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.

signaling, neutrophils were loaded with the Ca^{2+}-sensitive fluorescent dye fluo-4, and fluorescence was measured in response to stimulation with 1 mM thapsigargin using a Tecan plate reader.
Results: Neutrophils were collected from four people without CF and 12 people with CF with a range of CF transmembrane conductance regulator mutations including F508del, L467P, 4209TGTT >A, G551D, and N1303K. Eleven of the12 were undergoing elexacaftor/tezacaftor/ivacaftor treatment at the time of collection. In non-CF neutrophils, thapsigargin induced a 39 ± 5% increase in cytosolic Ca^{2+} concentration over 10 minutes. CF neutrophils displayed a 53 ± 6% increase in cytosolic Ca^{2+} concentration. The rate of increase in cytosolic Ca^{2+} concentration and area under the curve (AUC) over 10 minutes were significantly greater in CF neutrophils than non-CF controls ($p < 0.01$ and $p = 0.04$, respectively), suggesting dysregulated Ca^{2+} signaling. ELD607, but not scrambled peptide exposure, reduced AUC by 30% ($p < 0.01$) in CF neutrophils. Non-CF neutrophils displayed a similar (29%) reduction in AUC that was not statistically significant, probably because of the small sample size.
Conclusions: Collectively, these data indicate that CF neutrophils display exaggerated Ca^{2+} signaling in response to thapsigargin that can be inhibited by ELD607. We predict that ELD607 treatment will benefit people with CF by rebalancing Ca^{2+} signaling in CF neutrophils and reducing inflammation.
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Impact of elexacaftor/tezacaftor/ivacaftor therapy on pathological reprogramming of lung-recruited neutrophils conditioned by cystic fibrosis airway fluid

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Background: Cystic fibrosis (CF) is caused by mutations of the CF transmembrane conductance regulator (CFTR) anion channel, leading to airway polymicrobial infections and neutrophilic inflammation. Prior studies from our group showed that blood neutrophils undergo metabolic, transcriptional, and functional reprogramming upon entry into the CF lung lumen, leading to primary granule exocytosis, immune modulation of T cells and macrophages, and metabolic licensing (dubbed the "GRIM" fate). Although the advent of elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) therapy directed at the CFTR channel has improved outcomes for people with CF, it is unknown if and how it may affect neutrophil reprogramming in their lungs.
Methods: We previously showed that GRIM neutrophils can be mass produced in an in vitro model [1] in which human blood neutrophils are recruited through a differentiated epithelial layer into CF airway supernatant (CFASN) purified from sputum of treatment-naïve patients. Here, we generated and tested the effect of airway supernatant from sputum of patients on ELX/TEZ/IVA modulator therapy (CFMOD) for comparison based on phenotyping (flow cytometry), functional (*P. aeruginosa* killing), and metabolomic ($^{13}C_6$-glucose tracing) analyses of transmigrated neutrophils.
Results: CFMOD-recruited neutrophils contained IVA, demonstrating exposure to the modulator (Figure 1). CFASN/CFMOD-recruited neutrophils showed greater primary granule exocytosis; less *P. aeruginosa* killing; and greater adenosine monophosphate, guanosine monophosphate, and metabolites related to citric acid cycle activity than neutrophils recruited to a control chemoattractant (leukotriene B4). $^{13}C_6$-glucose flux data showed similar rates of extracellular glucose use in all transmigration conditions but higher total glycolysis to lactate in CFASN/CFMOD-transmigrated neutrophils.

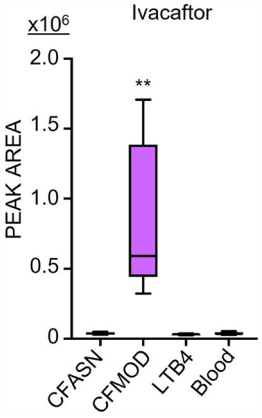

Figure 1. Ivacaftor, one of the modulator drugs, is measured in the cystic fibrosis modulator therapy (CFMOD)-recruited neutrophils but not in the CF airway supernatant (CFASN), LTB4 (chemoattractant control), or blood neutrophils. Excerpt from our pilot metabolomics data on lung-recruited neutrophils showing intracellular metabolites. One-way repeated-measures analysis of variance (<0.05).

Conclusions: Pathological reprogramming of neutrophils upon transmigration into CF airway fluid is not substantially altered by use of ELX/TEZ/IVA modulator therapy. Ongoing transcriptional (ribonucleic acid-Seq) and epigenetic (deoxyribonucleic acid methylation profiling) studies will bring further understanding of this process and suggest potential targets for immunomodulatory therapy to be used as potential adjunct treatment to modulator therapy.
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Mechanisms by which cystic fibrosis transmembrane conductance regulator may influence SARS-CoV-2 infection

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Background: People with cystic fibrosis (PwCF) have chronic, pronounced respiratory damage and have been considered among those at highest risk for serious harm from SARS-CoV-2. Numerous clinical studies have reported that individuals with CF in North America and Europe, although highly susceptible to COVID-19, do not have mortality levels that exceed those of the general population.
Methods: To understand features that might influence lethality of COVID-19 in PwCF, we tested potential relationships between CFTR and viral pathogenesis. As one approach to evaluate impact of CF transmembrane conductance regulator (CFTR) on COVID-19 severity, independent sets of

blood samples from virally infected individuals were genotyped. Blood was obtained from 424 U.S. patients hospitalized with severe COVID-19 and a much larger European cohort of 7147 healthy individuals and 2587 individuals with severe COVID-19. Deoxyribonucleic acid in both studies was probed for the F508del variant. In other experiments, we investigated the possibility that lack of CFTR might alter viral binding and propagation. We used human bronchial epithelial cell (HBEC) monolayers from individuals without functional CFTR for this purpose. Finally, we examined effects of CF airway secretions and features such as viscosity, pH, and protease/anti-protease imbalance during SARS-CoV-2 infection.

Results: We found no evidence of a relationship between deficient CFTR function (based on carrier status for the severe F508del defect) and clinical outcomes from COVID-19. In addition, viral propagation studies using airway epithelial monolayers (a model that reproduces many aspects of in vivo tissue biology) were not influenced by homozygous absence of CFTR. We show that levels of angiotensin converting enzyme-2 receptor messenger ribonucleic acid (mRNA) appear normal in CF primary epithelium, whereas transmembrane serine protease 2 mRNA is variable but lower ($p < 0.001$) in a manner that correlates with viral infectivity ($R^2 = 0.76$). Dependence of viral proliferation on features of CF mucosal fluid—including pH (viral replication optimum at pH 7–7.5), viscosity (diminished propagation in highly viscous apical media), and protease/anti-protease imbalance were identified as likely contributors to efficiency of SARS-CoV-2 replication and pathogenesis.

Conclusions: These findings using patient data, CF and non-CF primary airway epithelia, and CF airway secretions fail to demonstrate a causal relationship between loss of CFTR and susceptibility to severe COVID-19. Notwithstanding the caveat that addition of virus in small buffer volumes disrupts airway surface liquid depth and composition, our findings also argue against a role for CFTR during acute infection of airway cells in vitro. On the other hand, chronic disruption of periciliary liquid, diminished pH, altered protease/anti-protease homeostasis, and increased fluid viscosity (sequelae that occur in CF lungs) were implicated as contributors to impaired SARS-CoV-2 propagation. Such studies provide a basis for future work to test relationships between CFTR and severity of COVID-19.

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Association between myeloperoxidase activity and methionine oxidation products in bronchoalveolar lavage and risk of bronchiectasis in infants and toddlers with cystic fibrosis

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Background: Lung inflammation characterized by exuberant neutrophil influx and high concentrations of myeloperoxidase (MPO) occurs shortly after birth in cystic fibrosis (CF). MPO is the only enzyme in humans that produces hypochlorous acid (HOCl), a strong and promiscuous oxidant, under physiological conditions. We previously associated methionine sulfoxide (MetO), a HOCl byproduct, with MPO, neutrophils, and bronchiectasis using bronchoalveolar lavage fluid (BALF) in a cross-sectional study of early CF. Here, we sought to determine the relationship between MPO and oxidized metabolites and risk of bronchiectasis development in people with CF across a range of ages.

Methods: BALF was prospectively collected from people with CF younger than 6 who developed bronchiectasis at 9 years ($n = 14$) and matched people with CF who did not have evidence of bronchiectasis at 9 years ($n = 14$). MPO enzymatic activity was measured using immunocapture and Amplex Red oxidation assay. Methionine oxidation products were detected using high-resolution mass spectrometry via Q Exactive High Field operated in positive and negative modes after separation using a HILICON iHILIC-(P) Classic high-performance liquid chromatography column. We analyzed Spearman correlations of metabolites with MPO, subsetting for aged 0 to 2 and 3 to 6 and averaging repeated measures.

Results: MPO activity was significantly greater in CF children who developed bronchiectasis than in those who did not (mixed effects $p < 0.001$). MetO and dehydromethionine (dhMet), another methionine oxidation product of HOCl, were significantly higher in BALF of CF children who developed bronchiectasis than in those who did not (mixed effects $p < 0.001$). dhMet, but not MetO, was also significantly correlated with MPO activity in children younger than 2 (Spearman $r = 0.6031$, $p = 0.001$) and aged 3 to –6 (Spearman $r = 0.7351$, $p < 0.001$), without subsetting for future bronchiectasis development. Subsetting for bronchiectasis development revealed correlation between MPO and dhMet in 3- to 6-year-olds who developed bronchiectasis ($r = 0.600$, $p = 0.02$).

Conclusions: MPO is active in the airways of CF infants and toddlers and is higher in patients who develop bronchiectasis later in life. Methionine oxidation products MetO and dhMet, which are linked to MPO activity via HOCl, are associated with future bronchiectasis development. These data extend prior observations with MetO to dhMet and reveal its association with interindividual variations in MPO activity. Further study of these pathways may reveal strategies for treating, preventing, and monitoring inflammation in CF.

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Preinfection knockdown of Muc5b reduces severity of chronic *Pseudomonas aeruginosa* infection in the cystic fibrosis transmembrane conductance regulator knockout rat

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Background: One of the major gel-forming mucins in mucus is Muc5b, which is secreted from submucosal glands in strands and bundles to clear bacteria and other debris from the airway. In cystic fibrosis (CF), these mucins are overexpressed by hypersecreting cells and glands, contributing to mucus plugging and preventing normal clearance of pathogens such as *Pseudomonas aeruginosa*. Our *Cftr*$^{-/-}$ (CFTR knockout [KO]) rat model exhibits submucosal glands that, when mature, lead to development of a mucus defect that recapitulates the human CF lung environment. Data from our lab show that this mucus defect, apparent at 6 months of age in the KO rat model, is correlated with chronic infection with *P. aeruginosa*. Using our novel rat model, we want to assess the effect of altering mucin secretion into the airways on acute and chronic *P. aeruginosa* infection outcomes.

Methods: CFTR KO rats aged 6 months and older received two doses of 20 µg/300 µL Muc5b small interfering ribonucleic acid (siRNA) or scramble siRNA via intratracheal inoculation with 48 hours between treatments. Rats were then intratracheally inoculated with 10^6 colony forming units (CFUs) of the *P. aeruginosa* mucoid clinical isolate PAM57-15 48 hours after last treatment and euthanized 3 or 14 days after infection. Muc5b concentration in the bronchoalveolar lavage fluid (BALF) was determined using dot blot. Inflammatory cells in the BALF were quantified using Diff-Quik staining. Enzyme-linked immunosorbent assays were used to assess levels of important cytokines in BALF. Bacterial burden was assessed by homogenizing and plating lung tissue. Lung tissue was prepared for routine histopathology.

Results: There was no difference in infection outcomes between the treatment groups 3 days after infection. At 14 days after infection, Muc5b concentrations were significantly lower after Muc5b siRNA treatment than in the control. Although there was no difference in macrophage percentages in the BALF between the Muc5b siRNA and control at 14 days after infection, the percentage of neutrophils was significantly lower in the Muc5b siRNA treatment group, indicating less inflammatory cell influx. By 14 days after infection, CFUs in the lung were significantly lower in the Muc5b siRNA–treated group. Histopathological studies of lung and tracheal tissue using Alcian blue periodic acid-Schiff staining at 14 days after infection show reduced plugging of submucosal glands in the