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episode of SARS-COV2 infection, which required adjustment of the dose administered.

**Conclusions:** Our data confirm that ELX/TEZ/IVA treatment is safe, well tolerated, and effective in PwCF. ELX/TEZ/IVA improved pulmonary function and nutritional status and remarkably reduced hospitalization rate. Our data indicate that introduction of ELX/TEZ/IVA in CF care will radically change the natural history of and management approach to the disease.

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**Single-cell ribonucleic acid sequencing reveals pulmonary ionocytes subtypes in proximal ferret airway epithelium**

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**Background:** Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the CF transmembrane-conductance regulator (CFTR) gene leading to loss of or defective CFTR chloride channel function. CFTR is a multi-transmembrane protein that functions as a chloride/bicarbonate channel.

**Objectives:** Our previous work in ferrets demonstrates that CFTR is a well-conserved ubiquitous protein complex expressed on the ER membrane, suggesting that this subtype may proliferate, and mechanisms could lead to a new strategy to enhance mutant CFTR activation in vivo.

**Methods:** Single-cell ribonucleic acid sequencing was performed using the 10x Chromium platform to identify and characterize pulmonary ionocytes in ferrets. We performed western blot and immunofluorescence studies to confirm the expression of ion channels that is consistent with a specialized functional role in ion transport function in the proximal lung epithelium.

**Conclusions:** Our data indicate that introduction of ELX/TEZ/IVA in CF care will radically change the natural history of and management approach to the disease.

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**Ivacaftor boosts cystic fibrosis transmembrane conductance regulator–mediated nasal potential difference in cystic fibrosis transmembrane conductance regulator knockout mice treated with human cystic fibrosis transmembrane conductance regulator messenger ribonucleic acid lipid nanoparticle**

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**Background:** We have previously used lipid nanoparticles (LNPs) to deliver human cystic fibrosis (CF) transmembrane conductance regulator (hCFTR) messenger ribonucleic acid (mRNA) that partially restored CFTR function in CFTR knockout (KO) mice and CF cell models. This is important because 10% of people with CF who lack the specific mutations are not eligible for treatment with the highly effective modulator therapy that is significantly beneficial and effective in people with the F508del CFTR mutation. There is also some evidence of drug intolerability or low response to modulator therapy in eligible patient populations, so alternate approaches to restore CFTR function are needed. A limitation of mRNA LNP is lower functional expression of CFTR protein than in wild-type cells. Chloride ion transport is a function of the product of the number of CFTR channels at the cell surface and the open probability of each channel. Thus, increasing cell surface protein expression or enhancing CFTR open probability is a viable therapeutic strategy to augment hCFTR-mRNA–mediated chloride transport rescue in CF cell and animal models.

**Methods:** We performed western blot and immunofluorescence studies to test the efficacy of various LNP formulations for increasing CFTR protein expression in vitro and in vivo. Nasal potential difference (NPD) was measured in CFTRKO mice after instillation of hCFTR mRNA-LNP formulations before and after treatment with ivacaftor.

**Conclusions:** The newer formulations increased expression of protein in vitro, and ivacaftor perfusion increased hCFTR activity overall by 130.51% (standard error of the mean 27.06%) (n = 11).

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membrane. It is recognized as a membrane domain insertase and plays a critical role in mediating the biosynthesis of multi-transmembrane proteins [1,2], especially the ion channels, but whether EMC regulates CFTR biogenesis is not clear.

Methods: In this study, we generated intestinal epithelial cell–specific EMC subunit 3 (EMC3) knockout (KO) mice by crossing EMC3 flox mice with Villin-Cre mice. Intestinal crypts were harvested and analyzed by reverse transcription polymerase chain reaction, mass spectrometry and western blotting. Intestinal organoids were used to study CFTR function through cyclic adenosine monophosphate (cAMP)- and calcium-dependent pathways and to monitor calcium flux between wild-type (WT) and KO mice.

Results: Although EMC3 KO mice were viable after birth, they were smaller than their WT littermates, indicating a functional impairment of intestinal epithelium in the EMC3 KO mice. Molecular analysis of the protein profile collected by mass spectrometry of intestinal crypts from these mice revealed downregulation of the EMC complex, CFTR, and many other ion transporters. Western blot confirmed significant lower CFTR protein in EMC3 KO villi, whereas CFTR transcription was not altered. Forskolin- and cpt-cAMP-stimulated intestinal organoid fluid secretion was greatly reduced and delayed (Figure 1A). We found that EMC3 deficiency completely inhibited carbachol-mediated intestinal organoid fluid secretion (Figure 1B), whereas muscarinic receptors (carbachol receptors) were upregulated at transcriptional and translational levels in EMC3 KO villi. These data indicate that downstream signaling of muscarinic receptors was compromised in EMC3 KO epithelial cells, and muscarinic receptor–specific agonist Oxotremorine M failed to induce intracellular calcium flux or fluid secretion in EMC3 KO organoids. Pathway analysis of the above mass spectrometry data supported these observations.

Conclusions: We conclude that EMC plays a critical role in vivo in CFTR biogenesis and activation, especially Ca²⁺-mediated CFTR activation. Targeting the EMC could enhance mutant CFTR expression and function.

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CRISPR-based cellular models with endogenous expression of HiBiT-tagged wild-type and mutant cystic fibrosis transmembrane conductance regulator enable high-throughput biology studies and screening for new transporter modulators

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Background: CRISPR/Cas9 technology is a powerful gene-editing tool for insertion of pathogenic mutations in wild-type cystic fibrosis transmembrane conductance regulator (WT-CFTR) that cause cystic fibrosis. Its