[1,2], the proteins translated from this variant are expected to mature and form partially active channels on the cell membrane. Addition of modulators is likely to enhance maturation and improve the function of these CFTR channels. This approach is expected to provide treatment for about 2500 patients carrying the N1303 K mutation.
Methods: Lead ASO selection was performed by transfecting ASOs designed to skip over exon 24 into 16HBEge N1303 K cells. After transfection, ribonucleic acid was extracted and analyzed for exon 24 skipping by reverse transcription polymerase chain reaction and quantitative polymerase chain reaction. The selected lead ASO was assessed using the Ussing chamber system in 16HBEge N1303 K and human nasal epithelial (HNE) cells derived from patients carrying the N1303 K mutation and compared with elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA).
Results: Proof-of-concept studies have shown that proteins lacking exon 24 are mature and functional. Several lead ASO candidates that efficiently promoted exon 24 skipping were identified. The effect of ELX/TEZ/IVA on 16HBE and HNE cells containing the N1303 K mutation was assessed alone and in combo with SpliSense ASOs in the Ussing chamber system.
Conclusions: Lead candidate ASOs promoting skipping over exon 24 have promising potential for production of functional CFTR channels that can be further enhanced by adding correctors. This strategy has potential to provide clinical benefit for people with CF carrying the N1303 K mutation that currently have no available therapy.

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Administration of SP-101 and doxorubicin results in robust and durable human cystic fibrosis transmembrane conductance regulator minigene transgene expression in the airways of ferrets and corrects human cystic fibrosis airway epithelia in vitro

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Background: SP-101 (AAV2.5T-SP183-hCFTRΔR) is a recombinant adeno-associated virus gene therapy vector composed of a capsid optimized for efficient apical transduction of human airway epithelial (HAE) cultures and regulatory elements that drive expression of a human cystic fibrosis (CF) transmembrane conductance regulator (CFTR) minigene (hCFTRΔR). SP-101 is intended as an inhalation treatment for people with CF, followed by inhaled doxorubicin, a small molecule able to enhance hCFTRΔR messenger ribonucleic acid (mRNA) expression when used in combination with SP-101. To understand the importance of doxorubicin, the dose relationship between SP-101 and doxorubicin for hCFTRΔR mRNA expression in the airways of ferrets and the functional correction of human CF airway epithelia in vitro were investigated.
Methods: SP-101 was applied to the apical surface of primary human CF-HAE cultures grown at the air-liquid interface. Doxorubicin was applied to the basolateral side. Forskolin-induced CFTR chloride conductance was measured via Ussing chamber assay 7 days after SP-101 and doxorubicin addition. SP-101 followed by doxorubicin was delivered as a fine-particle fraction aerosol to the airways of adult ferrets using a nebulizer attached to a plenum exposure system. Expression of hCFTRΔR mRNA and endogenous ferret CFTR mRNA were determined by quantitative polymerase chain reaction optimized for removal of viral genome deoxyribonucleic acid. Ferrets were chosen because SP-101 has demonstrated tropism to the airways of ferrets, and a CF ferret model exists that has many of the pathophysiological features of CF.
Results: Co-administration of SP-101 with doxorubicin restored forskolin-induced CFTR-mediated chloride conductance in CF-HAE cultures with Class I, II, and III mutations. Chloride conductance increased with increasing SP-101 and doxorubicin doses, reaching levels similar to those in non-CF or small-molecule modulator controls. SP-101 alone was insufficient to correct the chloride transport defect in CF-HAE. Similar dose-response relationships were observed in the airways of wild-type ferrets. Inhaled administration of increasing doses of SP-101 and doxorubicin resulted in dose-dependent increases in hCFTRΔR mRNA expression. The highest levels were observed in the lungs and bronchi, followed by trachea and nose. Expression levels of hCFTRΔR mRNA were comparable with those of endogenous ferret CFTR 14 days after administration. hCFTRΔR mRNA expression started as early as 48 hours (earliest time point investigated) and persisted for up to 3 months (longest time point investigated). Comparable hCFTRΔR mRNA expression was also evident in the airways of CF ferrets, indicating successful transduction despite preexisting mucus accumulation.
Conclusions: These data provide a strong rationale for co-development of inhaled doxorubicin to realize the full potential of SP-101 gene therapy for individuals with CF who do not benefit from treatment with small-molecule modulators.

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Development of in vitro transcribed messenger ribonucleic acid therapeutics for cystic fibrosis

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Background: Cystic fibrosis (CF) is a relatively common recessive disease, caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. Absent or nonfunctional CFTR leads to thick, sticky mucus in the lungs, which results in chronic bacterial infection and inflammation. In vitro–transcribed (IVT) messenger ribonucleic acid (mRNA) has emerged in the last few years as a new approach for CFTR protein replacement therapy, but problems of delivery must be overcome.
Methods: We have developed novel receptor-targeted nanocomplex (RTN) formulations consisting of liposomes and receptor-targeting peptide for delivery of CFTR IVT mRNA. The RTNs were first optimized in transfections of primary CF bronchial epithelial (CFBE) cells using reporter IVT mRNAs. We then assessed delivery efficiency of CFTR mRNA in CFBE cells at submerged culture and ALI culture and assessed uptake by qualitative reverse transcription polymerase chain reaction and demonstrated protein expression by Western blot.
Results: We first optimized the RTN formulations, comparing combinations of three cationic liposomes and five peptides for their biophysical properties and transfection efficiency. We identified a novel formulation (size 130 nm, polydispersity index 0.28, charge 38 mV) for mRNA delivery that achieved almost 100% cellular uptake efficiency and 90% transfection efficiency. There were no differences in transfection efficiency between primary normal human bronchial epithelial (NHBE) cells and CFBE cells. The same RTN formulation was able to deliver the mRNAs in air-liquid interface (ALI)-cultured cells and mouse lungs where luciferase expression in mouse lungs was approximately 200 times as high as plasmid deoxyribonucleic acid–encoded luciferase. CFTR mRNA was successfully delivered to NHBE and CFBE cells. In addition, we co-packaged the CFTR corrector VX-809 with CFTR mRNA into RTNs and found that this increased expression of CFTR protein in CFBE cells in submerged culture approximately 2 to 2.5 times. CFTR protein translated from the IVT mRNA was detected by immunoblotting from 4 to at least 48 hours after the CFBE mRNA transfections. CFTR protein expression was also shown in transfections of ALI culture of CFBE cells by functional analysis of ion transport in an Ussing chamber. The change in short circuit current was 10 times as large in cells transfected with CFTR mRNA as in untransfected cells before and after forskolin was added, and it was approximately 59% of normal epithelial cells.
Conclusions: CFTR IVT mRNA delivery is a promising novel therapeutic for CF. The flexibility of the RTN formulation allows co-delivery of CFTR mRNA with VX-809, which significantly improved CFTR expression.