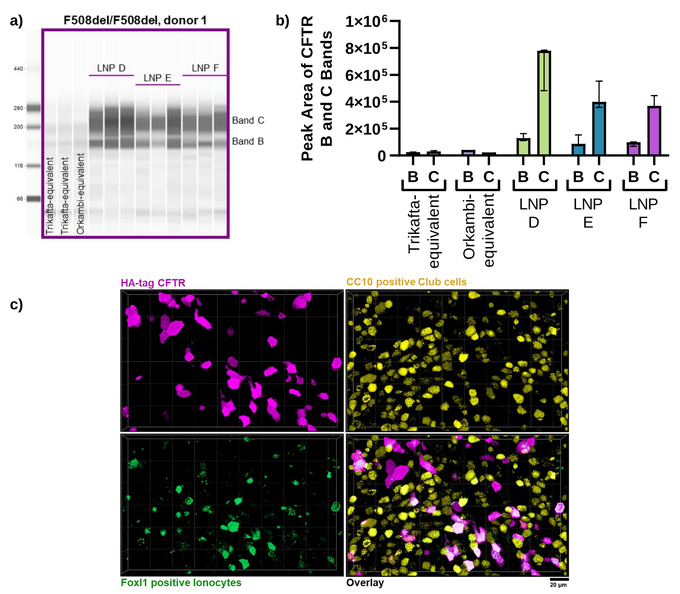
ReCode's CFTR mRNA formulated with different SORT LNPs was aerosolized on CF patient-derived hBE cells using a commercially available mesh nebulizer (**a**). **b)** Forskolin-induced Cl^- currents were measured by transepithelial current clamp (TECC24) recording: benzamil was used to inhibit epithelial Na+ channels, forskolin and VX-770 (potentiator) were added for CFTR activation and bumetanide for inhibition of $Na^+/K^+/Cl^{2-}$ transport. **c)** Chloride flux was measured as area under the curve and plotted as bar graph. Dotted red line shows the Cl^- flux values obtained from CFTR wild-type hBEs. Our data indicates that SORT LNP/CFTR mRNA treatment significantly increased CFTR function.

Different donors show different levels of CFTR rescue



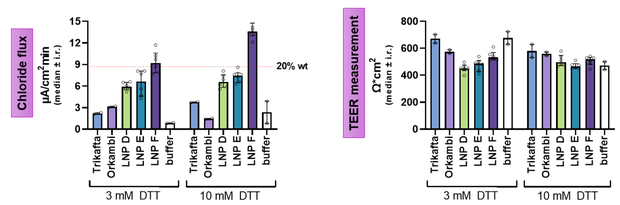
As described above, CFTR mRNA formulated with different SORT LNP compositions were aerosolized on CF patient-derived F508del/F508del hBEs. **a)** Twenty-four hours post-nebulization, transepithelial current was measured. **b)** Dotted red line represents the Cl^- flux values obtained from CFTR wild-type hBEs. Positive controls utilized: VX-445 + VX-661 + VX-770 ("Trikafta") and VX-809 + VX-770 ("Orkambi"). The buffer in which all LNPs were stored was used as negative control. All samples were treated with VX-770. Here, we investigated six different donors and observed that the level of CFTR functional rescue varied. Two main groups were detected, one with significant CFTR functional improvement (donors 1, 2, 4, and 6) and a second group with a minimal increase in function (donors 3 and 5) (bar graphs).

HA-CFTR was successfully detected in pulmonary ionocytes



F508del/F508del hBE cells were treated as described in previous figures. Cell lysates were obtained after conductance measurement. Proteins were analyzed by western-blot. **a)** Gel image of loaded samples. **b)** Quantification of bands B and C from image shown in **a)**. Note that CFTR was successfully detected in hBEs aerosolized with LNPs D, E, and F. Importantly, detection of "band C" suggests proper post-translational modification of CFTR. **c)** Representative IF images of F508del/F508del hBE cells treated with SORT LNP + HA-CFTR mRNA. Cells were fixed and labeled with anti-HA, anti-CC10 or anti-FoxI1 antibodies. Notice in some cells the co-staining of HA-tag and FoxI1 and a few with HA-tag and CC10.

Potential mucus effect on CFTR functional rescue



Ninety-six hours pre-nebulization, CF patient-derived hBEs were washed with 3 mM or 10 mM DTT. At 24h pre-treatment and on the day of treatment, cells were washed with 1x PBS. Cell cultures were then aerosolized as described above. The graphs here show the chloride flux and the transepithelial electrical resistance (TEER) measured at 24h post-treatment. Our results indicate that a more stringent wash with 10 mM DTT correlated with higher levels CFTR functional rescue (left graph, Chloride flux), while no adverse effect was observed on the integrity and permeability of the cell monolayer (right graph, TEER measurement).

CONCLUSION

Our results demonstrate the capability of the ReCode **SORT** LNPs to deliver LNP-formulated CFTR mRNA as an aerosol and increase CFTR function in well-differentiated CF hBE cultures. These preclinical data support further investigation and provide a practical approach to address a significant fraction of the patient population that does not benefit from current CFTR modulator therapy.

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