Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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: Pulmonary ionocytes (PIs) are distinguished by high expression of the transcription factor FOXI1, V-ATPases, and other ion channels such as cystic fibrosis (CF) transmembrane conductance regulator (CFTR). Consistent with this expression pattern, ionocytes in fish and frogs have confirmed roles in regulating salt and fluid movement, but in vivo functions of ionocytes in mammalian respiratory systems are unclear. Here we transcriptionally profiled ferret airway epithelial cells together with *FOXI1*-Cre^{ERT2} lineage-traced PIs to infer potential roles in mammalian airway function.
Methods: We initially profiled 70 000 proximal tracheal basal cell–derived epithelial cells from wild-type (WT) (n = 4), *FOXI1-knockout (KO)* (n = 4), and *FOXI1*-Cre^{ERT2}::ROSA-TG (n = 3) ferrets using 10x droplet-based 3′ small conditional ribonucleic acid sequencing. Methods for enrichment of the population of rare PIs in our dataset involve upstream isolation of traced (enhanced green fluorescent protein-positive [EGFP+]) ionocytes by fluorescence-activated cell sorting (FACS).
Results: We partitioned ferret airway epithelial cells (FAECs) into seven distinct clusters that we annotated based on known gene expression signatures for basal, secretory, ciliated, hillock, neuroendocrine, tuft, and ionocyte cells. PIs were not captured from FOXI1-KO airway cultures, consistent with FOXI1 being required for PI specification. We profiled 256 PIs from WT and *FOXI1*-Cre^{ERT2}::ROSA-TG ferret airway cultures and defined their consensus expression signature. Ferret PIs express genes for six ATP6V1 family proton pumps, chloride channels (CFTR, CLCNKB, BSND), four family solute carriers (SLCs), sodium channels (SCNN1G, SCNN1A), potassium channels (KCNK5, KCNK1), consistent with unique functions in ion transport. Consistent with other species, CFTR was highly expressed in ferret PIs. We also observed low levels of CFTR in other cell types. Re-clustering of PI transcriptional signatures suggested three subtypes. Type A ionocytes contained 58 differentially expressed genes, many of which were associated with oxidative phosphorylation, oxidative stress response, and tricarboxylic acid (TCA) cycle signaling pathway using ingenuity pathway analysis. Type-A ionocytes were also associated with cell cycle activation of chromosomal replication, suggesting that this subtype may proliferate, and a subset of *FOXI1*-Cre^{ERT2}::ROSA-TG–traced ionocytes in air-liquid interface cultures were EGFP + Ki67+. Type B ionocytes contained 228 differentially expressed genes, including the cation transporter ATP13A5, solute carrier genes SLC38A2 and SLC20A1, potassium channel KCNK5, and major histocompatibility complex II antigen presentation genes (CD74, H2-Aa, H2-Eb1). The Type B ionocytes were also enriched genes relevant to immune processing. Type C ionocytes showed 35 differentially expressed genes having biological functions closely associated with cell movement, a finding consistent with dynamic movement of appendages in time-lapse live ionocyte imaging. The highly expressed SLC34A2 channel in Type C ionocytes also suggests unique function in phosphate homeostasis.
Conclusions: Transcriptional profiling of PIs in ferrets indicates that they are enriched for expression of ion channels that is consistent with a specialized functional role in ion transport in the proximal lung epithelium. We identify putative subtypes of ionocytes, Types A, B, and C, that indicate further cellular specializations in mitochondrial function and replication, antigen presentation, and cell motility, respectively.

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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 (h*CFTR)* 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 hCFTR open probability is a viable therapeutic strategy to augment h*CFTR*-mRNA–mediated chloride transport rescue in CF cell and animal models. In this study, we aimed to increase net ion transport as measured by the nasal potential difference in vivo of mRNA-mediated CFTR rescue using two approaches. First, we varied the LNP formulation to test whether the newer formulations result in greater CFTR protein expression in vitro and in vivo. Second, we tested whether combining *hCFTR* mRNA delivery with the CFTR-potentiating small-molecule drug ivacaftor (VX-770), which increases CFTR open probability, will increase activity of the resultant hCFTR protein.
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.
Results: 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).
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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Endoplasmic reticulum membrane protein complex is required for cystic fibrosis transmembrane conductance regulator biogenesis and activation in vivo

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Background: Cystic fibrosis (CF) transmembrane-conductance regulator (CFTR) is a multi-transmembrane protein that functions as a chloride/bicarbonate channel and plays an important role in maintaining water homeostasis on the apical surface of epithelial cells throughout the whole body. Mutations in the *cftr* gene leading to loss of or defective CFTR function causes CF. Among the efforts to enhance mutant CFTR expression and function on the plasma membrane, intensive studies have focused on the cellular mechanisms mediating CFTR degradation, but CFTR biogenesis machineries are not well understood, and better understanding of these mechanisms could lead to a new strategy to enhance mutant CFTR expression. Endoplasmic reticulum (ER) membrane protein complex (EMC) is a well-conserved ubiquitous protein complex expressed on the ER

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 Ca^{2+}-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 enables 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

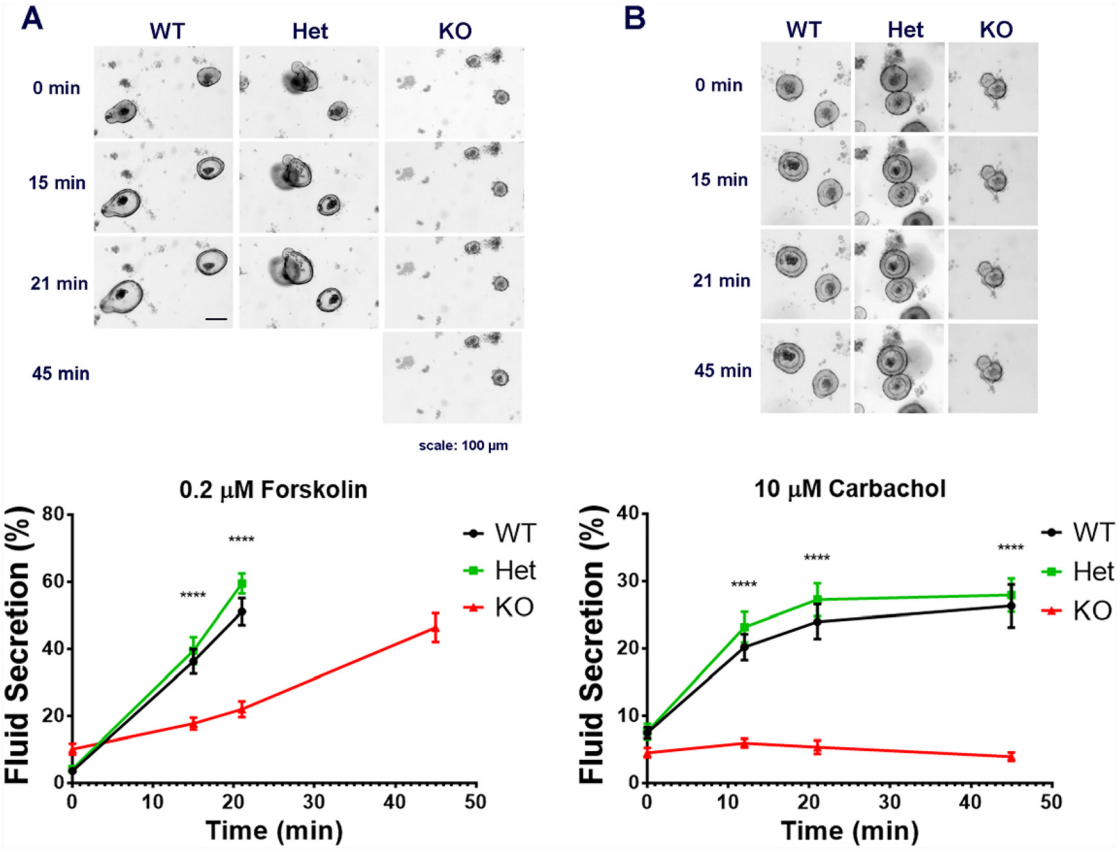

Figure 1. (abstract 667): Endoplasmic reticulum membrane protein complex subunit 3 (EMC3) deficiency impaired cystic fibrosis transmembrane conductance regulator (CFTR) activation. Kinetic study of fluid secretion was performed using mouse intestinal organoids from wild-type (WT), heterozygous (Het), and knockout (KO) mice. CFTR activation was induced by (A) 0.2 µM forskolin and (B) 10 µM carbachol. Statistical analysis was performed between WT and KO groups using unpaired Student t-test. The error bar is mean (standard error of the mean.